Catalyst

Facets of Biochemistry & Biomedical Sciences

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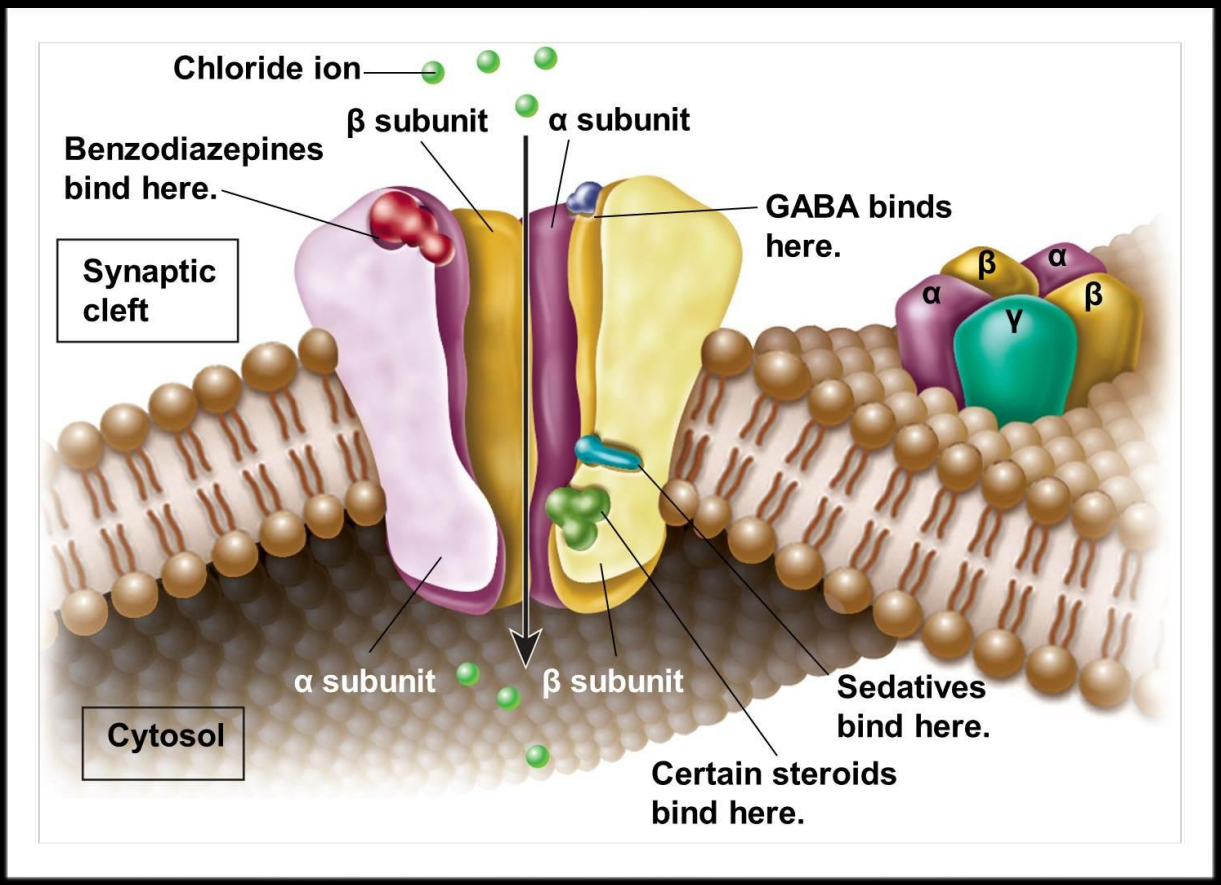

FEATURED IN THIS ISSUE:

Welcome

As graduate students, we identified the need for a Departmental publication, not only to voice our own ideas, but also to give us firsthand experience in the publication process. To meet this need, in collaboration with three undergraduate students, and Dr. Felicia Vulcu, we developed *Catalyst: Facets of Biochemistry and Biomedical Sciences*. Our goal with *Catalyst* was to have a completely trainee-run journal, publishing high-quality research, perspectives, and protocols, while highlighting the innovative work being done within the Department of Biochemistry and Biomedical Sciences (BBS) at McMaster. *Catalyst* is an open-access, peer-reviewed journal rooted in Open Journal Systems that aims to capture the essence of BBS life by showcasing work from undergraduate students, graduate students, postdoctoral fellows, faculty, and staff. There has been a drastic shift in the last decade within both academia and industry that demands trainees be well rounded – having skills beyond an excellent command of the bench. Through *Catalyst*, we hope to facilitate the development of invaluable communication, critical analysis, and leadership skills in all trainees involved in the journal.

In the fall of 2014, we put out a call for volunteers to join the *Catalyst* team, and were overwhelmed with the number of students, at all levels, interested in helping with this initiative. Now, we are happy to present our first issue, both in print and online, containing work from the most elite graduate and undergraduate students from the spectrum of life sciences programs at McMaster. In this issue, you will find publications discussing topics ranging from microbial biology to human disease to pedagogy.

Being actively involved in the process of developing and preparing a journal for publication, creating submission guidelines, and being part of the editing, revision, and publication process, has been an incredible experience. This work is not all our own, however, and would not have been possible without the help of our faculty advisor, Dr. Felicia Vulcu, who has been with us every step of the way. We'd also like to acknowledge Andrew Mocle, Nishanth Merwin, and Teagan Telesnicki, three undergraduate students whose ideas and hard work helped develop *Catalyst* into what it is today, as well as our editorial and review boards. We also want to thank you, our readers, for picking up a copy of our inaugural issue. Science is forever changing, and we feel privileged to be able to catalog a snapshot of the breakthroughs happening within our Department. We hope you are as excited about the first issue of *BBS Catalyst* as we are.

With many thanks,

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The Comparative Analysis of Wild-Type and *F242y/F242a* Mutant Porcine D- Amino Acid Oxidase

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Abstract
D-amino acid oxidase (DAO) is a flavoenzyme that utilizes D-amino acids from the peptidoglycan layer of bacterial cell walls to generate hydrogen peroxide, a known antibacterial agent. The objective of this study is to further explore the currently unknown potential of DAO as an antibacterial agent by examining its activity through active site mutations. In this study, *F242Y* and *F242A* porcine DAO mutants were created by site directed mutagenesis (SDM) and splicing by overlap extension (SOE) PCR respectively. Spectrophotometric assays were utilized to determine the effectiveness of wild type DAO in producing hydrogen peroxide with D-alanine, D- serine and L-alanine. It was determined that D- alanine, the natural substrate of DAO, contained the greatest hydrogen peroxide producing capability. D-serine also produced hydrogen peroxide but not to the extent of D-alanine, while L-alanine demonstrated no production. By utilizing the activity of wild type DAO as a baseline, we hope to create potent DAO mutants that are effective against a greater variety of peptidoglycan D-amino acids. Future implications of this research include the development of DAO injections or orally administered drugs that can combat currently resistant infectious diseases.

Abbreviations
DAO: D-amino acid oxidase protein, SOE-PCR: Splicing by overlap extension polymerase chain reaction, SDM: Site-directed mutagenesis, FAD: Flavin adenine dinucleotide, WT: Wild-type.

Introduction

D-amino acid oxidase (DAO) is an antibacterial agent commonly found in the peroxisomes of kidney, liver, and brain cells[1]. It is also located on the outer surface of neutrophils and catalyzes the oxidative deamination of free but essential D-amino acids from the peptidoglycan layer of bacterial cell walls[1]. DAO exhibits the highest affinity toward hydrophobic amino acids such as D-alanine and D-proline compared to more acidic or basic residues[1]. The oxidative deamination of D-amino acids by DAO occurs in a two-step process. First, a hydride equivalent is transferred from a D-amino acid to a co-factor molecule of Flavin Adenine Dinucleotide (FAD) in a deamination reaction to produce an α-keto acid and ammonia[2]. The reduced FAD is oxidized by oxygen gas resulting in the production of hydrogen peroxide, a reactive oxygen species[2]. Hydrogen peroxide produces free radicals that damage proteins, lipids, and DNA in bacterial cells, which ultimately leads to cell death[3]. DAO is thus utilized by human immune cells as a method to limit the survival of catalase-negative bacteria[3].

Although DAO is known to be involved in many catalytic processes, its biological role in mammals remains unclear[1]. DAO can potentially kill catalase-negative resistant bacteria by oxidizing free D-amino acids surrounding the peptidoglycan layer to produce hydrogen peroxide[1]. In our study, we further explore the currently unknown potential of DAO as an antibacterial agent by examining its activity through active site mutations at phenylalanine 242 (*F242*) of porcine DAO due to its close proximity to the D-amino acid substrate and FAD. Phenylalanine was hypothesized to have a significant role in the enzyme's catalytic ability, as it is a large amino acid and thus would encompass a larger volume of space in the active site. By changing the size of the active site, D-amino acids' ability to enter the active site would be altered. *F242Y* and *F242A* were investigated and it was predicted that the *F242Y* mutation would decrease the bactericidal effect because of the altered hydrophobicity of the active site, which would deny entrance of uncharged D-amino acids. On other hand, the *F242A* mutation would increase this effect due to the increased active site size while maintaining hydrophobicity thus allowing bigger amino acids to enter the cavity.

In this study, the mutant *F242Y DAO* gene was created through site directed mutagenesis (SDM) while the *F242A* mutant was created through splicing by overlap extension polymerase chain reaction (SOE- PCR). A spectrophotometric assay conducted on purified wt-DAO demonstrated that D- alanine, the natural substrate of DAO, produced the most hydrogen peroxide while D-serine, another known substrate of DAO, produced hydrogen peroxide but not to the extent of D- alanine. Furthermore, L-alanine was not able to produce hydrogen peroxide

due to DAO's inability to deaminate L-amino acids. These results are consistent with current findings and experiments with DAO[1]. Future work on this study will include the confirmation of the *F242A* mutant through sequencing. This will be followed by the expression and purification of both mutants for the spectrophotometric and bactericidal assays to determine their respective antibacterial activities.

Results

SOE-PCR and Ligation

Both of the first cycle reactions was done with the inclusion of a temperature gradient to cover a wider range of annealing temperatures in case of incorrect annealing temperatures. PCR was successful for the *F242Y* and the outside forward, inside reverse reaction was successful for the *F242A* mutation (Figure 1). Bands found at 750 bp for the AB reaction and 250 bp for CD suggested success. The CA+D reaction was unsuccessful due to the band found at 1000 bp. The CA+D reaction and the full overlap of F242Y was tested once more and yielded successful bands found at 750 bp and 250 bp for the CA+D reaction (Figure 2). The second round of SOE PCR was successful based on the bands found at 1000 bp at each temperature for the *F242A* and *F242Y* mutants (Figure 3). Transformation of pET26b-*dao* into *E. coli* DH5α was unsuccessful since there were no colonies present on the LB-Agar plate supplemented with ampicillin.

Site Directed Mutagenesis and DNA Cloning

Transformation into XL10-Gold ultracompetent cells was successful only for the *F242Y* mutant. The mutation was verified by sequencing the purified SDM product. F242Y pET3a-dao was successfully transformed into *E. coli* BL21 (DE3) as suggested by colonies present on the LB-Agar plate with ampicillin.

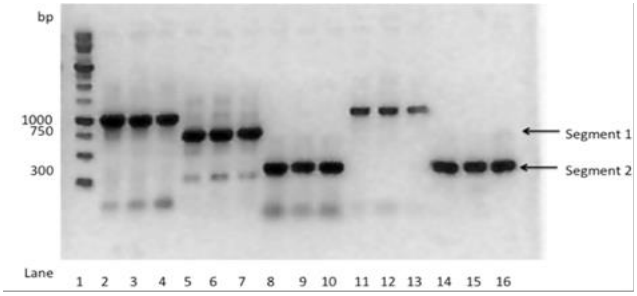

Figure 1. *Successful SOE PCR Amplification of Both Segments for F242Y and Amplification of Segment 2 for F242A.* GelRed was added to the agarose gel to help visualize bands underneath the UV Transilluminator. Full length of the DAO gene is 1026 base pairs and the segments generated from the first round of SOE PCR are 726 and 300 base pairs. Lanes of the gel are as followed: Lane 1-DNA Ladder, 2-WT DAO control at 52ºC 3-54.7ºC 4- 57ºC 5- A+BY reaction at 52ºC, 6- 54.7ºC, 7- 57ºC 8-CY+D reaction at 52ºC 9- 54.7ºC 10- 57ºC 11- A+BA reaction at 52ºC 12- 54.7ºC 13- 57ºC 14- CA+D reaction at 52ºC 15-54.7ºC 16- 57ºC

WT-DAO Expression and Characterization

SDS-PAGE of the samples taken from the Ni-NTA purification as well as cell lysate and the cell pellet was performed. Single bands at approximately 39 kDa were present within each elution fraction (Figure 4). The concentration of first wt-DAO elution fraction was obtained using the NanoDrop spectrophotometric method. An absorbance of 0.8 was observed and the extinction coefficient for DAO is 75 860, yielding a concentration of 1.54 mg/mL using the Beer-Lambert law.

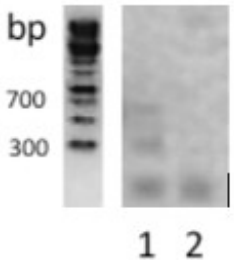

Figure 2. *Successful Amplification of F242A SOE PCR Segments.* 1% agarose gel stained with GelRed was used to separate PCR products. The first lane shows the first round of SOE PCR of the F242A mutant. The bands found at 726 and 300bp showed amplification of segment 1 and 2. Unsuccessful overlap of segments 1 and 2 of the F242Y mutation is shown in the 2nd lane.

Spectrophotometric Assay

Peroxide producing capability of wt-DAO was measured for either D-alanine, D-Serine or L-Alanine through a spectrophotometric technique. With the presence of horseradish peroxidase and o-dianisidine the absorbance at 460 nm is directly proportional to the production of H_2O_2 from the oxidative deamination of D- amino acids by DAO[4]. D-Alanine produced the highest absorbance that correlated to the largest amount of peroxide produced, while L-Alanine produced the least (Figure 5).

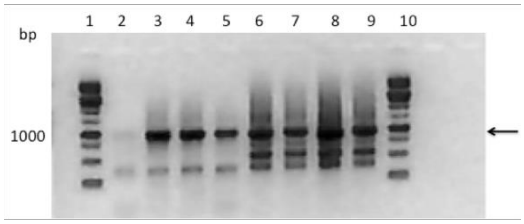

Figure 3. *Amplification of F242A and F242Y SOE PCR Mutants.* 1% Agarose gel stained with GelRed was used to separate PCR products. The arrow at 1000bp verifies the full overlap of DAO, which has a gene size of 1026 bp. Lane 1 and 10 is the 1kb DNA ladder, 2- F242A at 53°C, 3- 55°C, 4- 57°C, 5- 60°C, 6- F242Y at 53°C, 7- 55°C, 8- 57°C, 9- 60°C.

Bactericidal Assay

The bactericidal potential of wt-DAO was measured with D-alanine, D-serine, L- alanine, D-phenylalanine, and D-glutamate. Colonies found on the plate were too numerous to count for each dilution, so bactericidal activity of DAO could not be determined.

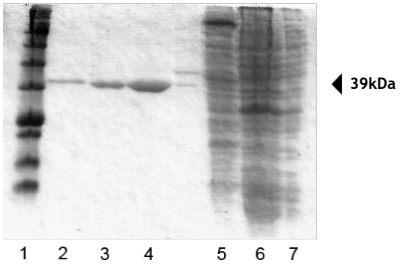

Figure 4. *Wt-DAO protein was expressed and purified from E.coli BL21 (DE3) using Ni-NTA column chromatography.* Purification fraction samples from Ni-NTA chromatography were mixed with Laemmli Sample Buffer and loaded into a 12.5% polyacrylamide gel. The gel was then stained overnight using Coomassie stain and visualized. Lane 1 contained the BLUeye Protein Ladder. 2- wt-DAO elution fraction 3. 3- elution fraction 2. 4- elution fraction 1. 5- wash fraction. 6- flow through. 7- *E.coli* cell lysates. band at 39kDa, as indicated by the arrow correspond to DAO protein.

Discussion

The aim of this study was to elucidate the antibiotic potential of DAO through the development of novel active site mutations. DAO derives its function from its structure which is a result of its amino acid sequence. Alterations to the sequence, especially within the active site, affect the structure and therefore its function. Utilizing functional based assays to comparatively analyze wt-DAO and mutants, the extent of the effects could be quantified.

Our experiment began with the cloning of DAO genes and creation of mutants. The first round of SOE PCR was successful as indicated by the presence of bands at approximately 750 and 250 bps corresponding to segments 1 and 2 of the gene respectively. Successful final round of SOE was indicated by presence of ~1000bp bands that correspond to annealing of our segments resulting in our full gene. All temperature gradients yielded identical results, which confirmed that annealing temperature was not an issue in our unsuccessful attempts. Incorrect primer use was the reason for bands at
~1000 bp in lanes 11-13 (Figure 1). For our final attempt of SOE, we used extra template DNA to ensure there was enough template DNA. This led to the presence of extra bands on our gel that was likely the result of non-specific annealing between homologous segments
(Figure 3). Since *Taq* polymerase catalyzes
DNA at the rate of 1kb/min, a one minute elongation time may not have been sufficient for it to polymerize some gene or gene segments. This may also be a source of error in our failed SOE attempts (Figure 2). Troubleshooting was performed by adding 2 uL of each gene fragment on a temperature gradient of 52°C,
54.7°C, 57°C and 60°C for each overlap reaction.

The SDS-PAGE performed for the Ni- NTA purification fractions indicate that wt-DAO was successfully expressed and purified. The single bands observed in the three elution fractions at approximately 39 kDa correspond to the molecular weight of porcine DAO in the literature[5]. The first elution fraction contained the greatest amount of protein, which was expected since most of the protein was displaced from the resin with the first addition of the elution buffer. In future studies, a western blot could be utilized to identify DAO protein with greater certainty instead of solely basing its identity on molecular weight.

The spectrophotometric assay was utilized as an indirect method to determine the antibacterial activity of wt-DAO. The absorbance at 460 nm is directly proportional to the production of H_2O_2 from the oxidative deamination of D-amino acids by DAO. As H_2O_2 is produced, it is converted to a fluorophore in the presence of horseradish peroxidase and o-dianisidine[4]. The absorbance for D-alanine increased more rapidly compared to D-serine and L-alanine and indicates that D-alanine is the preferred substrate for wt-DAO. D-serine exhibited a relatively lower rate of reaction compared to D-alanine as indicated by a smaller slope in the absorbance graph (Figure 5). This was expected since D-serine contains a hydroxyl group that can disrupt hydrophobic interactions and result in a decreased binding affinity for the DAO active site[2]. L-alanine did not exhibit any significant activity in the presence of DAO. L-amino acids cannot effectively transport their electrons to the cofactor FAD thus inhibiting the first step of oxidative deamination[2]. In future studies, additional amino acids, such as D-glutamate and D-phenylalanine, could be utilized to determine their affinities for the DAO active site. D-glutamate is of particular interest since it is located in bacterial cell walls and could be a target of DAO mutants.

In contrast, the bactericidal assay for wt-DAO was utilized as a direct method to determine its antibacterial activity. The assay produced inconclusive results as all serial dilutions produced too many colonies to count. It is hypothesized that D-alanine would have exhibited the fewest number of colonies while L- alanine would have exhibited approximately the same number as the control. Another 1:100 dilution in PBS would be required to obtain countable colonies in future experiments.

The utilization of DAO has great applications in today's society and presents enormous opportunity to combat one of the greatest problems affecting our generation – antibiotic resistance. The mutant *F242Y* and *F242A* genes can be utilized in future research in order to produce the mutant proteins so that their activity can be determined. This can help in understanding the key amino acids that facilitate its function, and the role of DAO as an antibiotic to combat known resistant bacteria. This unique approach in combating antibiotic resistance is promising, as it does not use synthetic drugs in which bacteria may confers resistance. Rather, it utilizes the radical damage which hydrogen peroxide produces to proteins, lipids and DNA. The main

results that were obtained from the study were the activity of the wt-DAO protein through the spectrophotometric assay. This provides a baseline measure of DAO activity which can be used for future comparisons to that of the mutant. This research can be applied to many different fields crossing the disciplines of biochemistry, molecular biology, genetics and medicine. It can further be used to novel ways to fight the threat of antibiotic resistance including potential DAO topical creams or orally administered drugs to kill bacteria.

Materials and Methods

Wild type pET3a-*dao* was kindly provided by the Dr. Coombes lab (McMaster University, Hamilton).

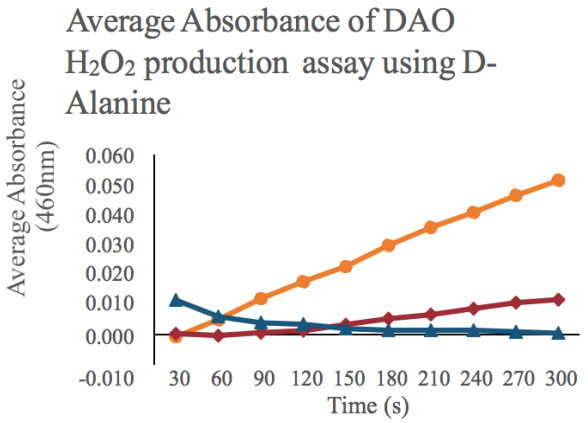

Figure 5. *Wt-DAO can successfully catalyze D-alanine and D-serine, but not L-Alanine*. Wt-DAO was added to a solution containing FAD, o-dianisidine, phosphate buffered saline and D-Alanine, D-Serine or L-Alanine. The absorbance of the reaction was measured at 460 nm using a spectrophotometer. DAO was able to metabolize D-Alanine and produce peroxide much faster than with D-serine. There was no positive change in absorbance in the reaction with L-alanine; DAO was not able to metabolize L-alanine.

F242Y and F242A mutagenesis and cloning

F242Y and *F242A* mutant DAO were created via SDM and SOE-PCR. All of the mutagenesis products were confirmed by sequencing. All primers (Table 1) used were synthesized at the McMaster MOBIX lab (Hamilton, ON). Two fragments of SOE-PCR was produced with wt- pET3a-*dao*, 100 µM of primer A, CA or CY for one segment and primer D, BA or BY for the second fragment. dNTP (FroggaBio), PCR buffer (FroggaBio), 2.5 units of i-Taq™ DNA polymerase (FroggaBio) and nuclease-free water (GeneDireX) were added for a total reaction volume of 50 µL. The Second cycle of SOE-PCR began with the previously amplified products, reagents and Primer A and D. The resultant products from both cycles were separated on a 1% agarose gel and purified using the PureLink® Quick Gel Extraction and PCR purification Combo Kit (Life Technologies) using the centrifuge method. The purified mutants, along with pET26b plasmid were digested with *NdeI* and *XhoI* in buffer O (Fermentas). The digested genes were separated on a 1% agarose gel and purified with PureLink® Quick Gel Extraction and PCR purification Combo Kit using the gel extraction method (Life Technologies). The digested mut- *dao* genes and pET26b were mixed with 0.5 units of T4 DNA ligase (Life technologies, Carlsbad, CA), T4 DNA ligase buffer (Life technologies), and nuclease-free water (GeneDireX) and incubated at room temperature overnight. The ligation reactions were added to two 40 µL of *E. coli* DH5α chemically competent cells (Life technologies,). The transformation was completed using the heat shock method (Sigma-Aldrich). Liquid LB broth (4.6 g of Tryptone, 2.3 g of Yeast, 4.6 g of NaCl; Sigma-Aldrich) was then added to each tube and placed in a cell shaker at 37°C for 30 minutes and spread onto LB plates supplemented with 100 µg/mL kanamycin (Sigma-Aldrich) and placed in 37°C incubator for 24 hours. One colony from the LB agar plate was inoculated into a liquid LB supplemented with kanamycin and grown overnight. F242A and F242Y pET26b-dao was isolated using alkaline lysis method with the PureLink® Quick Plasmid Miniprep Kit via centrifugation (Life Technologies).

Site directed mutagenesis was conducted with pET3a-*dao*, 100 µM of primer E, F, G and H. The QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies) was used for SDM.

Table 1. Collection of primers used for SOE-PCR and SDM, (5'-3')

Primer Name	Sequence
A,Outside forward	CATATGATGCGTGTGGTGGTGATTGG
BA, F242A inside reverse	CCCCACCTGGGCGGTGCCTCCAAGTGTC ACT GCCTGCAG
BY, F242Y inside reverse	CCCCACCTGGTAGGTGCCTCCAAGTGTC ACT GCCTGCAG
CA, F242A inside forward	GGAGGCACCGCCCAGGTGGGGAACTGG AAT GAGATAAATAATAT
CY, F242Y inside forward	GGAGGCACCTACCAGGTGGGGAACTGGAA TG AGATAAATAATAT
D, Outside reverse	CTCGAGTCAGAGGTGGGATGGTGGC
E, F242Y forward SDM	GTGACACTTGGAGGCACCTACCAGGTGGG GA ACTGGAAT
F, F242Y reverse SDM	CCCCACCTGGTAGGTGCCTCCAAGTGTC ACT GCCTGCAG
G, F242A forward SDM	GTGACACTTGGAGGCACCGCCCAGGTGGG GA ACTGGAAT
H, F242A reverse SDM	ATTCCAGTTCCCCACCTGGGCGGTGCCTC CA AGTGTCAC

DAO protein purification

E.coli BL21 (DE3) cells containing pET3a-*dao* plasmids were inoculated into liquid LB containing ampicillin and incubated at 37°C in the cell shaker. At 0.49 OD_{600}, 0.1 M IPTG (Sigma-Aldrich) solution

was added to the culture and incubated at 37°C for the next 3 hours. The cells were then pelleted and lysed using the BugBuster® Protein Extraction reagent (Novagen) containing 25 units/mL Benzonase Nuclease (Novagen) and protease inhibitor (Sigma-Aldrich). The supernatant was loaded into a nickel-nitrilotriacetic acid (Ni-NTA) bead column (Sigma-Aldrich) and eluted with an imidazole gradient of 10mM - 250mM. The supernatant was collected and stored at -20°C. Elution fractions were characterized on a 12.5% polyacrylamide gel via Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS- PAGE).

Spectrophotometric Assay for DAO Activity

5 mM D-amino acid (D-ala, D-ser, L- ala), 1X potassium phosphate buffer, 1.3 µg o- dianisidine, 0.33 µg horseradish peroxidase, 100 µM FAD and 0.308 mg of purified DAO, all obtained from Sigma-Aldrich, were added to a glass test tube in triplicates. Reaction's absorbance is measured at 460 nm every 30 seconds for 5 minutes with a spectrophotometer. The absorbance values were averaged and compiled into a graph using Microsoft Excel.

Bactericidal Functional Assay

E. coli DH5α was cultured in LB broth (5 g yeast extract, 10 g Tryptone, 10 g NaCl; Sigma-Aldrich) until an OD_{600} of 0.6 while shaken at 37°C at 200 rpm. A 1:100 dilution of the culture was added to a solution containing 0.5 mM amino acid, 200 µL of 1.54 mg/mL DAO and 10 µL of 10 µM FAD for a final volume of 200 µL. Each reaction was placed into a well on a 96 well plate, in triplicates, followed by a 10^{-6} serial dilution. 7 uL of each dilution was then pipetted onto LB agar plates and incubated at 37°C overnight. Colonies were counted the next day.

Acknowledgements

We would like to thank our mentor Brian Tuinema for his guidance on the project and Shari-Ann McCollins, Meagan Heirwegh and Felicia Vulcu for the lab space and their assistance. Some protocols were modified from material completed in BIOCHEMISTRY 2L06: Inquiry in Biochemical Techniques, Fall 2014, McMaster University'. We would also like to thanks Dr. Brian Coombes for providing us with the plasmids used in this study.

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Investigating the Mechanisms of Antimicrobial Resistance

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Abstract
Since their discovery, antimicrobial agents have revolutionized the treatment of infectious disease. Increased use of these agents, however, contributed to the concomitant development of microbial resistance, rendering these treatments less effective, or often useless[1]. Despite considerable pressures on the scientific community, few new classes of antimicrobial agents have been discovered since the antibiotic era (1950-1970). Microbes' astonishing ability to adapt to antibiotics poses a serious threat to the modern health care system[2]. In order to reduce the prevalence of resistance and develop new antimicrobial agents, it is crucial to understand the origins of antibiotics, the development of resistance through evolutionary mechanisms, and the biochemical mode of action of antibiotics along with their associated resistance pathways. This review investigates the various mechanisms of antibiotic resistance, from both an evolutionary and biochemical standpoint. Microbes are able to adapt and mutate at unparalleled rates through mechanisms such as horizontal gene transfer and high reproduction rates[3]. Acquired resistance mechanisms include modifying enzymes, point mutations in the target site of antibiotics, and reduced uptake of antibiotics[4]. This paper concludes by considering responses to the current crisis in microbial resistance, such as preventative measures and the development of new antibiotics.

Introduction

The introduction of antibiotics to treat infectious disease was one of the most significant medical advances of the 20th century. Initially, it was easy for society to disregard the few early cases of antibiotic failure in favour of their astronomical cure rates. As a result, many consider antibiotic resistance a recent problem[2]. However, human use of antibiotics, together with microbial resistance, predates their modern use. For example, ancient remains of Nubian people from 350-550 CE have been found to contain traces of antibiotic compounds, and traditional Chinese medicine has incorporated the use of plant-based antibiotics for thousands of years to treat an array of illnesses[2]. Resistance to major antibiotics such as penicillin and streptomycin was also reported almost immediately after their discovery[2]. Despite this, persistent and widespread ignorance of resistance and the misuse of antibiotics have created a contemporary medical crisis.

The antibiotic revolution started in the lab of Paul Ehrlich (1854-1915). With the observation that common dyes affected certain strains of bacteria differently due to their chemical composition, he developed a novel screening program to search for compounds that targeted syphilis, a prominent disease of that time[5]. In1909, Ehrlich successfully identified the antibiotic arsphenamine (Salvarsan). It was considered the "magic bullet" for syphilis and was the first recorded compound with antimicrobial activity[6]. Coincidently, Ehrlich was also studying resistance in several organisms. He noticed that the practice of delivering drugs by increasing the dosage until it was therapeutically effective was leading to an increase in resistance; he therefore recommended maximal dosages for treatment. By 1913, although the exact mechanisms for resistance were unknown, the idea of drug resistant bacteria was common knowledge of the scientific community[7].

Alexander Fleming (1881-1955) is famous for his serendipitous discovery of penicillin; however it was not until Ernst Chain (1906-1979) and Howard Florey (1898-1968) published a paper on the antimicrobial value of penicillin and optimized purification techniques[8] that the antibiotic era dawned[2]. For decades, many new classes of antibiotics were synthesized or purified, resulting in a dramatic drop in infectious fatalities and the belief that the battle against microbes had been won[6]. One of the most shocking aspects about widespread use of antibiotics in this era was the scientific community's negligence, as the ability of bacteria to rapidly adapt was known, but not acknowledged[9]. The antibiotic era was followed by a dry spell of three decades during which resistance continued to grow and the rate of antibiotic discovery plummeted as pharmaceutical companies switched to more lucrative fields. This left a void in treatment options for patients with microbes resistant to drugs available at the time (Wright, G. (2014). In-person Interview.). Finally, in 2000, the dry spell was broken by the development of linezolid, the first of the class of oxazolidinones, followed by daptomycin in 2003. These antibiotics could kill bacteria resistant to a variety of older antibiotics, making them extremely attractive to the medical community[10]. Society needs to use these historical lessons about misuses of these drugs to protect the effectiveness of antibiotic treatment.

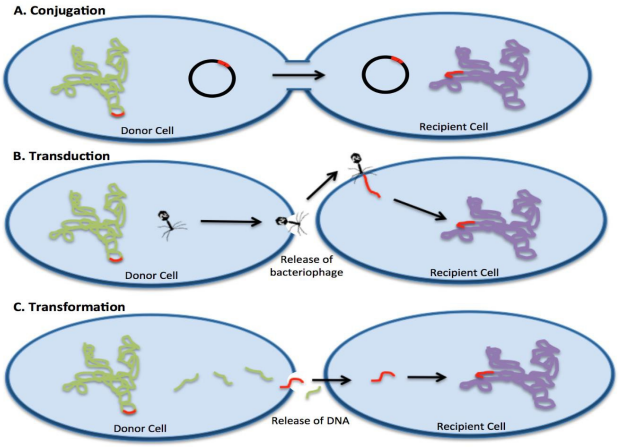

Figure 1. *Horizontal Gene Transfer Mechanisms.* (A) Bacterial Conjugation occurs though direct contact of the cells allowing the formation of a bridge in which replicated plasmids containing the r genes move. (B) Transduction is facilitated by the infection of bacteriophages that integrates the r genes into the hosts DNA along with bacteriophage DNA. (C) Bacterial transformation occurs when DNA containing r genes from one cell is released into the environment and is taken up by the other cell. R genes are indicated in red, donor DNA in green, and recipient DNA in purple[11].

Antibiotic resistance is now a common term, and society is starting to experience its significant implications. Researchers are feeling the pressure to learn about mechanisms of resistance in order to develop novel treatments and stay ahead of the microbes. However, to date, microbes have demonstrated an uncanny ability to adapt to antibiotics due to their high reproducibility, ability to acquire foreign DNA, and their high spontaneous mutation rates[1]. Consideration of key processes involved in the bacterial transfer of genes and the associated biochemical mechanisms of antibiotic resistance is vital to understanding resistance now and for future generations.

Evolutionary Mechanisms of Antibiotic Resistance

Resistant genes (r genes) have been discovered in soil samples that are over 300 000 years old, indicating that they were present in microbes that could not have come in contact with modern antibiotics (Wright, G. (2014). In-person Interview.). R genes make up the antibiotic resistome (a collection of antibiotic resistant genes) and can exist in any microbe as either cryptic or expressed (Brown, E. (2014). In-person Interview.). Since bacteria reproduce at unparalleled rates, r genes spread rapidly to produce entire colonies of resistant organisms. Microbes have developed a plethora of mechanisms, not only for developing resistance, but also for sharing it amongst themselves[9].

Table 1. Summary of target function of antibiotics and associated classes of antibiotics that participate in each type of inhibition[4,14,15]

Process	Target	Reason for Selective	Common Examples
Interference with Cell Wall Biosynthesis	Enzymes that synthesize and cross-link peptidoglycan or prevent the peptide substrate from reacting with the transpeptidases or transglycosylases	Mammalian cells lack peptidoglycan	β-lactams (penicillin, cephalosporins) Glycopeptides (vancomycin, teicoplanin)
Bacterial Protein Synthesis Inhibition	Bacterial ribosomal subunits (50s, 70s and 30s)	Protein synthesis in eukaryotic cells occurs via the 80s ribosome	Macrolides, Tetracyclines, Aminoglycosides, Chloramphenicol, Linezolid, and Clindamycin
Interferences with Nucleic Acid Synthesis	DNA gyrase and RNA polymerase that prevent DNA replication	Not as selective as mechanisms are shared between eukaryotic and prokaryotic cells	Quinolones (Fluoroquinolones), Rifampicin, and Metronidazole
Inhibition of Metabolic Pathways	Folate Biosynthesis	Folic acid synthesis in bacteria differs radically	Sulfonamides, Trimethoprim
Disruption of Membrane Function	Membrane, Efflux Pumps	Not as selective as mechanisms are shared between eukaryotic and prokaryotic cells	Polymycins and Amphotericin B

Mutation

The simplest mechanism by which microbes develop resistance is through spontaneous mutation. All cells have mechanisms to protect against mutations, such as proofreading enzymes that reduce the average mutation rate from 10^{-5} to 10^{-7} errors for every nucleotide synthesized. For bacteria, because of their high replication rate, this results in approximately three mutations every hour[9]. Rarely will a single mutation allow a cell to evade death from an antibiotic; however, given ample time and the occurrence of stepwise mutations, microbes have an increased chance of success[9].

Mutation can also result from the selective pressures initiated with the use of antibiotics, as they encourage the spread of r genes through favourable selection, and induce mutations through SOS mechanisms. When bacteria are under stress they begin to synthesize an SOS gene sequence, producing more proteins involved in DNA synthesis and repair. These proteins are not as precise and result in a higher mutation rate[9]. Occasionally, mutator mutations can occur. These are errors in proteins that synthesize or repair DNA, and result in an increased mutation rate of up to 100 fold[9].

Bacterial Promiscuity

Horizontal gene transfer (HGT) is the ability for bacteria to share DNA between organisms through methods other than reproduction as shown in Figure 1. In order for HGT to have an effect, bacteria must be able to uptake and incorporate foreign DNA[3]. Conjugation is the most common HGT mechanism and is dependent on the presence of plasmids. In conjugation, plasmids from the donor microbe are replicated and one copy is transferred into the recipient cell, which can then be amalgamated into the chromosomes of the recipient (Figure 1A)[3]. Transduction, another mechanism of HGT, is facilitated by bacteriophages (viruses that use bacteria as hosts to reproduce). Occasionally, bacterial DNA is integrated into the DNA of the bacteriophage progeny. When these progeny infect other bacterial cells, the initial bacterial DNA is also transferred into the new host. The bacteria can live with these new genes for some time if the virus enters a lysogenic state (Figure 1B). Finally, transformation occurs when DNA is released from one cell and taken up by another through the cell membrane to be incorporated into the host cell (Figure 1C)[9]. Awareness of gene transfer through the microbe community allows us to better understand how r genes are transmitted and can aid in anticipating future resistance. In order to avoid returning to the pre-antibiotic era, we must anticipate and prepare for resistance, as the bacterial evolutionary mechanisms in place will continue to operate.

Biochemical Mechanisms of Antibiotic Resistance

How Antibiotics Work

The mode of action (MOA) of all antibiotics is to interfere with vital bacterial function thereby inhibiting their growth, while maintaining minimal toxicity to the patient[12]. Antimicrobial agents can be either bacteriostatic (agents that inhibit multiplication) or bactericidal (agents that directly kill the bacteria)[4]. Antibiotics achieve their low toxicity to eukaryotic cells by targeting cell components or processes specifically unique to bacterial cells (e.g. peptidoglycan, bacterial ribosomal subunits, folate biosynthesis, etc). The processes that the major classes of antibiotics disrupt are summarized in Table 1[13].

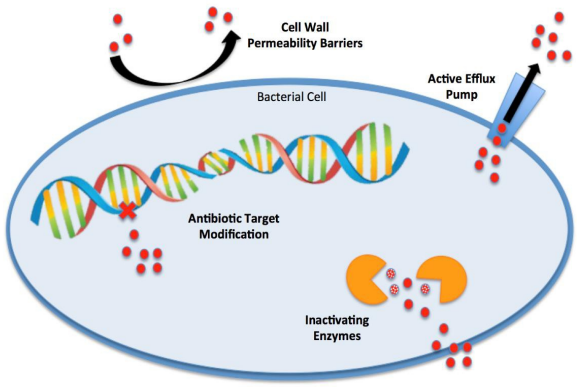

Figure 2. *Biochemical Mechanisms of Antibiotic Resistance*. Diagram of common mechanisms employed in bacteria associated with antibiotic resistance. Mechanisms include target modification, inactivating enzymes, active efflux, and modification of cell wall permeability. Mechanisms are not standalone within bacteria and within various strains of bacteria, thus adding complexity such as multiple drug resistance and cross-resistance. Antibiotics are indicated as red circles.

Development of Resistance

The major mechanisms of resistance correspond to the main MOA of antibiotics and include enzymes that inactivate the microbial agent, mutations in the target site that reduce the binding ability of the antibiotic, and devices that decrease the amount of antibiotic through reduced uptake or increased efflux[16]. These major classes of mechanisms and corresponding examples are outlined in Figure 2 and Table 2. Acquired resistance mechanisms are amplified by the selective pressures resulting from the misuse of antibiotics and present a major threat to the future of antibiotic therapy[13].

Target Modification

One major method by which bacteria acquire resistance is through point mutations in select genes[18]. These mutations can arise spontaneously, or through the mechanisms of HGT as discussed previously. Mutations commonly occur at the binding site of the antibiotic target protein and result in an altered structure or conformation to reduce binding affinity of the antibiotic[7,14]. For example, target modification used by the bacterium vancomycin-resistant enterococci (VRE) reduces the binding affinity of vancomycin by 1000 fold, rendering it nearly ineffective[13].

Enzyme Inactivation

Certain enzymes within bacteria have the ability to alter the structure of antibiotics so as to render them ineffective[4,19]. The precise mechanism varies with both the bacterial strain and the antibiotic, however most mechanisms are similar amongst similar classes of antibiotics[19]. A characteristic example is enzymes that inactivate β-lactams, such as penicillin. β-lactamases hydrolyze the β-lactam ring, which prevents the inhibition of the final cross-linking step in peptidoglycan formation by the antibiotic and renders the antibiotic ineffective. Other

enzymes have the ability to add chemical substituents to an antibiotic, reducing target binding affinity[4]. For example, certain enzymes act on the amino and hydroxyl groups of aminoglycosides to add substituents such as a phosphate groups. With the added substituents, the drug can no longer bind to its ribosomal target to inhibit protein synthesis[19].

Table 2. Summary of the common types of resistant mechanisms and examples of known antibiotics affected by each form of resistance[4, 12, 17, 18]

Types of Resistance	Examples
Target Modification	An amino acid substitution by VRE alters the D-Alanyl-D-Alanine structure in vancomycin, which prevents cell wall crosslinking. Vancomycin binds to the D-Ala-D-Ala terminus via hydrogen bonding at its active site, inhibiting the formation of peptidoglycan. The mutation alters an amide linkage to an ester linkage, which reduces the number of hydrogen bonds and introduces electronic repulsion, restricting antibiotic binding.
Enzyme Inactivation (Inactivation of β-lactams)	β-lactamases break and open the β-lactam ring (essential to inactivate transpeptidases in the cross-linking formation process), which renders the antibiotic ineffective.
Enzyme Inactivation (Add Chemical Substituents)	Enzymes add phosphate groups on the amino and hydroxyl group of aminoglycosides, which inhibit the antibiotic from binding to ribosomes to inhibit protein synthesis.
Enzyme Inactivation (Via Point Mutations)	Mutations in the gene that encodes for the enzyme dihydrofolate reductase eliminates the binding affinity of trimethoprim and its enzyme inhibition action.
Active Efflux	A resistance mechanism seen in an array of antibiotics, such as tetracycline, as it is not a standalone mechanism.
Cell Wall Permeability Barriers	Mutations in the antibiotic binding proteins in bacterial cell wall can reduce affinity to antibiotics such as β-lactams and glycopeptides to reduce the overall permeability of the cell wall.

Lastly, enzyme inactivation can occur via point mutations. Mutations result in amino acid substitutions that alter the structure and chemical properties of the antibiotic binding site, which produce drug resistant forms of key enzymes that regulate vital metabolic activity[13]. An example of this is resistance to trimethoprim, whereby mutations in the gene encoding the enzyme dihydrofolate reductase eliminate the binding affinity of the drug and thus its enzyme inhibition action[14].

Active Efflux and Reduced Permeability

Bacterial cells can develop resistance through active efflux and reduced permeability, which lower the intracellular concentrations of antibiotics. Active efflux occurs as a result of the overexpression of genes that code for efflux pumps when the antibiotic is detected in the cell[4]. The antibiotic is then removed from the cell at a faster rate than it enters, resulting in ineffective intracellular concentrations[14]. Cell wall permeability can be reduced through mutation of the antibiotic binding proteins in the cell wall, leaving it less susceptible to lysis, or through the reduced formation of crosslinks[4]. Both of these mechanisms often act as an enhancement that accompanies other types of resistance mechanisms. In addition, bacteria with alterations in permeability and efflux mechanisms must be able to withstand the associated reduction in nutritional intake[13].

Resistance is Complex

In addition to these complex individual mechanisms, researchers are also faced with phenomena such as cross resistance (developed resistance to antibiotics that are chemically related) and multiple drug resistance (acquired resistance to unrelated chemical compounds due to the synergistic effects of the outlined mechanisms)[13]. As well, individual classes of antibiotics can be resisted simultaneously through multiple mechanisms[20]. Understanding the biochemical mechanisms behind resistance is crucial to improving existing antimicrobial reagents and for designing new classes of antibiotics that are not hindered by currently known mechanisms of resistance.

Conclusion and Future Directions

Antimicrobial resistance is an ancient complication. R genes have been circulating the resistome for nearly 3.8 billion years, and will continue to accumulate with time[12]. The development of antibacterial resistance was inevitable because of the sheer number of microbes, their short replication time, and the gene mutation frequency of bacteria. Bacteria have complex mechanisms allowing them to share selective resistant genes that code for mechanisms such as efflux pumps, target modification, reduced permeability, and modifying enzymes that allow the bacteria to avoid the harmful effects of antibiotics[16].

Humans play an integral role in the development of resistance. The misuse of antibiotics dramatically increases the rate of resistance through selective pressures[9]. Society must adhere to strict use protocols to limit the spread of antibiotic resistance. These include dose concentration and duration guidelines[9], ensuring antibiotics are prescribed only

for bacterial infections, and eliminating the use of sub-threshold doses of antibiotics for agricultural practice[15].

The latter is especially important because transmission of resistant genes from animals to humans quickly results in the antibiotic becoming ineffective in human treatment[1]. While agricultural use of antibiotics may have many economic benefits in terms of yield and product quality, the negative impact of this practice on the future of health care may be monumental.

In order to develop new antibiotics, we must look beyond the scope of what is known. One new avenue under investigation is the use of analog structures of natural molecules that have co-evolved with bacterial resistance to produce semi-synthetic derivatives[1]. Another approach is to synthesize molecules that are more closely related to their natural counterparts. This may result in chemical libraries of molecules that are able to penetrate cells and avoid efflux. It is predicted that antibiotics will become very specific towards the target site and the type of infection. This type of treatment goes alongside the development of highly technical diagnostic assays to determine the characteristics of a particular infection (Brown, E. (2014) In-person Interview).

A full appreciation of both the history and emergence of antibiotic resistance, in addition to the evolutionary and biochemical mechanisms of the development of resistance will enable researchers to better understand areas of success and failure for future improvement of the treatment of infectious disease. It is only through this understanding that we can avoid an era in which humans are once again at the mercy of microbes.

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Altering Testing Styles in Biology Courses to Emphasize the Application of Course Content

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As I sat down to write my first biochemistry midterm, I anxiously scrawled out my name on the Scantron sheet. We were reminded for the umpteenth time to write with a number 2 pencil and then our start time finally arrived. I still remember the first question, what is the nucleotide sequence targeted by the EcoRI restriction enzyme? My heart sank.

I had not thought it prudent to dedicate space in my grey matter to memorize this detail. To me, it did not seem like a detail worth testing. Why would I have to memorize these six little letters, when I could look up the answer within seconds? Is it not more important to know that these target sequences can be used to isolate DNA fragments? While this question caught me by surprise, I only had myself to blame because this type of question is quite common.

There is a common perception among McMaster students that courses that fall under the biology umbrella are notorious for asking very fact oriented questions while underemphasizing what those facts may actually be used for. I have heard the exams be likened to a bland game of Jeopardy. Both require you to answer questions about esoteric topics, but little critical thinking is actually necessary. Notably these opinions are not just the product of disgruntled imaginations, nor are they isolated to McMaster. A study surveying 77 introductory biology courses across the U.S. revealed that many examination questions targeted lower cognitive levels, or in other words, the factual based questions that I previously mentioned[1].

In my experience, there are two types of questions that make up an exam in biology. The first is the factual type. These questions test your knowledge about the details of the system and as long as you have memorized the textbook, you should know the answer. An example of this could be "what neurotransmitter is used in the sympathetic nervous system?" These questions are straightforward and do not require much critical thinking. The other type of question requires students to apply their knowledge. I feel these questions more effectively test the students understanding of the content because it usually requires students to think about how the pieces of knowledge are interconnected and relate to the whole picture. An example could be: "The sympathetic nervous system in this organism is defective. Describe what factors could be defective and how it would lead to the observed phenotype". These questions usually incorporate multiple aspects about a topic and they motivate the student to think about how they are linked to each other.

Ultimately the goal of learning the class material is not just to be able to recite it on a Scantron sheet, but instead, to be able to synthesize the knowledge and apply it to novel situations to try and make new discoveries. Education should aim to create intellectually self- sufficient adults so that we may make sound and independent judgments about the world around us[2]. It is to the benefit of society as a whole to cultivate higher level thinking skills so that we may find new solutions to present day issues like climate change or diseases like cancer[3].

That is why the factual type questions are not ideal. The emphasis on the recall of material promotes the superficial understanding of knowledge. However, in order to solve more complex problems in the real world, students must be able to think about how what they have learned may be applicable in a new situation[3]. I am not advocating that courses should ignore the underlying facts altogether. Facts must be taught in order for the overarching concepts to have any relevance[4]. However, I do believe that testing methods in many biology courses have remained pedantic by putting such a large focus on the details.

One method in moving away from the fact-focused style of testing is to decrease or remove multiple choice questions from exams. These questions are pervasive in higher education institutions, and from my experience McMaster is not an exception. Indeed, a 2014 review on the topic of assessment discussing trends and opportunities highlighted this issue[5]. Multiple choice questions are effective in having students discern scientific truths, but they lack the ability to promote critical thinking[6]. Instead there should be an increase in the proportion of written answer questions where the students are presented with a problem and they must propose a solution. The open-ended nature to these questions is more effective in having the student showcase their knowledge in context and it encourages the student to think about how what they have learned applies to new situations[6].

While writing multiple choice questions capable of targeting higher level thinking skills is another viable solution, this method may not yield the most

fruitful results. Designing higher level multiple choice questions is often challenging and most faculty have not received proper training for this task[7]. Furthermore, given that the majority of multiple choice questions currently target lower level thinking skills, students now associate multiple choice questions with memorization and their study habits reflect this perception. A study comparing the study habits of students tested with only multiple choice questions and students who received written answer in addition to multiple choice questions showed that students receiving both types of test questions more frequently used cognitively active study habits. Even when explicitly told that the multiple choice questions were designed to test higher level thinking skills, students expecting only multiple choice questions still used cognitively active study habits less frequently than the students in the other cohort[8].

That is why I believe that there should be a shift to increase the proportion of application questions to reflect a more holistic approach to biology. This change can help create the next generation of scientists to further humanity's quest for knowledge.

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Recent Biochemical Developments in Epilepsy: Case Study of Glutamate and GABA

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Abbreviations
GABA: gamma-aminobutyric acid, $GABA_A$: gamma-aminobutyric acid receptor A, $GABA_B$: gamma-aminobutyric acid receptor B, AMPA: alpha-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid, NMDA: N-methyl-D-asparate, K^+: potassium ion, Na^+: sodium ion, Cl^-: chlorine

Introduction

Epilepsy is a neurological disorder characterized by recurrent seizures that cause changes in attention or behaviour[1]. Epileptic seizures occur due to abnormal, excessive, and hyper-synchronous neuronal activity in the brain[2]. Currently, antiepileptic drugs are ineffective for 30% of all epileptic patients and most only provide short-term relief and have high levels of inter-patient variability in treatment effectiveness[3-5]. These treatments do not directly address the core causes of epilepsy, which include acquired damage to neural circuits, congenital abnormalities, and genetic deficiencies[5]. This is attributed to a lack of understanding of the molecular pathways responsible for epileptogenesis[6]. Because it affects approximately 50 million people worldwide, it is critical to understand the underlying biochemical mechanisms contributing to this condition to develop more effective therapeutics[7].

Current Role of Biochemistry In Epilepsy Research

The majority of our current understanding of epilepsy has come from clinical and epidemiological studies that provide us with minimal information on the etiology of the disease[8]. With developments in technology, biochemical investigations have helped to improved our understanding of the fundamental mechanisms underlying epilepsy, including the key neurotransmitters involved[2]. Below, we will discuss how novel biochemical investigations into the neurotransmitters glutamate and gamma-aminobutyric acid (GABA) have advanced the field of epilepsy research.

The Role of Glutamate and GABA in Epilepsy

The major excitatory neurotransmitter is glutamate and its activity has been linked to epilepsy[9]. The ligand-gated ion channel (ionotropic) class of glutamate receptors include alpha-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid (AMPA), kainate, and N-methyl-D-aspartate (NMDA) receptors. Upon ligand binding, these glutamate receptors cause a net influx of Na^+ ions that depolarizes the neuron. Ionotropic receptors contribute to hyper-excitation of neurons, which plays a key role in epileptic seizures[10].

The major inhibitory neurotransmitter is GABA and its receptors are $GABA_A$ and $GABA_B$. $GABA_A$ are ligand gated Cl^- ion channels that cause Cl^- influx and $GABA_B$ are coupled with secondary messengers that cause K^+ efflux. Both receptors generate inhibitory postsynaptic potentials in neurons[10].

Normally, the Glutamate-GABA axis is held in equilibrium by physiological mechanisms. Glutamate and GABA released into the synapse are recycled by neighbouring astrocytes via active transport into the cell and catabolism into glutamine[11]. Deregulation of this axis results in increased excitability, excitotoxicity, and epileptic activity. A loss of function in glutamine synthetase, which metabolizes glutamate into glutamine, has been linked to neuronal hyper-excitability[11]. Abnormal glutamate release by glial cells can also cause spontaneous excitation of surrounding neurons[12]. Moreover, certain seizure activity has been attributed to insufficient GABA synthesis, such as those seen in glutamic acid decarboxylase cofactor deficiencies[13]. Overall, excessive stimulation of glutamate receptors or under stimulation of GABA receptors results in hyper-excitation of neurons and epileptic activity[14].

The integral role of the Glutamate-GABA axis in regulating neuronal excitability makes it a potent therapeutic target in the treatment of epilepsy. However, there has been a lack of momentum in translating the vast amount of biomedical research into effective therapies, primary due to poor clinical trial results[15]. Many recent findings have provided insight into the mechanism underlying the Glutamate-GABA axis and its relation to neuronal excitability. These findings provide new ways of approaching the treatment of epilepsy.

Successes in Translating Glutamate/GABA Studies into the Clinical Setting

Current biochemical research focuses on targeting glutamate and GABA receptors to maintain a balance in excitatory and inhibitory

neurotransmission[14]. To date, the role of many different classes of ligand-gated glutamate receptors in epilepsy have been studied. AMPA and NMDA glutamate receptors are the primary mediators of excitation in the central nervous system and have been widely targeted for the treatment of epilepsy[16]. AMPA and NMDA antagonists, such as Perampanel and Felbamate, have shown moderate anticonvulsant effects[17].

A newly emerging field of research on a less widely studied class of glutamate receptor, known as kainate receptors, has recently generated significant interest. Topiramate was FDA approved in 2012 as a kainate receptor antagonist for the treatment of epilepsy. In a meta-analysis of seven double-blind, randomized controlled trials of adults with treatment-resistant partial-onset seizures, 41% of topiramate-treated patients exhibited a ≥50% reduction in seizure frequency compared to 15% in the placebo control[18]. Furthermore, benzodiazepines, a class of drugs that act as positive allosteric modulators of the $GABA_A$ receptor, have been approved as efficacious anticonvulsants[19]. By strengthening inhibitory GABA signals, benzodiazepines such as diazepam and clonazepam reduce risk of epileptic seizures[20]. A randomized trial involving 258 adults showed that diazepam reduced duration of seizures by 33%[21].

The extensive understanding of the glutamate and GABA receptors provide a strong biochemical foundation for drug discovery and greatly improve the probability of designing glutamate/GABA-related antiepileptics with high clinical success. One major limitation of biochemical approaches is that they may not accurately predict clinical success. However, these models provide the basic understanding necessary to develop effective therapeutics. The lack of potent drugs may be attributed to incomplete biochemical investigations before moving them to clinical trials[15].

Conclusion and Implications

Current generation antiepileptics have high inter-patient variability in terms of treatment effectiveness, and matching a patient to a successful drug largely depends on trial and error[3,5]. To create broad spectrum, lasting antiepileptics, future ventures should attempt to further grasp the underlying biochemistry before initiating clinical trials. The past decade of research into the protein structure and function of glutamate and GABA receptors has provided scientists with opportunities to target these receptors for therapeutic purposes. The current challenge stems from the ubiquitous presence of glutamate and GABA receptors which are important in maintaining proper neural activity. It is crucial to develop highly specific drugs that target these receptors in areas of the brain most prone to epileptiform activity. Importantly, molecules targeting ligand-receptor interactions are not the only effective therapeutic targets. Biochemistry provides us with the tools to understand the synthesis, synaptic release, and termination of glutamate and GABA, allowing us to develop a wide range of therapeutics targeting different stages of these neurotransmitters' life cycle.

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