Preservation of Dinosaur Biomolecules in Fossils

J. Lai
Integrated Science Program, McMaster University

Summary

Over the past 20 years, multiple lines of evidence have produced an increasing amount of evidence supporting the hypothesis that organic materials such as proteins can be preserved in fossils for much greater periods of time than previously thought. Here, we assess the evidence presented and argue that it provides convincing support for the presence of biomolecules within multi-million-year old fossils.

Received: 01/23/2015 **Accepted:** 02/27/2015 **Published:** 03/03/2015
URL: https://journals.mcmaster.ca/iScientist/article/view/974/992

Keywords: Dinosaur Biomolecules, Fossils, Immunohistochemistry, Collagen

While biomolecules in fossils can provide scientists with much useful information, these molecules are vulnerable to breakdown following the organism's death. In 1993, Mary Schweitzer first announced the evidence suggesting the presence of collagen and other soft tissue in a *Tyrannosaurus rex* fossil, in an abstract submitted to the fifty-third annual meeting of the Society of Vertebrate Paleontology (Schweitzer, 1993). At the time, the consensus among scientists was that soft tissue could not be preserved for such a long time, and Schweitzer's discovery was widely disputed. Over the subsequent 20 years, Schweitzer and other scientists have continued examining dinosaur fossils for evidence of biomolecules and have produced abundant support for their hypothesis that biomolecules can be preserved within dinosaur bones.

Scientists have produced multiple lines of evidence to support the hypothesis that biomolecules, including collagen and heme, are preserved within some dinosaur fossils. These include examination of samples using various forms of microscopy, analysis using several chromatographic and spectrometric techniques, and immunological analyses. Further, the evidence collected has allowed mechanisms for these molecules' preservation to be proposed. Here, we assess the multiple lines of evidence presented and argue that it provides convincing support for the presence of biomolecules within multi-million-year old fossils. If it proves to be true that biomolecules are indeed preserved in such old fossils, there may be useful applications to not only paleontology, but also molecular biology and astrobiology.

Microscopy

One of the first ways in which the dinosaur bones were examined was through the use of microscopy. In the original 1993 abstract, it was noted the examination with a microscope showed details resembling cells and collagen (Schweitzer, 1993). Further analysis used scanning electron microscopy, transmission electron microscopy, confocal microscopy, and electron diffraction pattern analysis to demonstrate that the bone material had not undergone much replacement or infilling post-mortem, and showed fibrous structures suggestive of collagen (Schweitzer, et al., 1997). Similarly, scanning electron microscopy of a dinosaur eggshell revealed structures that were interpreted to be similar to those found in eggshells of extant birds (Schweitzer, et al., 2005). These techniques were used in a later paper, in addition to atomic force microscopy, which allowed the scientists to observe an approximately 70nm repeated banding pattern in dinosaur cortical and medullary bone, which matches with collagen in extant bone (Schweitzer, et al., 2007). While the presence of fibre-like structures can be somewhat ambiguous, the atomic force microscopy measurement, with its quantitative match to collagen-related structures, appears to provide some evidence of extraordinary preservation, although these techniques do not provide much certainty to the possibility of preserved biomolecules: they only demonstrate macroscopic preservation of structure.

Spectroscopy and Chromatography

Various spectroscopic and chromatographic techniques were also used to analyse the dinosaur fossils. In multiple studies, gas chromatography, mass spectrometry, and high performance liquid chromatography were used to examine the bones for biomolecules, with results suggesting the presence of nucleic acids and peptides (Schweitzer, 1993; Schweitzer, et al., 1997; Schweitzer, et al., 2007; Asara, et al., 2007). In particular, Asara, et al. (2007), used mass spectrometry to propose peptide sequences found in the sample. In the case of the study which examined dinosaur eggshells, energy dispersive X-ray elemental analysis was used, which also suggested the presence of biomolecules (Schweitzer, et al., 2005). Amino acid analyses were also performed in some of these studies, and not only did they show the presence of amino acids in the samples (Schweitzer, et al., 1997; Schweitzer, et al., 2007), but they also displayed peaks consistent with profiles for collagen and expected changes due to exposure to the environment (Schweitzer, et al., 2007). This evidence is much more convincing and makes a good case for the preservation of biomolecules such as peptides within the fossils, as it definitively shows that these biomolecules can be found within the fossil. While the possibility of contamination was suggested (Buckley, et al., 2008), this was accounted for through the use of controls, including performing the same analysis on analytical equipment used, as well as the sandstone matrix in which the fossil was found. These did not show the presence of peptides, unlike the fossil, suggesting that the detected biomolecules are not due to contamination (Asara and Schweitzer, 2008).

Immunochemistry

Perhaps the most convincing evidence has come from immunological tests. In several studies, Schweitzer and her colleagues created antisera by inoculating some rabbits with fossil material and other rabbits with chicken proteins (Schweitzer, et al., 2005; Schweitzer, et al., 2007). ELISA tests were then performed, testing the antisera against both fossil and chicken antigens. Both antisera reacted to both antigens, demonstrating the similarity between the two sets of antigens (Schweitzer, et al., 2005). This suggested that the dinosaur material contained a material which bore antigenic similarity to collagen. Further evidence that the material in the bone is in fact collagen comes from in situ immunohistochemistry studies. Antisera raised against avian collagen reacted with dinosaur antigens in situ, and the scientists were also able to reduce the reactivity with some success using collagenase on the bone sample (Schweitzer, et al., 2007).

Proposed Mechanisms of Preservation

With the increasing amount of evidence supporting the idea that biomolecules have been preserved in the fossil record, researchers have begun to examine possible mechanisms that might have allowed these molecules to avoid degradation. When the discovery was first announced, it was proposed that the affinity of biomolecules like nucleic acids and peptides for the hydroapatite in bone protected them from being degraded by removing them from solution (Schweitzer, et al., 1994). This was later elaborated upon when it was proposed that organic molecules and apatite crystals in bone stabilized one another synergistically (Schweitzer, et al., 2007). Later on, it was proposed that during diagenesis, biomolecules undergo reactions that increase the resistance to being degraded: for example, proteins might be converted into hydrophobic hydrocarbons, a hypothesis suggested by a petroliferous odour encountered by researchers while preparing the eggshell for analysis (Schweitzer, et al., 2005). The role of the depositional environment, including the porosity of the parent rock and the presence of calcite cements, in preserving biomolecules in fossils was also noted. Besides the protection of peptides through reaction with bone material (Schweitzer, et al., 2007), suggested mechanisms include protection of organics by biofilms (Peterson, Lenczewski and Scherer, 2010) or by iron (Schweitzer, et al., 2013). These mechanisms either protect enzyme recognition sites or remove oxygen to prevent oxidative degradation (Schweitzer, et al., 2013).

Implications

Previously, ancient materials have not been subjected to much analysis for organic compounds due to the belief that these materials could not survive for such long periods of time (Schweitzer, et al., 2013). There could therefore be perhaps many fossils in which organic material could be retrieved that previously had not been considered. These organic materials, such as peptides, can be extremely useful as a tool in paleontology, allowing scientists to reconstruct phylogenetic relationships based on similarities between peptide sequences in extant and extinct organisms (Schweitzer, et al., 2009). Several of the proposed mechanisms by which organic molecules may be preserved also have notable implications. In a study in which the role of iron-oxygen chemistry is examined, the authors note that use of iron chelators greatly increased immunoreactivity of the samples (Schweitzer, et al., 2009). This suggests that many more sources of ancient organics could be discovered using this method or similar methods to remove chemicals that may be protecting the organics (Schweitzer, et al., 2009). As well, it was noted in another study that certain segments of collagen proteins tended to be preserved better, especially those that are hydrophobic and lack acidic amino acids (San Antonio, et al., 2011). This could provide important information to molecular biologists regarding the relationship between peptide sequences and their function: the scientists showed that functionally crucial parts of the collagen protein were more stable over geologic time (San Antonio, et al., 2011).

These findings have many implications not just for paleontology and molecular biology but also for astrobiology. Advancements made by studying the preservation of organic molecules over geologic time can be applied to the search for extraterrestrial organic molecules and life. By understanding conditions in which organics may be preserved longer and methods by which these preserved materials can be detected and analyzed, scientists may have more tools with which to search for life elsewhere.

Conclusion

Only a few decades ago, the suggestion that peptides could be recovered from dinosaur fossils would have found little support within the scientific community. Today, growing evidence, outlined and assessed here, suggests that this does in fact occur, and research in this field continues today, potentially providing new intriguing directions in paleontology, molecular biology, and even astrobiology.

References

Asara, J. M. and Schweitzer, M. H., 2008. Response to comment on "protein sequences from mastodon and *Tyrannosaurus rex* revealed by mass spectrometry". *Science*, 319(5859), p.33d.

Asara, J. M., Schweitzer, M. H., Cantley, L. C. and Cottrell, J. S., 2008. Response to comment on "protein sequences from mastodon and *Tyrannosaurus rex* revealed by mass spectrometry". *Science*, 321(5892), p.1040c.

Asara, J. M., Schweitzer, M. H., Freimark, L. M., Phillips, M. and Cantley, L. C., 2007. Protein sequences from mastodon and *Tyrannosaurus rex* revealed by mass spectrometry. *Science*, 316(5822), pp.280-285.

Buckley, M., Walker, A., Ho, S. Y. W., Yang, Y., Smith, C., Ashton, P., Oates, J. T., Cappellini, E., Koon, H., Penkman, K., Elsworth, B., Ashford, D., Solazzo, C., Andrews, P., Strahler, J., Shapiro, B., Ostrom, P., Gandhi, H., Miller, W., Raney, B., Zylber, M. I., Gilbert, M. T. P., Prigodich, R. V., Ryan, M., Rijsdijk, K. F., Janoo, A. and Collins, M. J., 2008. Comment on "protein sequences from mastodon and *Tyrannosaurus rex* revealed by mass spectrometry". *Science*, 319(5859), p.33c.

Peterson, J. E., Lenczewski, M. E. and Scherer, R. P., 2010. Influence of microbial biofilms on the preservation of primary soft tissue in fossil and extant archosaurs. *PLoS ONE*, 5(10), p.e13334.

San Antonio, J. D., Schweitzer, M. H., Jensen, S. T., Kalluri, R., Buckley, M. and Orgel, J. P. R. O., 2011. Dinosaur peptides suggest mechanisms of protein survival. *PLoS ONE*, 6(6), p.e20381.

Schweitzer, M. H., 1993. Biomolecule preservation in *Tyrannosaurus rex*. *Journal of Vertebrate Paleontology*, 13(Supplement to 3), p.56A.

Schweitzer, M. H., Cano, R. J., Starkey J. R. and Horner, J. R., 1994. Multiple lines of evidence for the preservation of collagen and other biomolecules in undemineralized bone in *Tyrannosaurus rex*. *Journal of Vertebrate Paleontology*, 14(Supplement to 3), p.45A.

Schweitzer, M. H., Chiappe, L., Garrido, A. C., Lowenstein, J. M. and Pincus, S. H., 2005. Molecular preservation in Late Cretaceous sauropod dinosaur eggshells. *Proceedings of the Royal Society B*, 272(1565), pp. 775-784.

Schweitzer, M. H., Johnson, C., Zocco, T. G., Horner, J. R. and Starkey, J. R., 1997. Preservation of biomolecules in cancellous bone of *Tyrannosaurus rex*. *Journal of Vertebrate Paleontology*, 17(2), pp.349-359.

Schweitzer, M. H., Suo, Z., Avci, R., Asara, J. M., Allen, M. A., Arce, F. T. and Horner, J. R., 2007. Analyses of soft tissue from *Tyrannosaurus rex* suggest the presence of protein. *Science*, 316(5822), pp.277-280.

Schweitzer, M. H., Zheng, W., Cleland, T. P., Goodwin, M. B., Boatman, E., Theil, E., Marcus, M. A. and Fakra, S. C., 2013. A role for iron and oxygen chemistry in preserving soft tissues, cells and molecules from deep time. *Proceedings of the Royal Society B*, [online] Available at: < http://rspb.royalsocietypublishing.org/content/281/1775/20132741> [Accessed 6 January 2015].

Schweitzer, M. H., Zheng, W., Organ, C. L., Avci, R., Suo, Z., Freimark, L. M., Lebleu, V. S., Duncan, M. B., Vander Heiden, M. G., Neveu, J. M., Lane, W. S., Cottrell, J. S., Horner, J. R., Cantley, L. C., Kalluri, R. and Asara, J. M., 2009. Biomolecular characterization and protein sequences of the Campanian hadrosaur *Brachylophosaurus canadensis*. *Science*, 324(5927), pp. 626-631.