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Amino Acids Recovered from Fossil Egg Shells of Dinosaurs

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Amino acids recovered from fossil egg shells of dinosaurs showed two kinds. One is antique and small in amount showing the D/L ratio nearly one. The other is more abundant and seems recent in origin. The antique ones might be residue of peptides at the shell formation.

Studies on amino acids as chemical(or molecular) fossils are interesting, for these organic compounds have been essential to life throughout its history on the earth. Fossil samples as old as several million years are generally suitable for amino acid analysis. However, if samples have been in good preservation during their burial period in sediments, it is possible to find amino acids from samples older than several million years. One of the good candidates for such samples are fossils of hard tissue.

We obtained fragments of fossil egg shells of Hypselosaurs priscus Matheron. These egg shells occurred in sediment(marl) near Rosset, France. The age of the sediment is known to be about 70 million years old(Lower to Middle Maastrichtian in Cretaceous period). There is no report of dating for these fossil egg shells. However, they are as old as the sediment, since they are autochthonous in the sediment with occasional occurrence of complete fossil eggs.[1] We searched for amino acids included in the egg shells at the shell formation, by examining the amounts of amino acids and several their enantiomeric ratios. Our results indicate the presence of antique amino acids as well as recent ones. We will describe these findings and discuss the antiquity of amino acids.

Fragments of the fossil egg shells we used were generally 1-2 cm large and 2 mm thick. These fragments were apparently contaminated with iron oxides. We removed brownish surface contaminants by a knife until the fragments showed either white or gray color. According to the two colors, fragments were separated into "white" and "gray" groups, although there was no sharp cut between the white and gray ones. These fragments were immersed in 0.01 M HCl and sonicated to remove further contaminants for "clean" fragments.

We used a set of about 2 g of either the white or gray, clean fragments for each analysis. A set of the fragments was placed in a glass tube and 6 M HCl was added gradually to dissolve these fragments. By addition of conc. HCl, the dissolved solution was brought to roughly 6 M HCl. The glass tube was sealed under reduced pressure, and the tube was placed in an oil bath at 110 °C for 22 h for acid hydrolysis. To the hydrolyzed solution, about 2 ml of conc. HF were added

to make calcium precipitate. Another 1 ml of conc. HF was added to the solution for the complete precipitation. The supernatant recovered after centrifugation was dried under reduced pressure, and the residue was taken up by an appropriate volume of 0.01 M HCl. A known portion of this HCl solution was used for quantitative analysis by an amino acid analyzer(Durrum 500). The rest of the HCl solution was dried, and the residue was treated first with isopropanol-1.5 M HCl at 90 °C and then, with TFAA/CH_2Cl_2 at 60 °C. The derivatives of amino acids, N-TFA-isopropyl-esters, were analyzed by a gas chromatograph equipped with an optically acitve glass capillary column(Chirasil-Val) to separate the enantiomers. These enantiomers were detected by a FTD detector and the enantiomeric ratios(D/L) were determined from the peak areas on the chromatograms. Confirmation of amino acids was made by a mass spectrometer(Shimadzu QP-1000) combined to the gas chromatograph(GC/MS).

Table 1. Amino acids recovered from fossil egg shells of dinosaurs (nmol/g)

	White-1	-2	Gray-2	-3
Asp	70.7	25.1	1.6	1.5
Thr	27.3	18.8	-	-
Ser	34.2	16.6	-	-
Glu	103.1	36.1	9.2	7.1
Pro	72.8	-	-	-
Gly	81.8	34.2	13.9	11.5
Ala	135.9	53.6	24.0	18.1
αAba	5.4	-	3.2	2.5
Val	53.3	20.8	5.6	5.0
Ile	28.9	12.5	-	-
Leu	106.6	22.0	-	-
Tyr	-[a)]	5.3	-	-
Phe	21.2	9.7	-	-
γAba	5.4	4.4	3.7	2.6
Lys	33.2	-	-	-

a) -: not detected, αAba: α-aminobutyric acid, γAba: γ-aminobutyric acid.

The amounts of amino acids recovered from the fossil egg shells are listed in Table 1. The amounts differ from sample(a set of fragments) to sample. Amino acids generally common to proteins were found rather abundantly in the white samples. On the other hand, the amounts were clearly less in the gray samples with no detection of several amino acids found in the white samples. This result indicates that the amino acids in the gray ones had been exposed to longer decomposition reaction than in the white ones.

The amino acid compositions in the white samples resemble data reported for dinosaur egg-shell proteins in which glycine, alanine, leucine, aspartic acid, and glutamic acid were abundant with a notable quantity of proline.[2)] These data were tentatively interpreted due to recent bacterial contamination in the shells. What peptides or proteins were included at the formation of the egg shells of dinosaurs are unknown, although the presence of Ca-binding peptide was suggested.[3)] Which amino acids had been introduced into the egg shells as contaminants during burial period are not known, either.

Biological peptides and proteins consist of L-amino acids. Analysis of the optical isomers showed the presence of several D-amino acids besides L-ones as seen in Fig. 1, and the enantiomeric ratios(D/L) are listed in Table 2. The D/L ratios in the three white samples are similar to each other and are fairly low (< 0.20)

except those of alanine. On the other hand, the ratios in the gray samples except No. 1 are quite high and are mostly close to one, the ratio of racemic mixture. Therefore, there is an indication that the gray samples contain amino acids which had experienced longer racemization reaction than the white samples.

The consideration of both the amino acid amounts and the D/L ratios in the white and gray samples leads to the following interpretation: The amino acids recoverd from the white samples are relatively much younger in origin, judging from those low D/L ratios. These are probably from recent biological contaminants. On the other hand, the amino acids from the gray samples seem much older than those from the white ones because of the high D/L ratios. The small amounts of amino acids with these high D/L ratios indicate that these amino acids represent only a small portion of the initial amino acids and the rest of the large portion had already decomposed.

The relatively high D/L ratios of alanine in the white sample Nos. 2 and 3, and the gray sample No. 1 need explanation. Serine and threonine are thermally less stable and decompose rather readly, and are usually absent in fossil shells of several million years old.[4] The decompositon of L-serine and L-threonine in foraminiferal shells principally followed

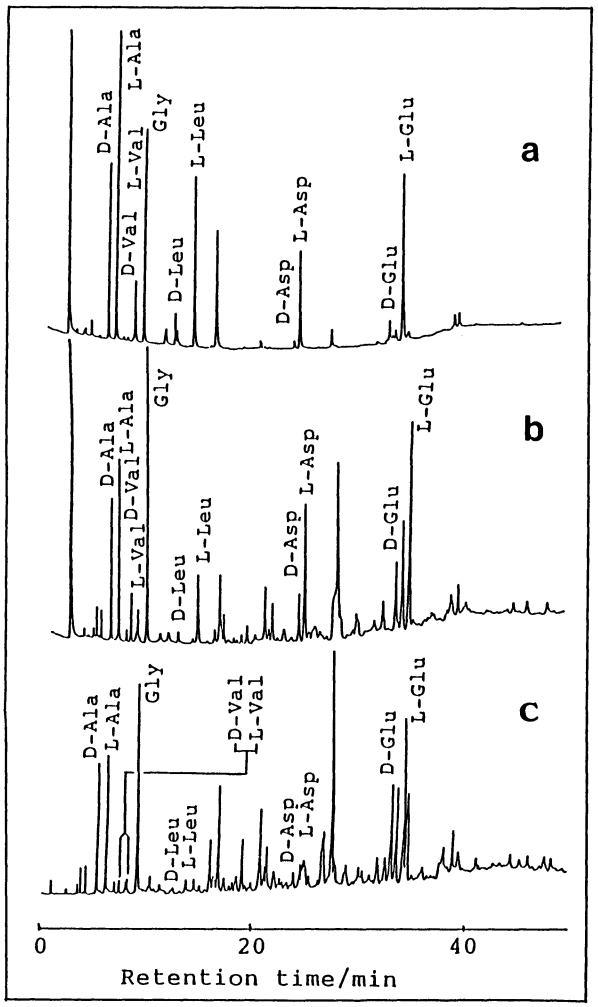

Fig. 1. Gas chromatograms of amino acids recovered from fossil egg shells of dinosaurs.
Chromatograms are sample a) white-1, b) gray-1, and c) gray-5.

Table 2. D/L ratios of amino acids recovered from fossil egg shells of dinosaurs

	White-1	-2	-3	Gray-1	-2	-3	-4	-5
Ala	0.05	0.53	0.62	0.75	1.02	0.91	0.96	0.98
Val	0.06	0.17	0.08	-[a)]	-	-	0.93	1.04
Leu	0.18	0.13	0.16	0.29	-	-	-	-
Asp	0.08	0.06	0.10	0.35	-	-	-	-
Glu	0.18	0.15	0.17	0.43	0.97	1.10	0.75	0.80

a) -: not determined.

dehydration reaction to yield racemic alanine and α-aminobutyric acid, respectively.[4] Our GC/MS(by CI) analysis showed the presence of racemic mixture of α-aminobutyric acid, though the amount was very minor(Fig. 2). Decomposition of L-serine in the egg shells partly accounts for the high D/L ratios of alanine observed.

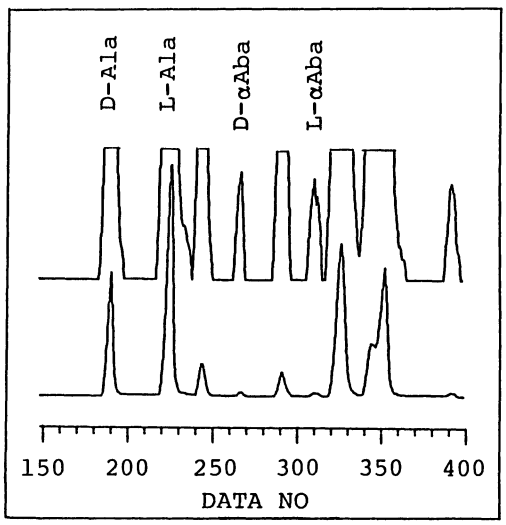

Fig. 2. Mass chromatograms of total ions for amino acids from fossil egg shells of dinosaurs.
αAba: α-aminobutyric acid, γAba: γ-aminobutyric acid.

It is desirable to estimate the age of those amino acids with high D/L ratios in the gray samples. Racemization half-life of three amino acids was reported as follows: 0.43 x 10^6 y for aspartic acid, 1.4 x 10^6 y for alanine, and ca. 6 x 10^6 y for isoleucine at 0 °C and pH 7.6.[5] However, the extent of racemization is also affected by the form of amino acids, either as free or bound form in peptide in addition to the conditions surrounding amino acids. The order of racemization rate reported[6] for the five amino acids in Table 2 as free form in aqueous solution is as follows; aspartic acid > alanine > glutamic acid > leucine > valine. It is generally understood that almost all amino acids in fossils become racemic by 10 to 20 x 10^6 y[7] from the study of racemization reaction using fossil Mercenaria shells of Miocene age.[8]

In the light of above information, alanine, valine, and glutamic acid from the gray sample No. 2 to 5 might be as old as 10 to 20 x 10^6 y or older. To conclude whether these amino acids are indigenous to the fossil egg shells has to wait for further examination of the fossil egg shells which have been preserved freshly during burial.

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