

## 1 - 4 . アミノ酸

### 1 - 4 - 1 . Introduction

It has been proposed that the massive extinction of organisms ranging from dinosaurs to marine plankton at the end of Cretaceous approximately 65 million years ago, had been caused by a global fallout of debris and dust due to an impact of an extraterrestrial body<sup>6, 7)</sup> or an extensive volcanism<sup>8, 9)</sup>. The hypothesis of a bolide impact was presented by the iridium enrichment<sup>6, 7, 10)</sup>, anomaly of  $^{87}\text{Sr}/^{86}\text{Sr}$ <sup>11)</sup> and occurrence of the shocked quartz<sup>12, 13)</sup> in the K/T boundary claystones, and the discovery of a possible impact site at Chicxulub, Mexico<sup>14, 15)</sup>.

Some findings by organic geochemical study were presented to support the bolide impact. One was an enrichment of soot, charcoal, PAHs and fullerenes at the K/T boundary as a result of global fires triggered by the impact<sup>17-22)</sup>. The other was unusually high occurrence of  $\alpha$ -aminoisobutyric acid and isovaline around the K/T boundary at Stevns Klint, Denmark<sup>26)</sup>. Since the two amino acids are known to be non-proteinaceous and be relatively abundant in carbonaceous chondrites<sup>23-25)</sup>, the finding supports the hypothesis of the bolide impact. However, the two amino acids were found at layers slightly above (30 cm) and below (50 and 100 cm) from the boundary and were absent in the boundary clay itself, while the iridium enrichment was found in the boundary clay. Therefore, there are some puzzling points why the enrichment of the two amino acids was found at different layers from that of iridium around the boundary, if they were provided by an extraterrestrial body, and why only the two amino acids survived from the 65 million year history of the layers without any protection. A similar kind of puzzling points was raised in a comment on the finding of the two amino acids<sup>54)</sup>. Based on the finding of the two extraterrestrial amino acids and to explain the puzzling point, a new hypothesis was proposed in which the two amino acids were cometary in origin and were deposited with the dust scattered in an orbit of a giant comet trapped in the inner Solar System<sup>55)</sup>.

These hypotheses are intriguing from an organic geo- and cosmochemical viewpoint, and therefore, the analyses for extraterrestrial amino acids in the K/T boundary sediments at Kawaruppu were carried out. However, such extraterrestrial amino acids were not detected at and near the K/T boundary. In this section, the results of analyses were described and discussed the possibility of the presence of the extraterrestrial amino acids in the K/T boundary.

### 1 - 4 - 2 . Experiments

#### 1-4-2-1. Samples

The K/T boundary sediments occur in the upper part of the Katsuhira Formation exposed in the bed of a shallow stream from the Mokawaruppu River, about 4 km upstream of Kawaruppu Town, Tokachi District, Hokkaido<sup>30, 31)</sup>. The sedimentary sequence at this locality consists predominantly of a marine, dark gray siltstone, bearing occasionally calcareous concretions. A distinct about 14 cm thick grayish-black claystone, which is similar in appearance to the well-known "boundary clay" of the classical K/T sections in Europe, is the only lithological break in this otherwise monotonous sedimentary succession. Thirteen sediment samples from different horizons in the K/T boundary claystone at Kawaruppu (0 - 13.7 cm), 5 samples above (40 - 495 cm) and 6 samples below (-395 - 0 cm) the boundary

claystone were analyzed amino acid compositions as well as *D*-, *L*-enantiomers of several amino acids. The locality and stratigraphic sequence of the samples are shown in Fig. 1-1.

#### 1-4-2-2. Analyses

One gram pulverized sample each was extracted with 3 ml water at 110 °C for 12 h three times. The extract was divided into two fractions, one for unhydrolyzed, and one for hydrolyzed with 6 mol l<sup>-1</sup> HCl at 110 °C for 12 h. Portions of the two fractions were analyzed quantitatively by amino acid analyzer (JEOL JLC-300). Rests of the fractions were dried and treated first with 2-propanol - 1.5 mol l<sup>-1</sup> HCl at 110 °C and then, with trifluoroacetic anhydride at 80 °C to make the derivatives of amino acids (*N*-trifluoroacetyl amino acid isopropyl esters). The residual pulverized sample after the water extraction was extracted with 6 mol l<sup>-1</sup> HCl at 110 °C for 12 h three times. Dissolved metal ions were removed from the HCl solution by extraction with 4-methyl-2-pentanone. After neutralization, the solution was centrifuged for 10 min at 2500 x g and precipitate was removed. The solution was treated by the same method as above to make the *N*-trifluoroacetyl amino acid isopropyl esters. These derivatives of amino acids were analyzed by a GC-MS.

The GC-MS was a Simadzu QP-1000 and QP-5000 with a Chirasil-*L*-Val glass capillary column (25 m x 0.25 mm i.d.) in order to separate the *D*-, *L*-enantiomers. The mass-spectra were acquired every 0.1 s for selective ions in the chemical-ionization mode at 200 eV with isobutane as reacting gas. Amino acids and their enantiomers were identified based on the retention time of standard amino acids on the mass fragmentograms of selective ions. The enantiomeric ratios (*D/L*) were calculated by their peak areas on the mass fragmentograms.

A procedural blank was carried out in parallel to the sample analysis, using 1 g of pre-ignited sand powder. Water was distilled, deionized and redistilled twice. Twice distilled 6 mol l<sup>-1</sup> HCl was used. All glassware was pre-heated at 500 °C for at least 3.5 h prior to use.

### 1 - 4 - 3 . Results

Only three amino acids were found in the unhydrolyzed fractions of the water extracts from the K/T boundary claystone samples. These amino acids were aspartic acid, serine and glycine at the concentration level of sub-nmol g<sup>-1</sup> (Table 1-4-1). No amino acids were detected in the unhydrolyzed fractions from the other rock samples. On the other hand, eight amino acids were found in the hydrolyzed fractions at the concentration from nearly 4 nmol to sub-nmol g<sup>-1</sup>, being relatively concentrated in the claystone and the layer 60 cm above the boundary (Table 1-4-2). However,  $\alpha$ -aminoisobutyric acid and isovaline were not found in any sample examined.

Mass fragmentograms of the hydrolyzed fractions of the water extracts are shown in Fig. 1-4-1. The enantiomeric ratios (*D/L*) estimated from the peak areas on the fragmentograms are listed in Table 1-4-3. In all samples, the *D/L* ratios were lower than 0.2 for all amino acids except for aspartic acid and serine. The ratios of aspartic acid were mostly from 0.36 to 0.40 except for one sample (0.08), while those of serine varied from 0.0 to 0.55. The number of amino acids identified by this method is larger than that by the amino acids analyzer. However,  $\alpha$ -aminoisobutyric acid and isovaline were not found in any sample examined.

Amino acids recovered by the HCl extraction of the pulverized samples after the water extraction were analyzed by the GC-MS method only. The amino acids recovered were smaller in number of kinds and amount than those by the water extractions, and they were all

in the *L*-form. Therefore, they were ignored in this study.  $\alpha$ -Aminoisobutyric acid and isovaline were not identified in any samples.

#### 1 - 4 - 4 . Discussion

A maximum of eight kinds of amino acids including diagenetically unstable serine and threonine were detected in the water extracts in this analyses of the K/T boundary rocks at Kawaruppu. The concentration of these amino acids were at most one nmol g<sup>-1</sup> level. If  $\alpha$ -aminoisobutyric acid and/or isovaline were present at a few nmol g<sup>-1</sup> (which is the level reported for the Stevns Klint sediments<sup>26</sup>) in the K/T samples at Kawaruppu, they could have been detected by the methods using the amino acid analyzer and the GC-MS fragmentograph.

A characteristic amino acid distribution in the sequence around the K/T boundary at Kawaruppu is the enrichment in the boundary claystone as seen in Figs. 1-4-2, 1-4-3. The enrichment is seen with neutral, acidic, and hydroxy amino acids and does not depend on the kinds of amino acids. Furthermore, the enrichment is not related to the carbon content as seen in Fig. 1-4-2b,c, although the content may include a small portion of carbonate carbon. Therefore, it is highly likely that the enrichment was due to the adsorption of amino acids by clay minerals in the claystone. No diffusion of amino acids was noted at above or below from the boundary claystone.

The *D/L* ratios in Table 1-4-3 show amino acids detected in many samples consist mainly of the *L*-form, although aspartic acid in seven out of eight samples contain the *D*-form about 1/4 to 1/3 of the acid, and serine does up to 1/2 in three samples. It is known that serine is thermally unstable, especially in the presence of water, and that aspartic acid racemizes much faster than the other amino acids<sup>56</sup>). A laboratory heating experiment shows that the half-life of aspartic acid racemization is 0.43x10<sup>6</sup> y at 0 °C under nearly a neutral pH, while those of alanine and isoleucine are 1.4x10<sup>6</sup> y and 6x10<sup>6</sup> y, respectively<sup>56</sup>). Therefore, the results shown in Table 1-4-3 highly indicate that the amino acids in the K/T boundary at Kawaruppu are not so old as 65 million years, but geologically much younger (probably less than a million years) in age.

The results of the present study are in contrast to those of the previous study of amino acids in the K/T boundary rocks at Stevns Klint as to finding of  $\alpha$ -aminoisobutyric acid and isovaline of extraterrestrial in origin and the diffusion of the amino acids from the K/T boundary<sup>26</sup>). The difference in the two studies probably explained by one or more of the following reasons. The first is that the locations of Kawaruppu and Stevns Klint are quite far apart. The fallout due to the impact body probably accumulated lesser at Kawaruppu than at Stevns Klint, since it has been reported that the iridium concentration at Stevns Klint is relatively high in the world<sup>10</sup>). Therefore,  $\alpha$ -aminoisobutyric acid and isovaline from the impact body accumulated lesser at Kawaruppu so that they were not detected. The second is that the 24 rock samples examined in this study did not cover any of the layer equivalent to the 30 cm above, and 50 and 100 cm below the K/T boundary at Stevns Klint. Diffusion of the two amino acids from the boundary to the three levels were proposed for the amino acid distribution at Stevns Klint<sup>26</sup>). On the other hand, amino acids are concentrated in the boundary claystone in Kawaruppu which does not suggest the process of diffusion, although it may be said that the amino acids did not have enough time for diffusion because they were incorporated probably much after than 65 million years ago. The third is that the two amino acids in the Stevns Klint layers might have been protected in sediments over 65 million years,

while those in Kawaruppu layers have degraded to less than this detection limit. Without any protection, it is difficult for amino acids to survive under geological conditions over 65 million years. Presently no appropriate explanation can be offered why only the two amino acids have been protected among many other extraterrestrial amino acids deposited at Stevns Klint. Based on the results in the present study, it is reasonable to conclude that there are no detectable amounts of extraterrestrial amino acids present in the K/T boundary at Kawaruppu, in addition to no solid evidence concerning the deposition of amino acids at the end of the Cretaceous.

Since the two amino acids were not detected in the K/T boundary sediments at Kawaruppu, the author can not present any evidence to discuss whether an extraterrestrial body impact had occurred or not at the end of Cretaceous. Nevertheless, failure to find the extraterrestrial amino acids at Kawaruppu possibly offer useful information as to the postulated cometary origin on the extraterrestrial amino acids found at Stevns Klint<sup>55</sup>). According to the postulate the two amino acids and their precursors in the comet dust would have been swept up by the Earth about 50 thousand years before and after the impact of a body which brought the iridium enrichment<sup>55</sup>). This cometary origin attempts to also explain the relatively richer amino acid concentration to iridium at the K/T boundary than that in an organic rich carbonaceous meteorite, because cometary dust is rich in CHNO elements. If the author follows this suggestion, it is highly likely that amino acid accumulation was homogeneous over world wide and for long period. If so, the extraterrestrial amino acids must have fallen at Kawaruppu at the same level of concentration as that at Stevns Klint. Therefore, the cometary dust hypothesis could not explain the source of these amino acids found at Stevns Klint.

Although,  $\alpha$ -aminoisobutyric acid and isovaline are rare amino acids in the biosphere, the two amino acids are included in fungal polypeptides, in which isovaline is optically active<sup>57</sup>). However, isovaline found at Stevns Klint was racemic and considered to be nonbiological in origin, since the racemization of the amino acid hardly proceeds even in the period of 65 million years<sup>26</sup>). These amino acids are, therefore, considered as extraterrestrial in origin. However, it may be possible to have the two amino acids of terrestrial origin in sediment. 5,5-Dimethylhydantoin (5,5-dimethyl-2,4-imidazolidione) and 5-ethyl-5-methylhydantoin (5-ethyl-5-methyl-2,4-imidazolidione) are detected in pollutants in the coal-gasification<sup>58, 59</sup>). In this reaction, 5-ethyl-5-methylhydantoin is synthesized as racemic by Bucherer-Bergs synthesis<sup>60</sup>). Acid hydrolysis of the two hydantoins yields  $\alpha$ -aminoisobutyric acid and racemic isovaline, respectively, as well as alkali hydrolysis<sup>60</sup>). Therefore, amino acids in the Recent Tokyo Bay sediments were analyzed to confirm this possibility.

## 1 - 4 - 5 . Conclusions

Amino acids of possible extraterrestrial ( $\alpha$ -aminoisobutyric acid and isovaline) and/or 65 million-year-old terrestrial in origin were not found in the K/T boundary at Kawaruppu, Hokkaido. The plausible explanations for the absence are; 1) the accumulated quantity at Kawaruppu was too small to be detected compared with that at Stevns Klint. 2) The samples analyzed in this study, did not include equivalent layers at Stevns Klint in which the two amino acids were found. 3) The two amino acids deposited but decomposed during diagenesis at Kawaruppu, whereas the two amino acids have been preserved at Stevns Klint. These explanations are valid if there was an impact event of an extraterrestrial body, either a

meteorite or a comet, at the end of Cretaceous. On the other hand, if the presence of the two amino acids was due to cometary dust fall before and after the K/T boundary, the two amino acids could have been found at Kwaruppu. Therefore, it is rather difficult to support the postulate of the cometary dust origin of the two amino acids.

Table 1-4-1. Amino acid concentrations in the unhydrolyzed water extracts in the K/T boundary sediments at Kavaruppu.

relative depth / cm	concentration / nmol g <sup>-1</sup>			
	total	aspartic acid	serine	glycine
495	n.d.	n.d.	n.d.	n.d.
225	n.d.	n.d.	n.d.	n.d.
120	n.d.	n.d.	n.d.	n.d.
60	n.d.	n.d.	n.d.	n.d.
40	n.d.	n.d.	n.d.	n.d.
12.7 - 13.7	n.d.	n.d.	n.d.	n.d.
11.7 - 12.7	0.26	0.26	n.d.	n.d.
11.0 - 11.7	n.d.	n.d.	n.d.	n.d.
10.6 - 11.7	n.d.	n.d.	n.d.	n.d.
8.7 - 10.6	n.d.	n.d.	n.d.	n.d.
7.5 - 8.7	n.d.	n.d.	n.d.	n.d.
6.3 - 7.5	0.58	0.14	n.d.	0.44
5.5 - 6.3	1.37	0.32	0.35	0.70
4.3 - 5.5	n.d.	n.d.	n.d.	n.d.
2.8 - 4.3	0.17	0.17	n.d.	n.d.
1.8 - 2.8	n.d.	n.d.	n.d.	n.d.
0.8 - 1.8	0.74	0.15	0.23	0.36
0.0 - 0.8	0.88	0.26	0.21	0.41
-3.0 - 0.0	n.d.	n.d.	n.d.	n.d.
-10	n.d.	n.d.	n.d.	n.d.
-85	n.d.	n.d.	n.d.	n.d.
-165	n.d.	n.d.	n.d.	n.d.
-235	n.d.	n.d.	n.d.	n.d.
-395	n.d.	n.d.	n.d.	n.d.
procedural control	n.d.	n.d.	n.d.	n.d.

n.d.: not detected.

Table 1-4-2. Amino acid concentrations in the hydrolyzed water extracts in the K/T boundary sediments at Kavaruppu.

relative depth / cm	concentration / nmol g <sup>-1</sup>								
	total	aspartic acid	threonine	serine	glutamic acid	glycine	alanine	valine	4-aminobutyric acid
495	1.15	0.18	0.06	0.13	0.19	0.60	n.d.	n.d.	n.d.
225	0.35	0.10	n.d.	n.d.	n.d.	0.25	n.d.	n.d.	n.d.
120	0.38	0.09	n.d.	n.d.	n.d.	0.28	n.d.	n.d.	n.d.
60	3.42	0.51	0.21	0.22	0.60	0.85	0.77	0.26	n.d.
40	1.86	0.32	0.14	0.15	0.28	0.58	0.39	n.d.	n.d.
12.7 - 13.7	5.30	0.56	0.22	0.69	1.10	2.29	0.44	n.d.	n.d.
11.7 - 12.7	2.84	0.23	n.d.	0.28	0.38	1.20	0.40	n.d.	0.36
11.0 - 11.7	2.25	0.28	n.d.	0.47	0.48	1.03	n.d.	n.d.	n.d.
10.6 - 11.7	7.92	0.80	0.35	1.12	1.57	2.92	0.69	0.49	n.d.
8.7 - 10.6	4.66	0.58	0.20	0.63	0.80	1.95	0.50	n.d.	n.d.
7.5 - 8.7	2.34	0.40	0.12	0.32	0.44	1.06	n.d.	n.d.	n.d.
6.3 - 7.5	4.97	0.41	0.21	0.67	0.79	1.90	0.47	0.22	0.29
5.5 - 6.3	3.89	0.36	n.d.	0.42	0.59	1.16	1.36	n.d.	n.d.
4.3 - 5.5	9.45	0.85	0.42	1.37	1.83	3.66	0.68	0.64	n.d.
2.8 - 4.3	1.52	0.20	0.10	0.21	0.25	0.76	n.d.	0.00	n.d.
1.8 - 2.8	6.01	0.52	0.29	0.85	1.11	2.31	0.60	0.33	n.d.
0.8 - 1.8	3.38	0.49	n.d.	0.49	0.68	1.71	n.d.	n.d.	n.d.
0.0 - 0.8	0.33	0.08	n.d.	n.d.	n.d.	0.26	n.d.	n.d.	n.d.
-3.0 - 0.0	0.30	0.00	n.d.	n.d.	n.d.	0.30	n.d.	n.d.	n.d.
-10	0.65	0.10	n.d.	0.08	0.14	0.33	n.d.	n.d.	n.d.
-85	0.54	0.08	n.d.	0.12	n.d.	0.34	n.d.	n.d.	n.d.
-165	0.86	0.13	n.d.	0.15	0.18	0.40	n.d.	n.d.	n.d.
-235	1.17	0.13	n.d.	0.18	0.23	0.63	n.d.	n.d.	n.d.
-395									
procedural control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected.

Table 1-4-3. Amino acid *D/L* ratios in the hydrolyzed water extracts in the K/T boundary sediments at Kavaruppu.

relative depth / cm	alanine	valine	leucine	aspartic acid	glutamic acid	threonine	serine	phenylalanine
	<i>D/L</i> ratio							
60	0.08	0.02	0.03	0.40	0.06	0.00	0.55	-
40	0.12	-	-	0.36	0.10	0.00	-	-
12.7 - 13.7	0.13	-	-	0.36	-	n.d.	0.38	-
7.5 - 8.7	0.11	0.04	0.02	0.38	-	0.00	0.17	0.17
2.8 - 4.3	-	0.06	0.05	0.40	-	0.19	0.52	-
0.0 - 0.8	0.06	-	0.02	0.37	0.07	0.00	0.54	0.18
-10	0.05	-	0.02	0.08	0.02	0.00	-	0.04
-85	0.17	-	-	0.36	0.08	0.00	-	0.14
procedural control	-	n.d.	-	n.d.	-	n.d.	n.d.	-

-: not detected the *D*-enantiomer, n.d.: not detected each enantiomers.



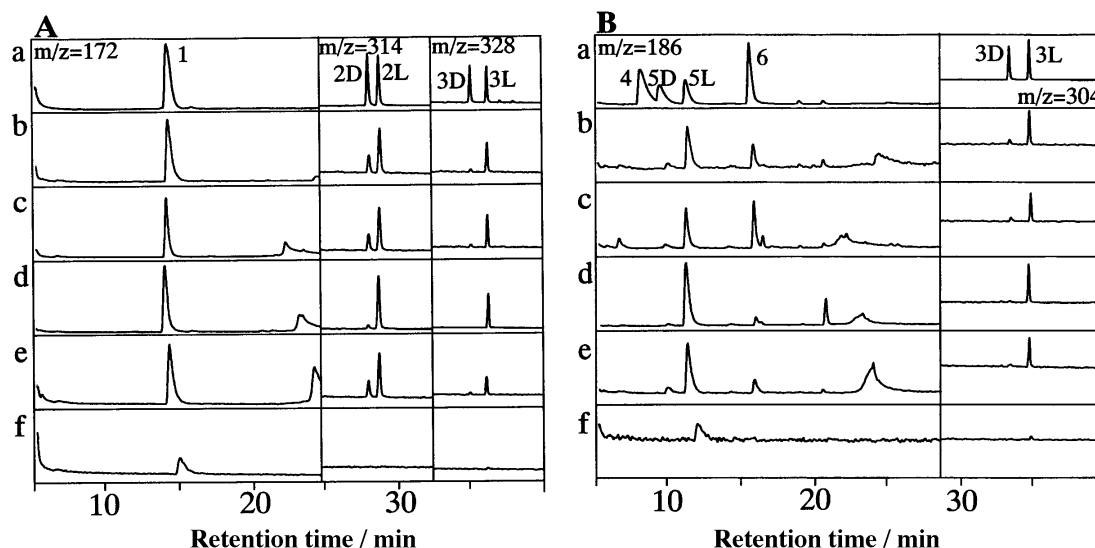


Fig. 1-4-1. Mass fragmentograms of *N*-trifluoroacetylisopropyl esters of amino acids in the hydrolyzed water extracts in the K/T boundary sediments at Kawaruppu. Selective ions are A:  $m/z = 172$ , 314 and 328, B:  $m/z = 186$  and 304, C:  $m/z = 256$ , D:  $m/z = 256$ , E:  $m/z = 270$ , and F:  $m/z = 354$  and 340. Samples are a: amino acids standards, b: below the boundary claystone (-85 cm), c: within the boundary claystone (0 to 0.8 cm), d: within the boundary claystone (8.7 to 10.2 cm), e: above the boundary claystone (+60 cm), and f: procedural control. Peaks are 1: glycine, 2: aspartic acid, 3: glutamic acid, 4: *N*-methylglycine, 5: alanine, 6: 3-aminopronanoic acid, 7: phenylalanine, 8:  $\alpha$ -aminoisobutyric acid, 9: 2-aminobutyric acid, 10: 4-aminobutyric acid, 11: isovaline, 12: valine, 13: 2-aminopentanoic acid, 14: 2-(aminomethyl)butyric acid, 15: 4-aminopentanoic acid, 16: 4-amino-2-methylbutyric acid, 17: 5-aminopentanoic acid, 18: isoleucine, 19: leucine, 20: 2-aminohexanoic acid, 21: threonine, and 22: serine, where D or L means *D*- or *L*-enantiomer.

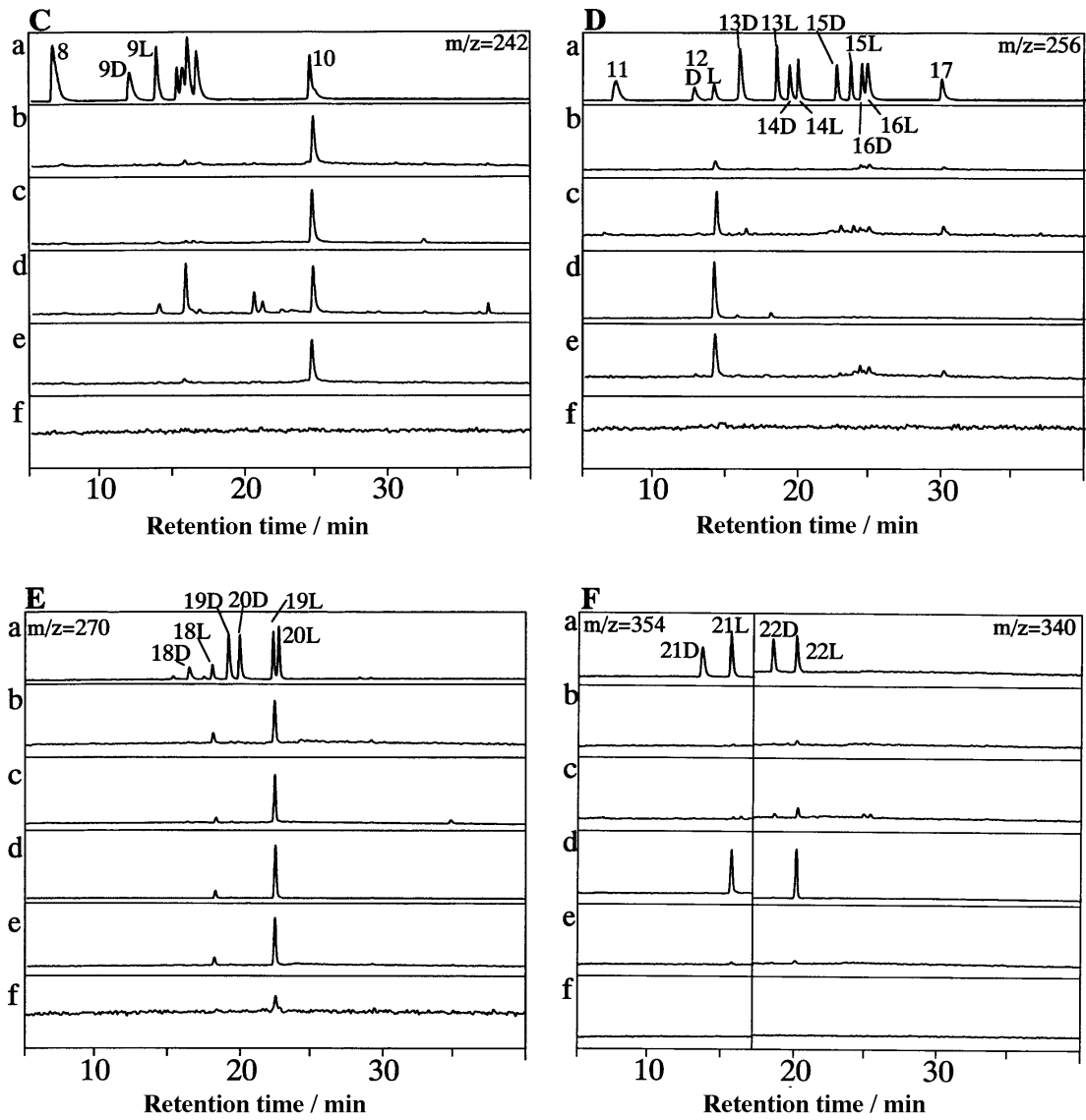


Fig. 1-4-1. (continued)



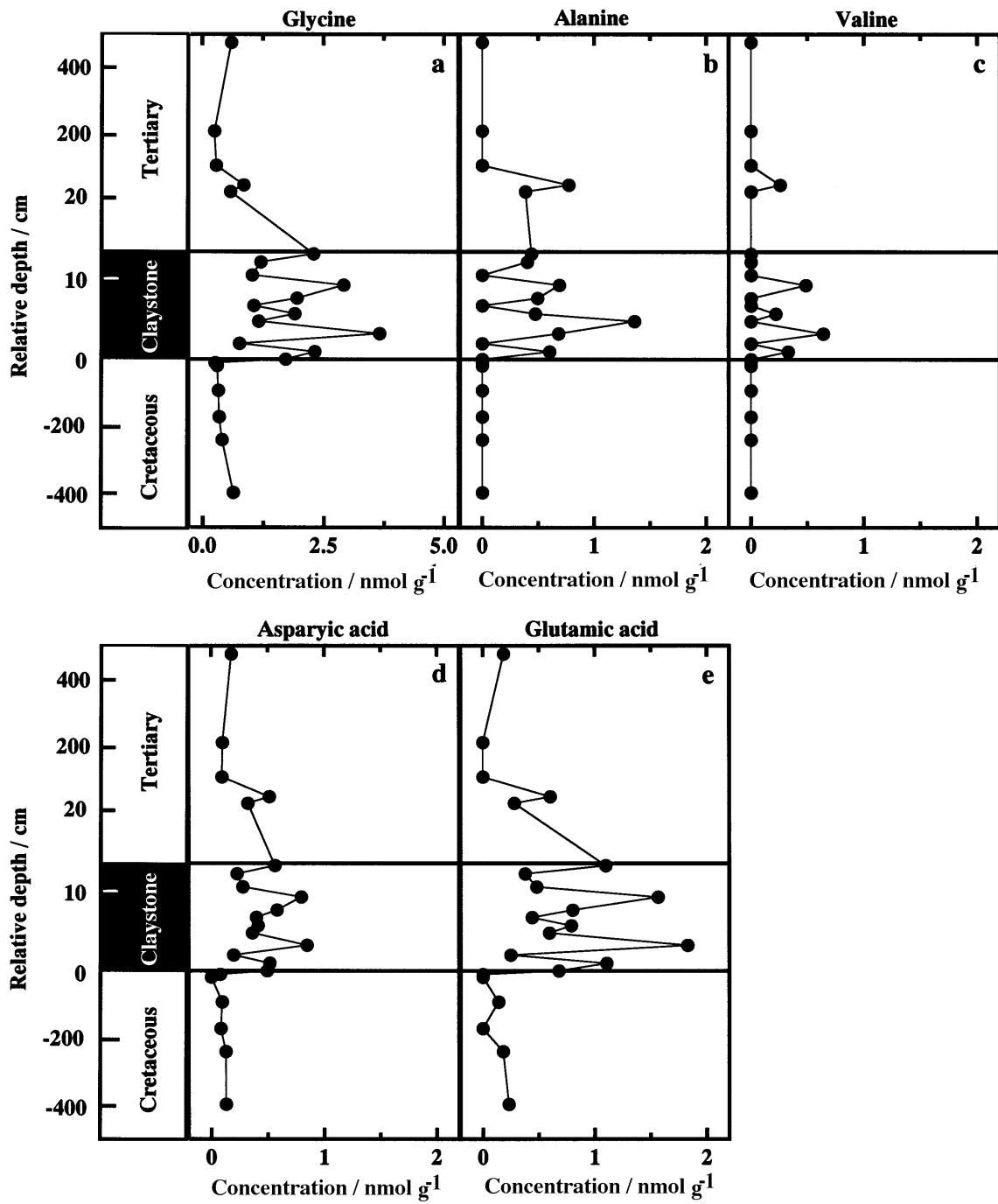


Fig. 1-4-3. Depth distributions of amino acids concentrations of the hydrolyzed water extracts in the K/T boundary sediments at Kawaruppu.

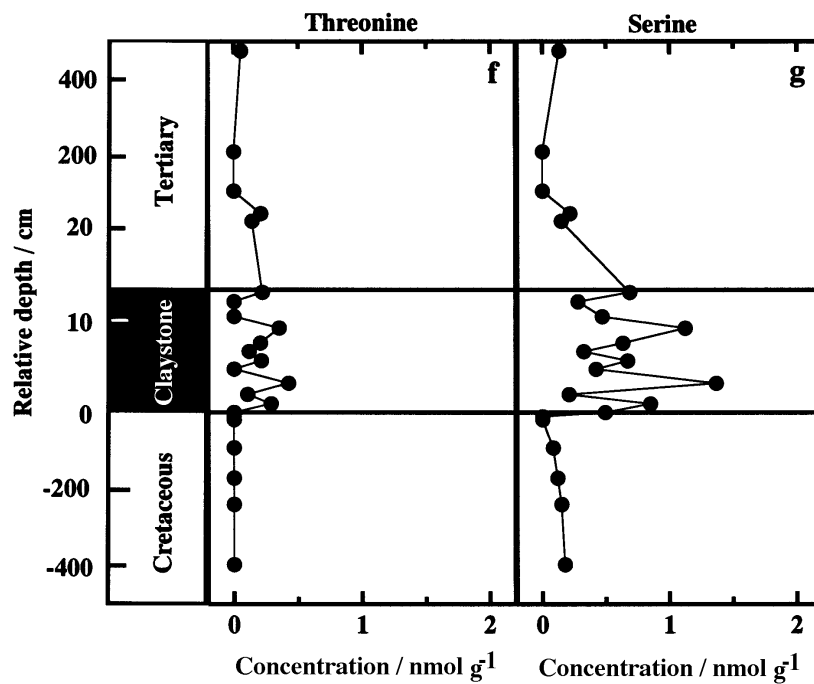


Fig. 1-4-3. (continued)