

CONCENTRATIONS AND DISTRIBUTIONS OF FREE AMINO ACIDS IN SEA AND LAKE ICE CORE OF ANTARCTICA

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Abstract The sea ice core (1.6m) and lake ice core (1.5m) were taken respectively from sea sampling site and Ace Lake near Davis Station, Vestfold Hills, Antarctica in November and October, 1988. The concentrations of amino acids in each 10cm of ice cores were determined by High Pressure Liquid Chromatography (HPLC). The results showed that the concentrations of amino acids revealed seasonal variation during the year. The highest concentration of amino acids, which was 30.92 $\mu\text{mol/ml}$, were found in the bottom of sea ice core, and it was approximately 45 $\mu\text{mol/ml}$ in the lake ice core. The lowest concentration was approximately 8.0 $\mu\text{mol/ml}$ which is presented in surface of the sea ice core, but it was 14.0 $\mu\text{mol/ml}$ which was found in 60cm section of lake ice core.

The seasonal variation process of concentration of amino acids were much similar to that of phytoplankton cells number in sea ice core, and the distribution and seasonal variation rate of individual amino acids were also much similar in each ice core sections. We suggest that the uniform spectrum of amino acids is probably derived from a peptide cell source and those amino acids were not utilized by organism.

Key words amino acids, HPLC, ice core, Antarctica.

Introduction

As a results of the metabolism of living organism and posthumous organisms decay, amino acids and peptides of various degree of polymerization are released into the water. Amino acids account for 30 — 70% of the organisms that was liberated by living phytoplankton and zooplankton in aqueous environment (Zlobin *et al.*, 1975). Studies on phytoplankton cultures have revealed a distinct similarity in constituent amino acids composition regardless of species (Cowey and Corner, 1963; Chau *et al.*, 1967). The amounts of free amino acids, which are contained in bacteria and phytoplankton should be greater than that dissolved in the water. They can be released to abnormally high amounts of dissolved free amino acids from broken and living cells when they were frozen in the ice core.

The counts of phytoplankton cells and measurements of chlorophyll-a indicate that the algae was abundant in the ice core community. There are two major peaks of phytoplankton

abundance in the ice core during the year, the first peak was occurred in May while the second peak occurred in November and December (Perrin *et al.*, 1987) in the same study area. We have studied amino acids and volatile fatty acids in sea water during May 1988 to February 1989 (Yang *et al.*, 1990 and Yang, 1992), the concentrations of those compounds were much lower during winter time but the higher concentrations of them presented in the summer algae bloom. So it is significant to know that variations of concentrations and distributions of amino acids are present in the sea ice core and lake ice core, and also what is different between the sea core and the Ace lake ice core. These results could be help to understanding biological ecology, physiology and biochemistry in ice zone.

The concentrations of amino acids in sea ice near Davis Station and Ace Lake ice core in Vestfold Hill, East Antarctica were measured by High Pressure Liquid Chromatography (PHLC) in 1988, This paper discusses the vertical distribution of amino acids in relation to seasonal variation of ice algae in sea ice core and Ace Lake ice core.

Material and Methods

Sea ice core was taken from 10 km north west of Davis Station, and Ace Lake ice core was taken from centre of Ace Lake, Vestfold Hill, Antarctica (Fig. 1).

Two ice cores were collected by a 7 cm diameter SIPRE ice auger, each 10 cm of core samples were melted at 20°C, and 5 ml melted water samples were filtered through Whatman GF/F glass filter (0.45 µm) which was pre-combusted at 450°C.

PHLC apparatus was used to determine amino acids. The system contained two Kortec ETP pumps, a Kortec mixing chamber, a Rainin microsorb 7125 injection valve with 500 µl loop, a water fluorescent detector Model 420-e. The columns are a Rainin microsorb C-18, 3micron, 50×4.6mm I. D. analytical column with Rainin gard column.

The amino acid derivatizing solution consisted of 50 mg *o*-phthalaldehyde (OPA) (Alltech company) dissolved in 1 ml methanol, add 750 µl 2-mercaptoethanol (Aldrich chemical company), and 150 µl Brij 35 (BDH 30%w/v solution in water) mixed with 5 ml 0.4mol/l Potassium borate (pH 10.4) in a 20 ml blank glass bottle over 24 hours.

Mobile phases were: (A) 0.1mol/l sodium acetate, 0.5%, Tetrahydrofuran and 9.5% methanol, adjusted to pH 6.8 with 0.1mol/l acetic acid. (B) methanol. The gradients used generally close to 5 to 65% (B) in 21 min., returning to initial conditions at a constant flow rate of 1ml/min.

400µl filtered samples was mixed with 100µl 1.0mol/l sodium acetate buffer in the 1ml vase, and add 100µl OPA derivatizing solution, with derivatizing time of 1min before injection. Injected volume was 500µl. The peaks were identified by their characteristic retention time and quantification was by peaks area linearly compared to standards. The typical standard with gradient of mobile phase and ice core samples runs are showed in Fig.

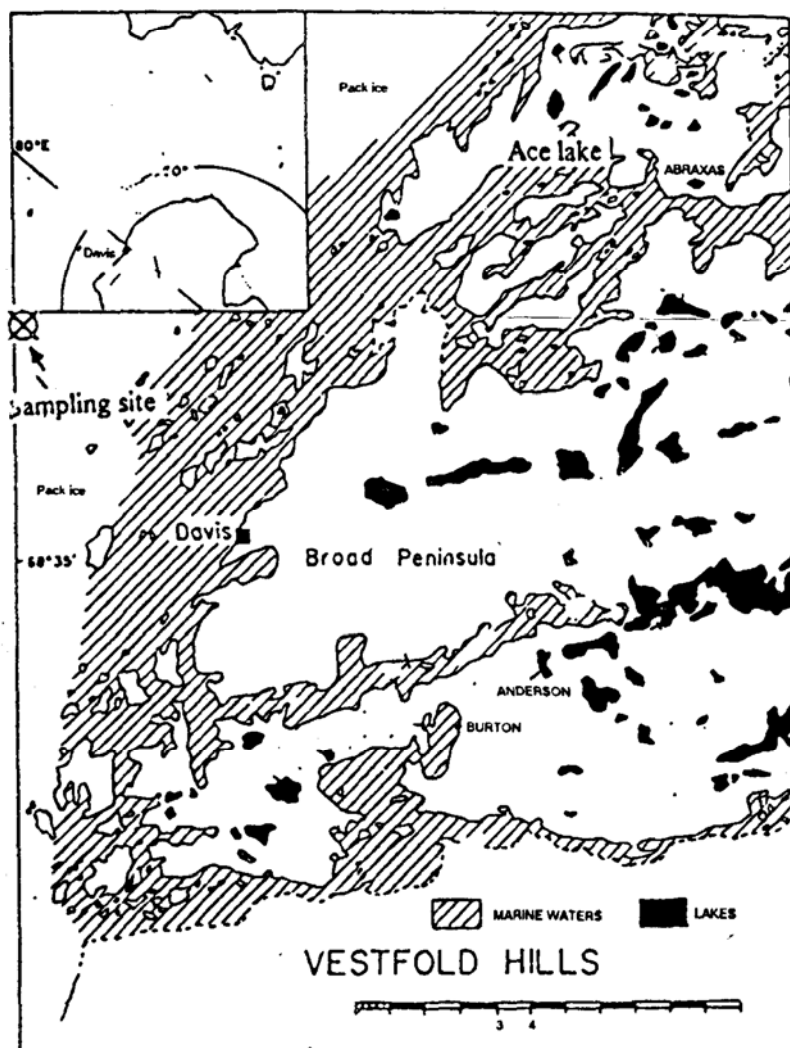


Fig. 1. Sampling station, sea ice core taken from 10 km north west of Davis Station, lake ice core taken from Ace Lake, Vestfold Hill.

2 and 3.

Results and Discussion

The results of amino acid measurement in sea ice core and Ace Lake ice core are summarized in Table 1 and 2. The results showed that the concentration of total amino acids in ice cores is much higher than that in the sea water. The concentration of total amino acids varied between $8\mu\text{mol/ml}$ and $30\mu\text{mol/ml}$ in sea ice core, but the highest concentration of that in sea water was only $0.302\mu\text{mol/ml}$ (Yang *et al.*, 1990).

Table 1. The concentrations of amino acids in the Ace Lake ice core.

No.	deep (cm)	Concentration (μmol/ml)																
		aspa.	glua.	asp.	ser.	val.	his.	gly.+thr.	orn.	arg.	ala.	try.+ABA.	met.	try.	phe.	iso.	leu.	lys.
1	0-10	1.506	0.513	1.823	3.959	0.453	2.261	2.619	1.741	0.278	0.151	3.198	0.384	0.339	0.725	0.474	0.610	0.288
2	10-20	1.705	0.569	1.613	4.471	0.577	2.849	3.067	2.398	0.418	0.208	1.517	0.343	0.432	0.917	0.997	1.129	0.788
3	20-30	1.390	0.507	1.586	4.123	0.507	2.381	2.953	2.142	0.410	0.171	1.280	0.327	0.363	0.848	0.894	1.088	0.875
4	30-40	1.988	0.706	2.202	5.062	0.719	3.493	3.667	3.561	0.688	0.262	1.799	0.245	0.501	1.985	1.336	1.573	1.209
5	40-50	1.709	0.650	1.711	4.880	0.556	3.095	3.644	3.511	0.509	0.379	1.707	0.214	0.408	0.947	1.013	1.215	1.015
6	50-60	0.860	0.377	1.070	3.454	0.348	1.726	2.574	1.885	0.365	0.193	1.008	0.145	0.219	0.542	0.611	0.824	0.678
7	60-70	3.370	1.001	3.490	6.058	1.433	5.776	4.508	5.186	0.961	0.532	2.247	1.891	0.989	2.804	1.989	2.258	1.508
8	70-80	2.181	0.808	2.253	5.064	0.748	3.441	3.638	3.304	0.688	0.314	1.964	0.407	0.542	1.194	1.388	1.573	1.178
9	80-90	1.670	0.618	1.720	4.504	0.562	2.735	3.136	2.509	0.535	0.200	1.529	0.276	0.422	0.894	1.102	1.266	0.969
10	90-100	1.863	0.741	2.136	5.061	0.688	3.552	3.730	3.653	0.629	0.337	1.802	0.160	0.421	1.850	1.177	1.411	1.068
11	100-110	2.531	0.838	2.393	5.051	1.043	3.559	3.455	3.186	0.682	0.344	1.655	1.901	0.733	1.877	1.472	1.599	0.920
12	110-120	1.975	0.673	2.019	4.894	0.946	3.179	3.399	3.004	0.667	0.305	1.459	1.334	0.591	1.681	1.207	1.379	0.904
13	120-130	1.016	0.436	1.123	3.719	0.389	1.880	2.607	1.731	0.416	0.197	1.020	0.366	0.263	0.589	0.664	0.834	0.701
14	130-140	1.154	0.476	1.409	3.831	0.354	1.978	2.504	1.703	0.487	0.141	1.009	0.207	0.255	1.118	0.710	0.875	0.605
15	140-150	1.034	0.346	1.142	3.543	0.445	1.597	2.534	1.693	0.291	0.211	0.808	0.598	0.469	0.396	0.598	0.758	0.596

Table 2. The concentrations of amino acids in the sea ice core.

No.	deep (cm)	Concentration (μmol/ml)																
		aspa.	glua.	asp.	ser.	val.	his.	gly.+thr.	orn.	arg.	ala.	try.+ABA.	met.	try.	phe.	iso.	leu.	lys.
1	0-10	0.440	0.311	0.321	1.610	0.132	0.526	1.041	0.756	0.141	0.779	0.505	0.047	0.191	0.243	0.185	0.172	0.371
2	10-20	0.529	0.278	0.568	1.227	0.099	0.762	0.799	0.674	0.227	0.830	0.530	0.030	0.137	0.162	0.142	0.397	0.369
3	20-30	1.454	0.681	1.578	4.049	0.432	2.638	2.735	2.213	0.834	2.589	1.020	0.092	0.287	0.225	0.875	1.609	0.950
4	30-40	1.057	0.680	1.146	3.140	0.358	1.963	2.109	1.496	1.189	3.716	1.037	0.166	0.237	0.607	0.643	0.878	0.664
5	40-50	1.593	0.750	1.799	4.402	0.515	3.037	2.923	2.387	1.043	1.840	1.409	0.104	0.351	1.560	1.012	1.265	0.769
6	50-60	1.581	0.596	1.647	4.447	0.625	3.150	3.249	2.957	0.743	0.313	1.459	0.088	0.374	1.006	1.025	1.125	1.100
7	60-70	1.810	0.606	1.844	4.667	0.648	3.253	3.132	2.884	0.963	0.754	1.488	0.083	0.421	1.045	1.051	1.300	0.976
8	70-80	1.113	0.432	1.205	3.682	0.544	2.202	2.688	2.184	0.533	0.461	1.020	0.090	0.286	0.756	0.684	0.875	0.791
9	80-90	1.120	0.395	1.122	4.119	0.498	2.352	2.746	1.964	0.498	0.452	1.001	0.110	0.332	0.771	0.679	0.754	0.547
10	90-100	0.803	0.311	0.896	3.221	0.447	1.705	2.264	1.656	0.452	0.433	0.820	0.090	0.213	0.523	0.529	0.677	0.545
11	100-110	1.591	0.605	1.624	4.452	0.578	2.771	2.988	2.459	0.569	0.294	1.356	0.087	0.368	0.865	0.921	1.168	1.022
12	110-120	1.268	0.471	1.338	4.203	0.559	2.571	2.873	2.473	0.680	0.635	1.202	0.184	0.308	0.767	0.727	0.925	0.575
13	120-130	1.694	0.628	1.716	4.827	0.548	2.758	3.334	2.775	0.521	0.307	1.490	0.165	0.343	0.777	0.896	1.201	0.902
14	130-140	1.303	0.426	1.401	4.164	0.549	2.480	2.884	2.535	0.679	0.623	1.234	0.226	0.313	0.756	0.768	0.961	0.688
15	140-150	1.393	0.529	1.460	4.310	0.538	2.677	2.832	2.267	0.876	0.594	1.232	0.189	0.358	0.805	0.812	0.943	0.706
16	150-160	2.338	0.872	2.207	4.830	1.019	3.403	3.096	2.790	1.104	0.635	1.399	1.125	0.618	1.819	1.250	1.450	0.911

The highest concentration of amino acids in the sea ice core was found in the bottom of

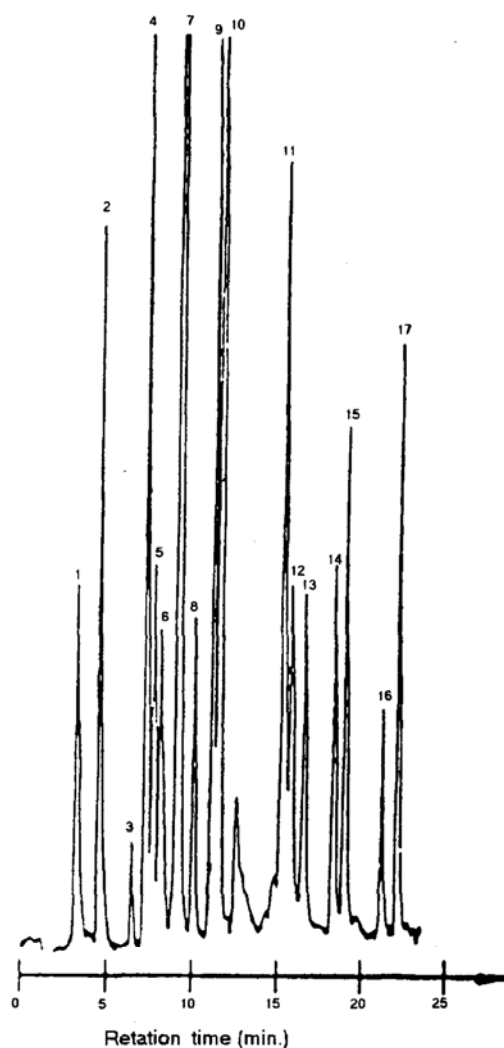


Fig. 2. HPLC chromatogram of standard amino acids condition: Flow rate, 1ml/min; Reaction time, 1min; Gain, 32; Injection volumn, 500 μ l with still water. (1. aspartic acid; 2. glutamic acid; 3. asparagine; 4. serine; 5. glutamine; 6. hisidine; 7. glucina + threonine; 8. arginine; 9. alanine; 10. turosine + r-ABA; 11. methlonine + valme; 12. tryptophene; 13. phenylalanine; 14. iso-leucine; 15. leucine; 16. L-ornithine; 17. lysine.)

ice core which was in November and the lowest concentration of it presented in the surface of the core which is near infiltration layer. The second peak of amino acids abundance presented in 50–70 cm section of the core which correspond to May – June. There was a decreasing process of the concentrations during 70cm to 100cm section, then the concentration was increased from 100cm until 160cm (Fig. 4).



Fig. 3. HPLC chromatogram of sea ice core sample between 10–20cm. condition: Flow rate, 1ml/min; Reaction time, 1min; Gain, 32; Injection volumn. 500 μ l. (1. aspartic acid; 2. glutamic acid; 3. asparagine; 4. serine; 5. glutamine; 6. glucina + threonine; 7. arginine; 8. alanine; 9. turosine + r-ABA; 10. methlonine + valme; 11. tryptophene; 12. phenylalanine; 13. iso-leucine; 14. leucine; 15. L-ornithine; 16. lysine.)

In the Ace Lake ice core, the concentration of total amino acids was higher than that in the sea ice core untill 120cm section of the core except for 60cm. The highest concentration was 46 μ mol/ml which presented in 70cm section of the core and the lowest concentration occurred in 60cm (Fig. 5).

The concentration variation process of total amino acids in the sea ice core was much similar as the that of ice alga cell numbers which were monitored by Perrin(1978). Fig. 6

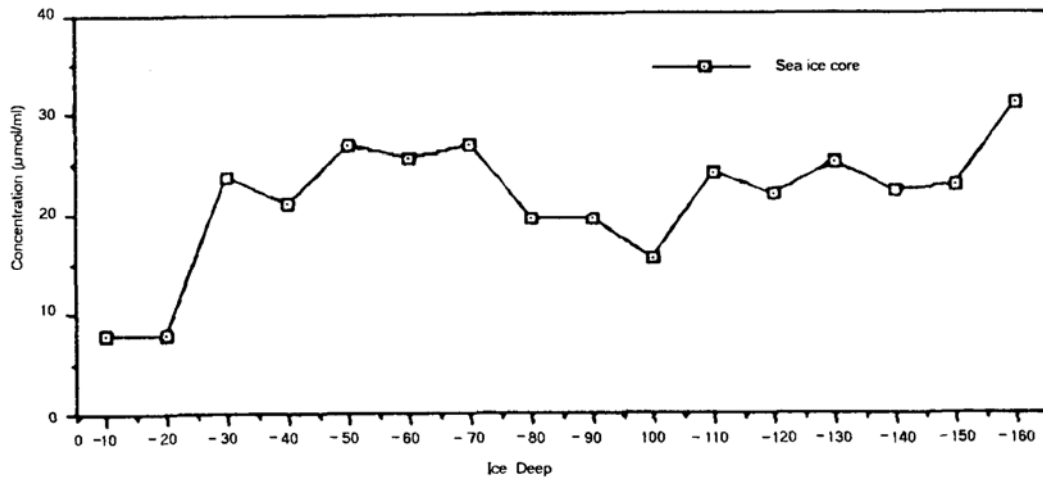


Fig. 4. The concentration variation of total amino acids in sea ice core.

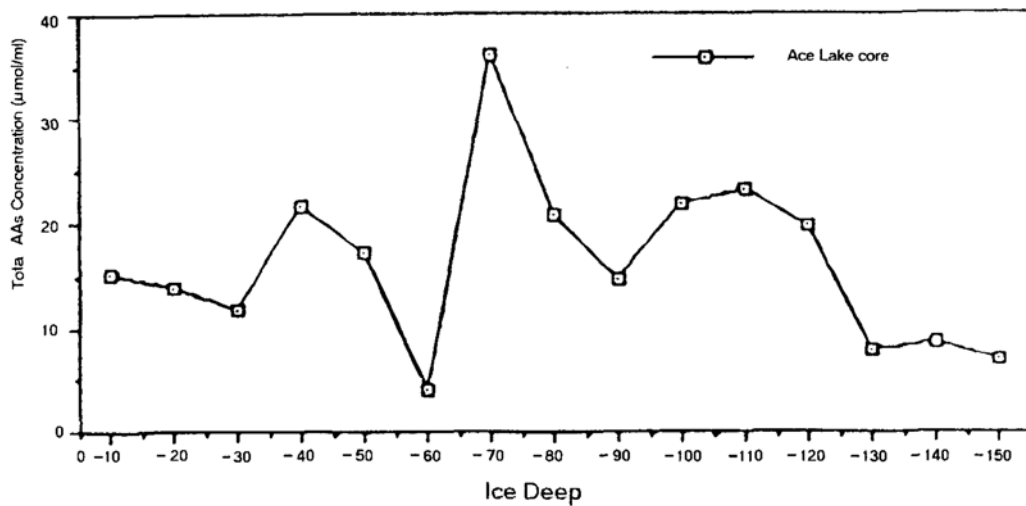


Fig. 5. The concentration variation of total amino acids in Ace Lake ice core.

showed a relationship between concentration of total amino acids and phytoplankton cell numbers in the sea ice core. There exist two major period of phytoplankton abundance in ice core during the year. The first one occurred in May and the second one was November—December. The higher concentration of and the similar variation process of amino acids as phytoplankton cell number indicated that phytoplankton cells release to amino acids as a primary activity and utilizing amino acids is secondary activity in the sea ice core community.

In the spectrum of amino acids in the sea ice core, the highest content was serine which is $38.25\mu\text{mol/ml}$, and 19.3% of the total amino acids. The secondary content of them was histidine, which is 11.2% of the total amino acids. Ornithine and glycine plus with

threonine were 10% and 12.4% respectively. Those compounds dominated amino acids in the sea ice core and the total content of them was approximately 62% of the concentration of total amino acids. The lowest content of amino acid was methionine, whose concentration was $2.8 \mu\text{mol/ml}$ and 0.8% of total amino acids only. Aspartic was the same as the asparagine, and the concentrations of them were middle level in the spectrum of amino acids. In the Ace Lake ice core, the total concentration of amino acids was higher than that in sea ice core, and the distribution of components of amino acids was similar to that in the sea ice core, but there existed some differences between arginine and alanine where the concentrations were lower than that in the sea ice core (Fig. 7). The similar distributions of components of amino acids showed that the way of source of amino acids in the Ace Lake ice core was probably the same as that in sea ice core, and amino acids in ice community was

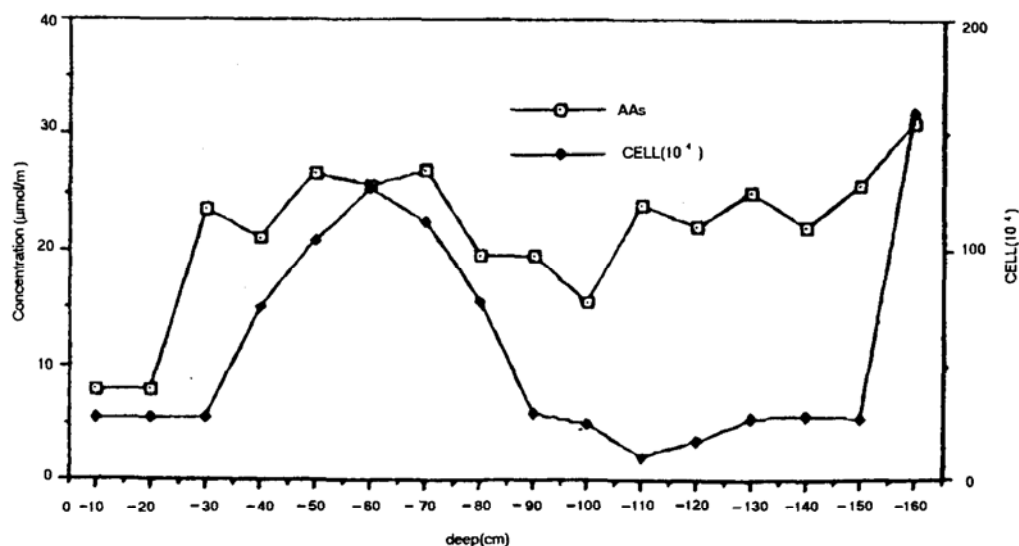


Fig. 6. The relationship between amino acids and phytoplankton cells (date from R. A. Perrin *et al.* 1978).

stabler than that in sea water.

The biological community in the Ace Lake is relatively simple. The only animal is a calanoid copepod, *Paralabidocera Antarctica* (Bayly, 1978), which reaches a maximum density of about 3 animals per litre. The major alga is a small green flagellate *Pyramimonas gelidicola* (Prasinophyceae). Smaller populations of a biflagellate algae, tentatively identified as a *Cryptomonas* species, an unidentified biflagellate algae and a large unarmored dinoflagellate related to *Gymnodinium* are also present (Burch, 1988; Volkman *et al.*, 1986). These algae from discrete populations distribute at different depths in the lake and migrate downwards in the summer months. Some species, such as Microflagellate, adapted to living in top of water column (under ice) during winter time (Burch, 1988). It is possible that these algae entered into the ice community and released amino acids though

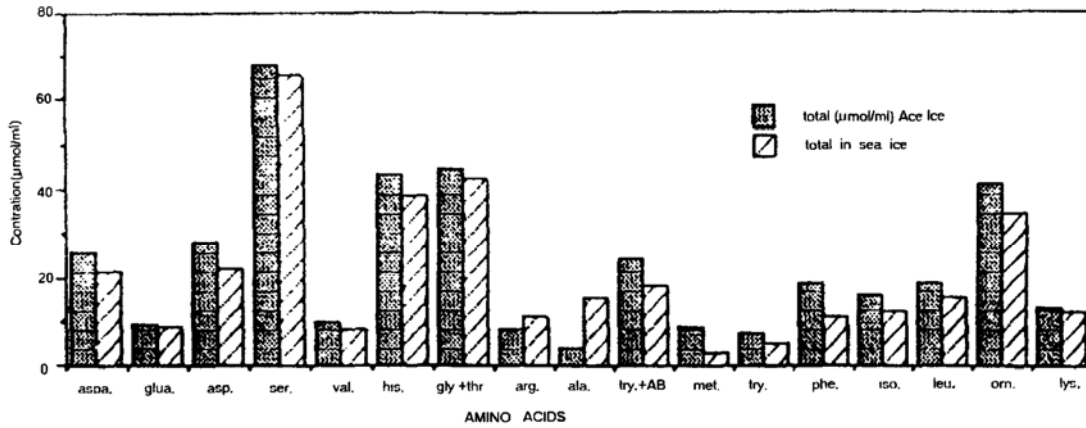


Fig. 7. The distribution of amino acid components in both ice cores.

there are no informations for the ice algae in ice core of this lake.

The proportion of each individual amino acid was much similar to the percentage of total

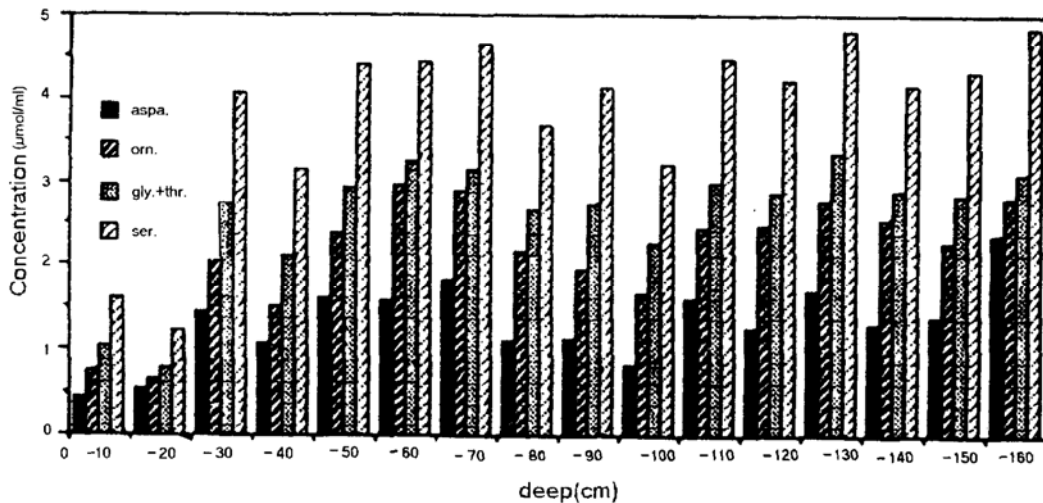


Fig. 8a. The variation of some higher concentration of amino acids in sea ice core.

amino acid in each ice sections (Fig. 8a, 8b, 9a, 9b and 9c). Those similar proportion distributions of concentrations indicated that spectrum of amino acids in the ice core is uniform. The uniform spectrum of amino acids were derived directly from peptides of ice algae cells and were unutilized by organism. This is why the concentration of amino acids in ice core was much higher than that in the sea water column in antarctic sea.

The ice bacteria are often occurring as paired or dividing cells, and an intimate relationship in the sea ice (David *et al.* ,1986), and also these bacteria produce amount of

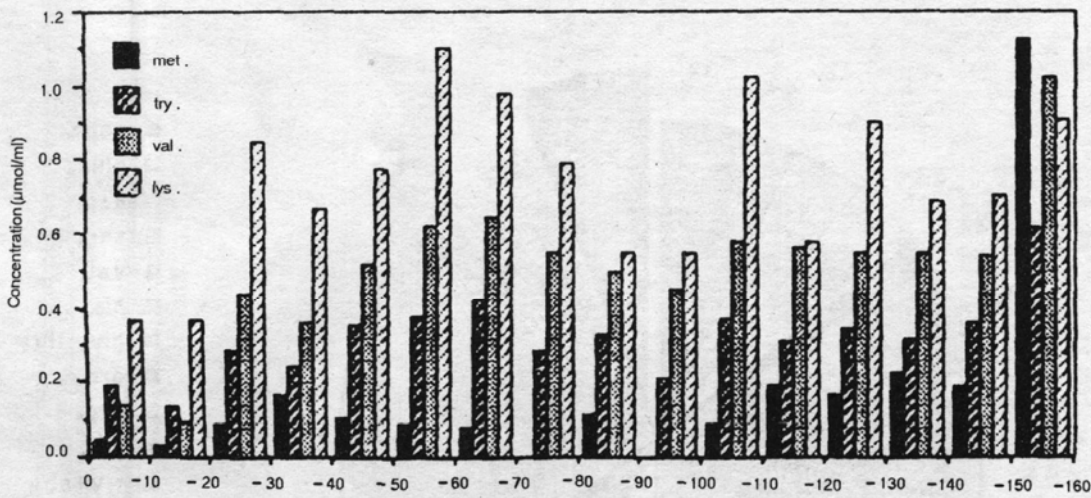


Fig. 8b. The variation of some lower concentration of amino acids in sea ice core.

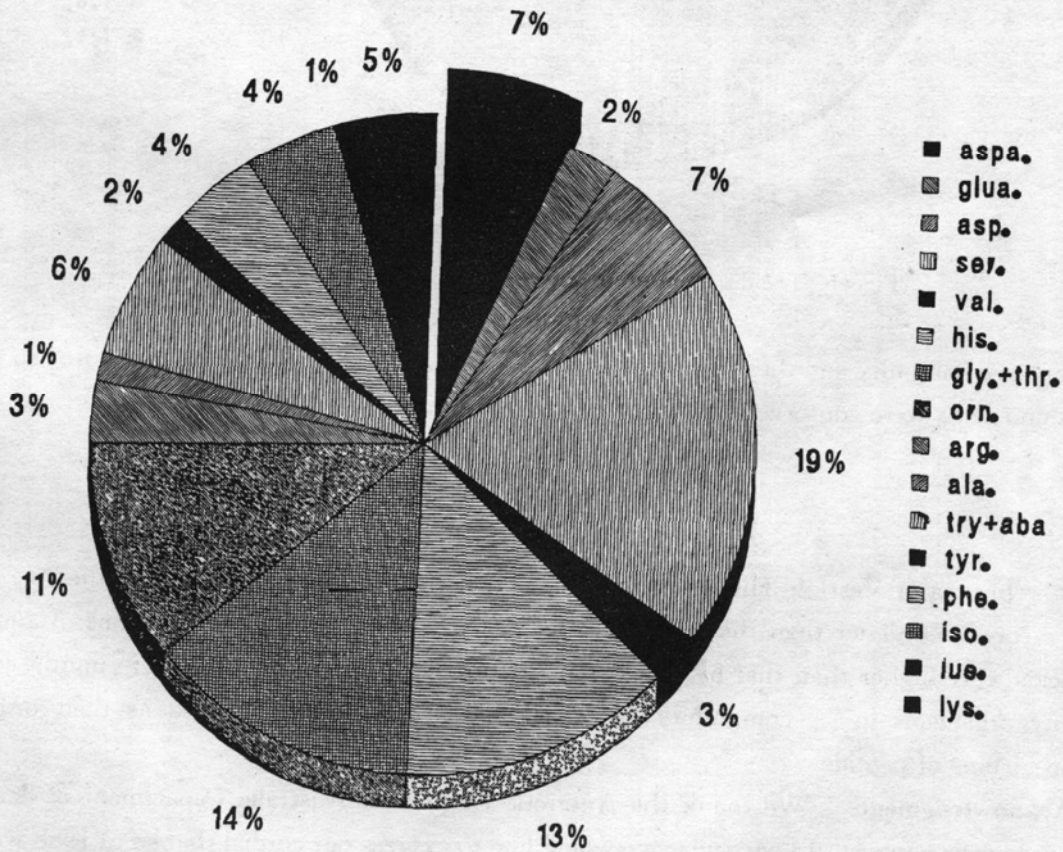


Fig. 9a. The distribution of amino acids in 50—60cm in sea ice core.

amino acids. However, we suggest that the amino acids were directly from peptide of cells in

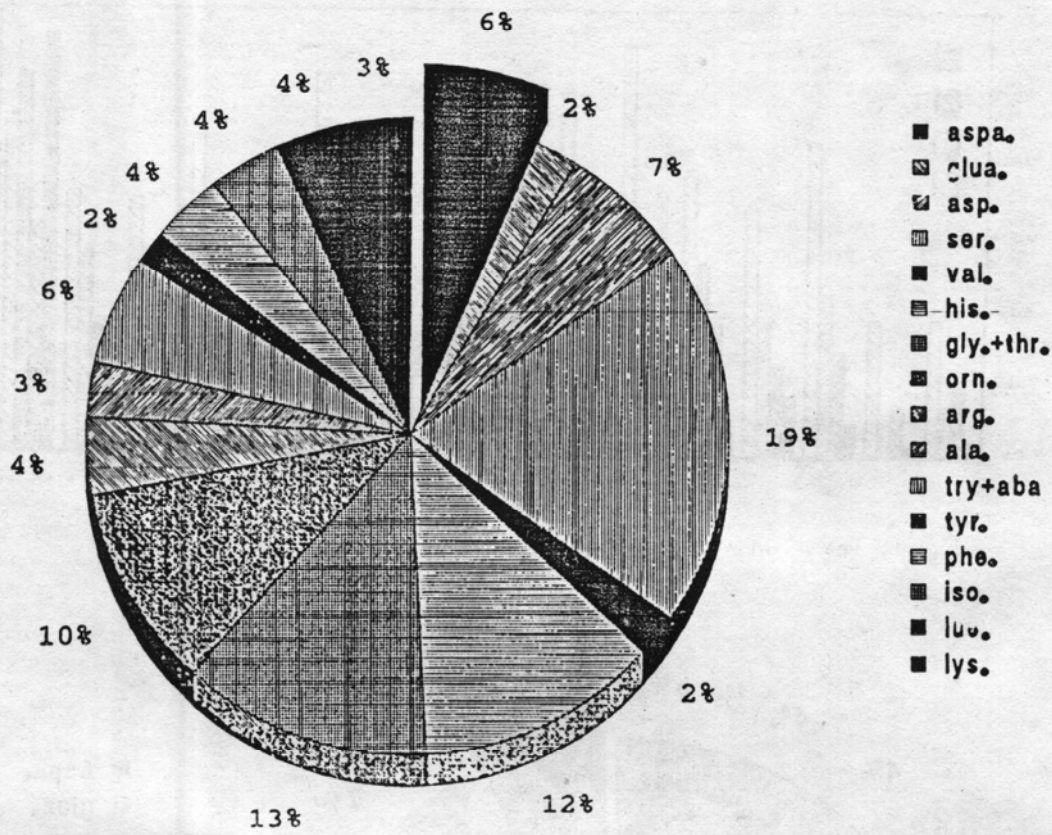


Fig. 9b. The distribution of amino acids in 140—150cm in sea ice core.

the ice community and these amino acids were unutilized by any organism in ice core, so that amino acids were conserved as their origin spectrum of peptide.

Conclusion

In area of Vestfold Hill, Antarctica, the concentration of amino acids in the Ace lake ice core was higher than that in the sea ice core, and also those concentrations of amino acids were higher than that in their water community. The amino acids were unutilized by any organisms in ice community, so that amino acids were conserved as their original spectrums of peptide.

Acknowledgments We thank the Antarctic Division of Australia Department of Science for logistic support. In particular we would like to express our cordial thanks to 1988 winter expeditioners at Davis station for assistance with field works.

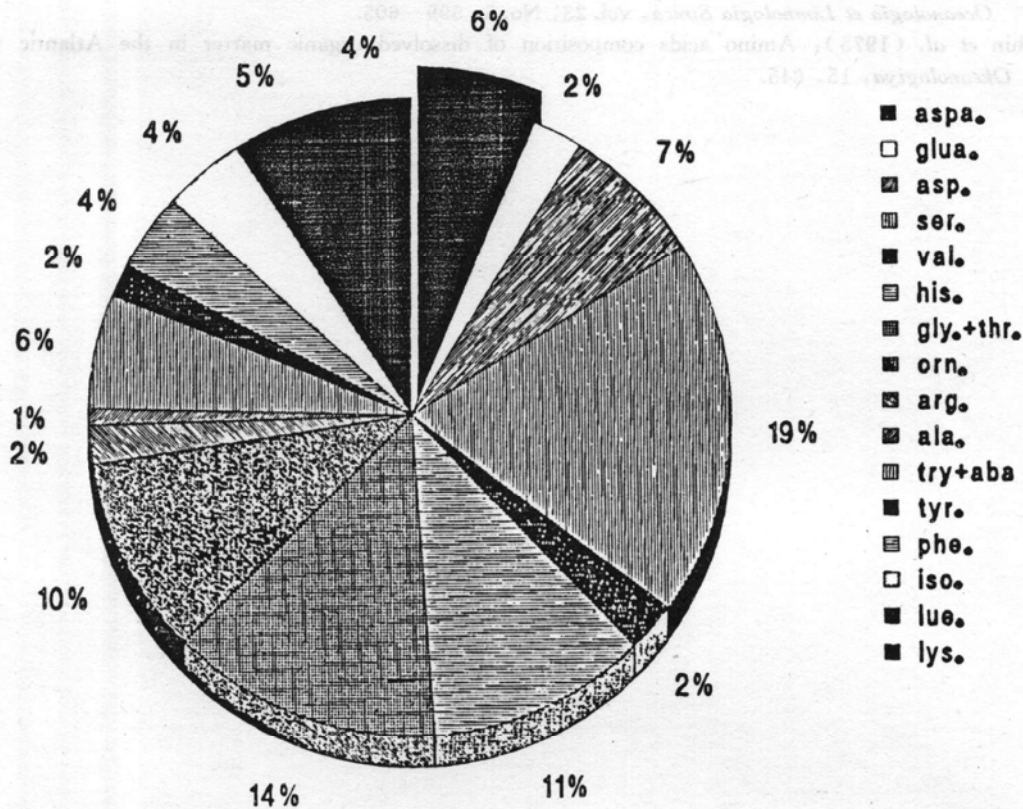


Fig. 9c. The distribution of amino acids in 20-30cm im Ace lake ice core.

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