

AN ANALYTICAL STUDIES OF FREE AMINO ACID AND
ITS RELATIONSHIP AMONG THE MAIN
GROUPS OF GREEN ALGAE

On the studies of chemical components and its relationship
to the phylogeny of marine algae. (III)

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李敏載·洪濼佑·李仁奎：綠藻 綠藻類의 유리 아미노酸 分布에 따른 系統學的 研究
藻類의 化學成分과 系統學的 相關性에 對하여 (Ⅲ)

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ABSTRACT

LEE, Min-Jai, HONG, Soon-Woo & LEE, In-Kyu, (Dept. of Botany, Seoul National Univ.) An analytical studies of free amino acid and its relationship among the main groups of green algae (III). Kor. Jour. Bot. V (3): 25-29, 1962.

Succeeding the previous papers, nineteen species of marine green algae and three species of fresh water green algae are analyzed to the free amino acid patterns by paper chromatogram, and it has been described as containing significant qualities of the pattern in relation to phylogenetic studies. Those seem to have a tendency of recognizable pattern on inter-Orders and inter-Phyla of marine algae. And the patterns of fresh water and marine green algae are also carried out referring to these studies.

INTRODUCTION

It was shown in the preceeding papers^{1) 2)} that the amino acid pattern on each Order and Phylum of marine brown and red algae, are presumed to have a certain significance in relation to phylogeny. Particularly, the characteristic occurrence of certain amino acids such as ornithine and citrulline, which are known as essential substances in the ornithine Kreb's cycle and their distributions are reported only in a few species^{3) 4) 5) 6)}, are broadened their appearances in many species by the studies of authors.

In addition to previous works, the studies concerning nineteen species of green algae are carried out in the present experiment and our results are discussed herein.

MATERIALS AND METHODS

The materials used in the present experiment are; 19 species belonging to 3 Orders, 5 Families of marine green algae and 3 species of fresh water algae. And its classified taxon is shown in Table I.

The localities and the date of collected materials, and the experimental methods are same as previous works.

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Table I. Classification of the given materials and its localities

Order	Family	Genus & Species	Localities*
Ulvales	<i>Monostromaceae</i>	<i>Monostroma arctium</i>	(P)
	<i>Ulva</i>	<i>Ulva pertusa</i>	(P) (K) (D)
		<i>U. conglobata</i>	(P)
		<i>Letterstedtia japonica</i>	(P)
		<i>Enteromorpha compressa</i>	(P)
		<i>E. linza</i>	(P)
		<i>E. intestinalis</i>	(P) (D) (K)
		<i>E. crinita</i>	(P) (D)
		<i>E. prolifera</i>	(P)
		Siphonocladiales	<i>Cladophoraceae</i>
<i>C. utriculosa</i>	(P)		
<i>C. uncinella</i>	(P)		
<i>C. rudolphiana</i>	(P)		
<i>C. gracilis</i>	(P)		
<i>Chaetomorpha moniligera</i>	(P)		
Siphonales	<i>Bryopsidaceae</i>		
	<i>Codiaceae</i>	<i>Codium contractum</i>	(P)
		<i>C. fragile</i>	(P) (K)
		<i>C. tomentosum</i>	(P)
		** <i>Spirogyra</i> sp. I	
<i>S.</i> sp. II			
<i>Zygnema</i> sp.			

* (P); Collected at the coast of Pusan, (K); at Kangnoong, (D); at Daechun.

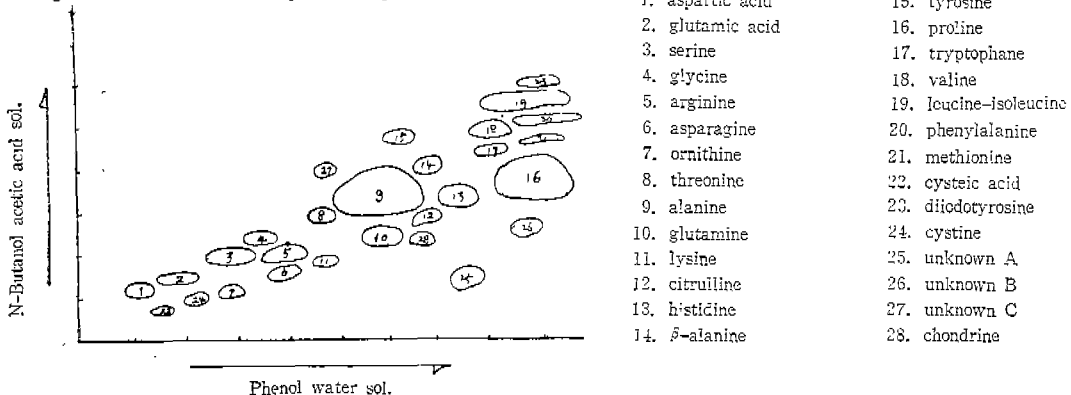
** Fresh water algae.

EXPERIMENTAL RESULTS

The total number of free amino acid constituents in 19 species of given materials are identified as 28 kinds, including 3 unknowns.

As in the following Figure, the distribution pattern is somewhat similar to brown and red algae except the unknown D, to be newly identified as a chondrine which occurs only in one species of the given green algae, *Letterstedtia japonica*.

Fig. I. The free amino acid pattern of green algae :



The revealed each amino acid in each species has peculiarity that β -alanine is detected conspicuously high in its frequency of occurrence, while the rest of them are more or less similar to the other Phyla of marine algae. And the detailed results are shown in Table II.

Table II. The free amino acid distribution on each species:

Material	Species													Total	Other species								
	<i>Monostroma arcticum</i>	<i>Ulva pertusa</i>	<i>U. conglobata</i>	<i>Letterstedtia japonica</i>	<i>Enteromorpha compressa</i>	<i>E. linza</i>	<i>E. intestinalis</i>	<i>F. crinita</i>	<i>E. prolifera</i>	<i>Cladophora densa</i>	<i>C. utriculosa</i>	<i>C. uncinella</i>	<i>C. rudolphiana</i>		<i>C. gracilis</i>	<i>Chaetomorpha monilifera</i>	<i>Bryopsis pulmosa</i>	<i>Codium contractum</i>	<i>C. fragile</i>	<i>C. tomentosum</i>	<i>Spirogyra</i> sp. I *	<i>S. sp.</i> II *	<i>Zygnema</i> sp. *
Aspartic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Glutamic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Serine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Glycine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Arginine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Asparagine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Ornithine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Threonine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alanine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Glutamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lysine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Citrulline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
β-Alanine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Tyrosine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Proline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Tryptophane	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Valine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Leucine-isoleucine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Phenylalanine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Methionine																+					+		
Cysteic acid				+														+					
Di-iodotyrosine	+							+	+														
Cystine																							
Unknown A	+																						
" B																							
" C				+				+															
Chondrine				+				+															
Total	19	17	21	20	17	19	19	17	19	19	20	20	18	19	20	21	21	21	22		20	19	17

* Fresh water algae

Furthermore, the distribution of amino acids and their relations among inter-Orders, inter-Families and inter-Genus are summarized in Table III.

In those results, it can be found that the free amino acids, which are detected throughout all the experimented species, are revealed as eleven kinds; aspartic acid, glutamic acid, serine, glycine, arginine, threonine, alanine, glutamine, proline, valine and leucine-isoleucine, while asparagine is appeared every species except one, *Letterstedtia japonica*, and β-alanine except *Cladophora utriculosa*. Citrulline and tyrosine are appeared in sixteen kinds of given species, and ornithine, lysine, histidine, tryptophane, phenylalanine, cystine and unknown A are distributed about half of the given species, while unknown B and C are comparatively less in their occurrences.

The rest of them, methionine, cysteic acid, diiodotyrosine and chondrine, are detected only in one or two species.

DISCUSSION AND CONCLUSION

It is interesting that our present result, finding occurrence of eleven kinds of free amino acids—essential according to our results—is opposite to our previous works dealing with twelve kinds in brown and red algae. In this investigation carried out in all green algae, neither tryptophane appeared in red algae,

Table III. The distribution of free amino acids in Order, Family and Genus.

Amino acid	Order	<i>Ulvales</i>				<i>Siphonocladiales</i>		<i>Siphonales</i>	
	Family	<i>Monostromaceae</i>	<i>Ulvaceae</i>			<i>Cladophoraceae</i>		<i>Bryopsisidaceae</i>	<i>Codiaceae</i>
	Genus	<i>Mono-</i> <i>siroma</i>	<i>Ulva</i>	<i>Letter-</i> <i>stedtia</i>	<i>Entero-</i> <i>morpha</i>	<i>Clado-</i> <i>phora</i>	<i>Chaeto-</i> <i>morpha</i>	<i>Bryopsis</i>	<i>Codium</i>
Aspartic acid		+	+	+	+	+	+	+	+
Glutamic acid		+	+	+	+	+	+	+	+
Serine		+	+	+	+	+	+	+	+
Glycine		+	+	+	+	+	+	+	+
Arginine		+	+	+	+	+	+	+	+
Asparagine		+	+	-	+	+	-	+	-
Ornithine		-	-	+	R	R	-	-	R
Threonine		+	+	+	+	+	+	-	+
Alanine		+	+	+	+	+	+	+	+
Glutamine		+	+	+	+	+	+	+	+
Lysine		+	R	+	R	R	+	-	-
Citrulline		-	R	-	+	+	+	+	+
Histidine		-	+	+	R	R	+	+	R
β -Alanine		+	+	+	+	R	+	+	+
Tyrosine		+	+	+	R	+	+	+	R
Proline		+	+	+	+	+	+	+	+
Tryptophane		-	R	+	R	R	-	-	+
Valine		+	+	+	+	-	+	+	+
Leucine-isoleucine		+	+	+	+	+	+	+	+
Phenylalanine		+	+	-	-	R	+	+	+
Methionine		-	-	-	R	-	-	+	-
Cysteic acid		-	R	+	-	-	-	-	R
Diodotyrosine		+	-	-	R	-	-	-	-
Cystine		-	-	-	R	R	-	-	-
Unknown A		+	R	-	R	R	-	+	+
" B		-	-	-	R	R	-	-	+
" C		-	R	+	R	R	+	-	+
Chondrine		-	-	+	-	-	-	+	-
The mean of Genus		19	19	20	18.2	19.2	19	21	21.5
" of Family		19	18.6			19.2		21	21.3
" of Order		18.7			19.2		21.3		
Total mean		19.4							

nor asparagine in brown algae are appeared throughout all the given species, but they are happened to occur random distribution in both, however, cystine, not the essential of brown and red, has appeared essentially in green algae, and these are revealed as ones of the conspicuous characteristics of green algae.

The average occurrence of these amino acids throughout the given species is somewhat higher than those of red and brown algae. In green algae, it is estimated as 19.37, while red algae is 19.03 and brown is 18.88 of amino acids. And their average frequency of inter-Orders, inter-Families and inter-Species is shown in Table III.

As in Table III, the present study may reconfirm our results of previous works; the average frequency of Order or Family increases in parallel with occurrence from low level groups to higher groups of algae. For instance, the average occurrence of Ulvaceae is the lowest of the five Families, 18.6, and Codiaceae has the highest frequency of 21.3, while Cladophoraceae, to be occupied between the above two Families, shows medium frequency as 19.2.

The previously reported unknown D was identified to be L-1-sulfoxythiazine-3-carboxylic acid, which was named "Chondrine" by Kuriyama et al⁷⁾ as a new amino acid, because its specific reaction, its Rf value and characteristic color with ninhydrin on paper chromatograms, was the same. Furthermore its dark bright blue color with ninhydrin was decayed soon and became brown by continuous heating. However, this amino acid was found in red alga, *Chondria crassicaulis*, its distributing frequency was high in brown algae.¹⁾ According to our research, only one species, *Letterstedtia japonica*, has this peculiar amino acid.

No lysine and tyrosine, in Mazur's report,⁸⁾ are found in Genus *Ulva*, but in our research the former was identified in *Ulva conglobata* and the latter was detected in both, *Ulva pertusa* and *U. conglobata*. A results of Ogino,⁵⁾ studying nine species of green algae, are somewhat different from our investigation, for example, in his work *Ulva pertusa* has only seven kinds of amino acids such as glycine, alanine, serine, glutamic acid, cystine, arginine and ornithine, but our present research reveals that its constituent is analysed to as much as seventeen kinds of free amino acids.

The frequency of occurrence of β -alanine is conspicuously high compared with that of brown and red algae, but citrulline and ornithine show medium frequency of the appearance between the above two, while diiodotyrosine shows the lowest in its appearance ratio.

To compare with the results of fresh water green algae, there is no distinctive difference on the pattern of essential amino acids between two groups, however, diiodotyrosine, unknown A, C and chondrine which are presumed to occur only in marine algae can not be found in fresh water algae, although ornithine and citrulline show random distribution.

要 約

前報에 이어서 19種의 海水綠藻類와 3種의 淡水綠藻類의 유리 아미노酸 分布에 따르는 藻類의 系統學的 相關關係를 考察하였다.

本實驗의 結果 前報에서 結論내린바 있는 유리 아미노酸의 個別 海藻에 나타나는 平均 出現頻度는 藻類系統關係와 더욱더 密接한 연관을 보여주어 그 平均値의 變化는 綠藻類의 進化 序列과 平行되어 增加하는 現象을 보여 주었다.

또 前報에서 未確認된 未知種 D의 아미노酸이 "Chondrine" 이란것이 밝혀졌다.

淡水綠藻와 海水綠藻의 比較分析한 結果는 大差가 없었으나 Diiodotyrosine, Chondrine 등이 淡水藻에서는 나타나지 않았음이 特異 하였다.

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