クロアワビ稚貝に対する摂餌誘引物質について

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Feeding Attractants in Chemical Constituents of Brown Alga for Young Abalone*1,2

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The attraction indexes to abalone of proteins, lipids, amino acids, volatile bases and betaines which were fractionated from the brown alga *Ishige okamurai* were higher than that of dummy as control. The indexes of the four constituents except the betaines were approximately equal to that of the whole extract and higher at different concentrations than that of dummy. In this context, it was estimated that the feeding attractants for abalone are the proteins, the lipids, the amino acids and the volatile bases.

Among a variety of two- and three-constituent combinations, the attraction indexes of combinations of the proteins-lipids and the proteins-lipids-amino acids or betaines were appreciably more effective than those of its single constituent and/or two-constituent combinations. These results reveal that the attraction is reinforced collaborately by two- and three-constituent combinations.

In the preceding paper[1] of a series of the studies on the feeding attractants for fishes and shells, it was estimated that the main probable attractant for young abalone *Haliotis discus* is proteins on the basis of the significance test of correlation between three-constituent combinations and an attraction index of whole extract.

To confirm this finding, nine chemical constituents including the proteins were fractionated from brown alga *Ishige okamurai* which is an attractive feedstuff for gastropod,[2] and were employed for the behavioral experiments to obtain their attraction indexes. Furthermore the indexes of a variety of two- and three-constituent combinations were also investigated.

Materials and Methods

Test Animals

One hundred specimens of young abalone of 2.3 cm in average shell length were obtained from the Senzaki Station of Aquaculture, on June 11, 1982. They were reared with green laver *Ulva pertusa* described previously[2] to 2.6 cm at the end of the behavioral experiments, on February 10, 1983. Fifty individuals were used for the experiments.

Fractionation and Determination of Chemical Constituent

Samples of *Ishige* were collected from the intertidal zone off Yoshimi and Yoshimo in Shimonoseki during May-June and stored at −40°C until use. The fractionation and determination of such chemical constituents as crude lipids, crude proteins, whole extract, aldehydes, volatile organic acids, volatile bases, amino acids, betaines, and sugars, were carried out by the method described in a preceding paper.[3]

Estimation of Attraction Index

The attraction index *a* (referred to as A. I. *a*) was estimated as described previously.[2] 7.5 m*l* of liquid constituents was soaked to gauze (25 × 25 cm) and 7.5 g of macerate or proteins was wrapped with gauze. They were employed as the attractant samples for obtaining the A. I. *a*. Only gauze was used as control dummy.

Results and Discussion

Table 1 shows the concentrations employed for the chemical constituents. These concentrations were used in the behavioral experiments unless specified.

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*2 Studies on the Feeding Attractants for Fishes and Shells-IX.

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Table 1. Concentrations of chemical constituents[3]

Constituents	Concentrations
Non-extractive constituents (mg/g)	
Lipids	30.6
Proteins N	3.2
Extractive constituents (mg/ml)	
Whole extractive* N	0.9
Aldehydes	0.2
Volatile organic acids	3.4
Volatile bases N	0.2
Amino acids	1.6
Betaines	0.9
Sugars	1.6

* Contained 0.4 mg/ml of lipids.

Table 2 shows the A.I.*a* of single chemical constituent. The trial system No. 1 was carried out to investigate the attraction of the proteins and whether or not the whole extract can be employed as a standard attractant in the other trial systems. The A.I.*a* of the whole extract was higher than those of the dummy and of the macerate which attracts the gastropod.[2] The A.I.*a* of the proteins was lower than that of the whole extract, but almost equivalent to that of the macerate. Accordingly, the whole extract was used as the standard solution in most of the other trial systems. In the trial systems of the organic acids and the lipids (No. 2), the bases and the aldehydes (No. 3), the bases and the amino acids (No. 4), and the betaines and the sugars (No. 5), the A.I.*a*'s were roughly equivalent to or higher in the lipids, the bases and the amino acids than in the whole extract. The A.I.*a*'s of these three constituents were appreciably higher than that of dummy, while the A.I.*a* of the betaines was slightly higher than that of the dummy. The trial system No. 6 was carried out to compare the attraction among the proteins, the lipids and the amino acids. The A.I.*a* was highest in the proteins and lowest in the amino acids, being intermediate in the lipids. From these results, it may be said that the attractants for abalone are the proteins, the lipids, the amino acids and the bases. To confirm this result, trial systems (No. 7–11) shown in Table 3 were carried out for the four kinds of constituents together with the betaines at three different concentrations. The A.I.*a*'s of the four constituents were considerably higher regardless of concentration than that of the dummy, but that of the betaines was higher only at the concentration ratio of 1.0. In this context, it was verified that the proteins, the lipids, the amino acids and the bases are the effective attractants as single constituent for abalone.

Table 4 shows the A.I.*a* of two-combined constituents between one of the proteins, the lipids or the amino acids, and one of other constituents. In the trial systems No. 12–14, the A.I.*a*'s were considerably higher in the proteins-amino acids, -betaines, -bases and -lipids than in single constituent of the proteins. In the systems No. 15–17, the A.I.*a*'s were higher in the lipids-amino acids, -betaines and -bases than in single constituent of the lipids. On the contrary, in the trial systems of the amino acids-other combinations (No. 18–20), the A.I.*a*'s were lower in any combination than in single constituent of the amino

Table 2. Attraction index *a*(A.I.*a*) of single chemical constituent

Trial system No.	Constituents	A.I.*a**	Trial system No.	Constituents	A.I.*a**
1	Dummy	4.5†	4	Dummy	4.4†
	Whole extract	8.0†		Whole extract	9.4†
	Macerate	6.3†		Bases	6.1†
	Proteins	6.7†		Amino acids	9.9†
2	Dummy	5.6†	5	Dummy	4.2†
	Whole extract	8.2†		Whole extract	12.0†
	Organic acids	5.6†		Betaines	5.3†
	Lipids	7.3†		Sugars	4.5†
3	Dummy	5.0†	6	Dummy	—
	Whole extract	5.4†		Proteins	9.6†
	Bases	8.8†		Lipids	8.4†
	Aldehydes	3.4†		Amino acids	7.4†

* Coefficient *a* (min) of a logistic curve $y=g/\{1+\exp[-r(j-a)]\}$ in the remaining time-course.[2]

†,†? $\Pr(\chi^2>\chi^2_{0.100})>0.100$ and slightly less than $\Pr=0.100$, respectively.[2]

Table 3. Attraction index *a* (A.I.*a*) at different concentrations

Trial system No.	Constituents	Conc. ratio	A.I.*a**	Trial system No.	Constituents	Conc. ratio	A.I.*a**
7	Proteins	0	3.2†	10	Bases	0	2.2†
		0.1	4.3†			0.1	6.2†
		0.5	9.1†			0.5	9.0†
		1.0	11.2†			1.0	5.5†
8	Lipids	0	6.3†	11	Betaines	0	10.1†
		0.1	13.6†			0.1	5.6†
		0.5	6.8†			0.5	6.6†
		1.0	15.4†			1.0	15.4†
9	Amino acids	0	5.9†				
		0.1	6.6†				
		0.5	6.3†				
		1.0	9.0†				

Signs, see footnotes of Table 2.

Table 4. Attraction index *a* (A.I.*a*) of two-constituent combinations

Trial system No.	Combinations	A.I.*a**	Trial system No.	Combinations	A.I.*a**
12	Dummy	—	17	Dummy	3.8†
	Proteins	4.3†		Lipids	6.3†
	Proteins-Amino acids	6.9†		Lipids-Sugars	4.2†
	Proteins-Betaines	7.1†		Lipids-Aldehydes	5.1†
13	Dummy	—	18	Dummy	3.8†
	Proteins	6.5†		Amino acids	9.9†
	Proteins-Bases	13.6†		Amino acids-Betaines	7.4†
	Proteins-Lipids	12.4†		Amino acids-Bases	7.4†
14	Dummy	5.4†	19	Dummy	6.7†
	Proteins	12.8†		Amino acids	8.4†
	Proteins-Sugars	6.5†?		Amino acids-Organic acids	7.3†
	Proteins-Organic acids	8.0†		Amino acids-Sugars	7.7†
15	Dummy	—	20	Dummy	—
	Lipids	2.8†		Amino acids	9.2†
	Lipids-Amino acids	6.9†		Amino acids-Sugars	7.0†
	Lipids-Betaines	6.6†		Amino acids-Aldehydes	8.8†
16	Dummy	—			
	Lipids	6.8†			
	Lipids-Bases	8.4†			
	Lipids-Organic acids	7.2†			

Signs, see footnotes of Table 2.

acids. From the results obtained above, it was estimated that the combinations of two-constituents, which are the effective attractants as single constituent, elicited, on the whole, collaborative attraction from abalone.

Furthermore the attraction of three-combined constituents was investigated. Table 5 shows the A.I.*a* of three-combined constituents between the proteins and two constituents among four kinds of effective attractants as single constituent, the lipids, the amino acids, the bases, and the betaines. The A.I.*a*'s were higher in the combinations of the lipids (No. 24 and 31), the betaines (No. 23 and 26), the amino acids (No. 27), and the bases (No. 32) combined with two-constituents than in the two-combinations. Especially the A.I.*a*'s were remarkably high in the combinations of the proteins-lipids-betaines (No. 23 and 31) and the proteins-

Table 5. Attraction index *a* (A.I.*a*) of three-constituent combinations

Trial system No.	Combinations	A.I.*a**	Trial system No.	Combinations	A.I.*a**
21	Dummy	3.8†	27	Dummy	3.8†
	Proteins	5.8†		Proteins	6.6†
	Proteins-Lipids	7.5†		Proteins-Bases	6.0†
	Proteins-Lipids-Amino acids	6.4†		Proteins-Bases-Amino acids	8.2†
22	Dummy	—	28	Dummy	3.0†
	Proteins	4.8†		Proteins	6.0†
	Proteins-Lipids	6.6†		Proteins-Bases	6.6†
	Proteins-Lipids-Bases	6.7†		Proteins-Bases-Lipids	6.0†
23	Dummy	—	29	Dummy	4.4†
	Proteins	4.0†		Proteins	5.3†
	Proteins-Lipids	5.8†		Proteins-Bases	8.0†
	Proteins-Lipids-Betaines	9.2†		Proteins-Bases-Betaines	7.0†
24	Dummy	3.8†	30	Dummy	—
	Proteins	5.0†		Proteins	5.8†
	Proteins-Amino acids	6.0†		Proteins-Betaines	5.2†
	Proteins-Amino acids-Lipids	15.4†		Proteins-Betaines-Amino acids	5.4†
25	Dummy	3.4†	31	Dummy	—
	Proteins	5.4†		Proteins	5.1†
	Proteins-Amino acids	6.0†		Proteins-Betaines	5.0†
	Proteins-Amino acids-Bases	5.6†		Proteins-Betaines-Lipids	11.9†
26	Dummy	—	32	Dummy	—
	Proteins	4.1†		Proteins	4.6†
	Proteins-Amino acids	4.6†		Proteins-Betaines	5.2†
	Proteins-Amino acids-Betaines	6.0†		Proteins-Betaines-Bases	8.6†

Signs, see footnotes of Table 2.

amino acids-lipids (No. 24). These results suggest that the lipids or the betaines with few exceptions (No. 28 and 29) reinforce collaboratively the attraction of two-combined constituents. In the previous paper,[1] it was reported that five groups of three-combined constituents were significant in the attraction by a statistical analysis and that the proteins might be one of the most probable attractants, because co-constituent in the combinations was the proteins. This supports the results shown in Tables 2, 4 and 5. From these experimental results described above, it was concluded, as a whole, that two- and three-combined constituents were collaboratively more effective than single one. In this connection, CARR[4] demonstrated the similar collaborative attraction for mud snail *Nassarius obsoletus*.

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