The shift assignment of the C-21 carbon can be rationalized by the observation that the substitution of a proton by a cyano group results in only a slight down-field shift at the attached carbon[10].

Moreover, the high resolution mass spectrum confirmed the above conclusion. The fragment ions m/e 462 (M^+ -100) (**8**), 220 (**10**) and 218 (**11**) were derived from the basic skeleton of saframycin group antibiotics. Of particular significance was the presence of the fragment ion m/e 243 (**9**) corresponding to the ion containing a cyano group at C-21 in the molecule.

Saframycin A therefore has the structure depicted in **4**, 21-cyanosaframycin B, or its antipode. However, assignment of the configuration of the cyano group has not been made.

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Amino acid composition of hemocyanin monomers from the horseshoe crab, *Tachypleus tridentatus*[1]

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Summary. The amino acid compositions of the 4 kinds of hemocyanin monomers from *Tachypleus tridentatus* are similar to each other. Whole hemocyanin from *T. tridentatus* is remarkably similar to *Limulus polyphemus* hemocyanin in the content of all the amino acids examined.

The minimum molecular weight of arthropod hemocyanins has been reported recently to be in the range from 65,000 to 83,000[2-5]. The electrophoretic patterns of hemocyanins from 4 kinds of horseshoe crabs revealed that each hemocyanin consisted of 4 or more kinds of monomers, and that there were electrophoretic similarities among the hemocyanin monomers from 4 Xiphosuran species, that is, *Tachypleus tridentatus*[4], *T. gigas*[6], *Carcinoscorpius rotundicauda*[6], and *Limulus polyphemus*[7]. In our preliminary study some hemocyanin monomers from *T. gigas, C. rotundicauda,* and *L. polyphemus* reacted with anti-*Tachypleus tridentatus* hemocyanin antiserum. In immuno-diffusion tests, they formed precipitin lines partially or totally identical with some immunoprecipitin lines caused by the reaction of the antiserum with hemocyanin monomers from *Tachypleus tridentatus*[8]. To compare the characteristics of hemocyanin monomers from the 4 kinds of horseshoe crabs is very interesting for the study of the systematic relationship of all 4 species in the order Xiphosura.

Amino acid composition of arthropod hemocyanins has already been reported[2,5,9]. However, mixtures of hemocyanin monomers were analyzed as a whole in those experiments. Recently, Jeffrey et al.[10] have reported the amino acid composition of 1 of 2 hemocyanin monomers from the Australian freshwater crayfish. Sullivan et al.[7] have reported the amino acid composition of 5 fractions of

Amino acid composition of hemocyanin samples

Amino acid[a]	Monomer of *Tachypleus* hemocyanin				Whole hemocyanin	
	HT 1	HT 2	HT 3	HT 4	*Tachypleus*[b]	*Limulus*[c]
Lysine	7.15	6.90	6.52	7.83	6.65	6.80
Histidine	9.78	9.07	9.91	7.28	9.04	9.26
Arginine	7.07	8.38	8.09	6.69	7.73	7.16
Aspartic acid	14.84	13.92	12.42	13.33	13.36	12.16
Threonine	3.89	5.08	4.36	5.06	4.46	4.73
Serine	4.42	4.14	4.02	4.42	3.91	3.88
Glutamic acid	11.07	12.85	13.93	13.47	12.89	13.44
Proline	4.50	4.01	3.83	4.21	4.02	3.79
Glycine	3.53	3.40	3.51	4.31	3.30	3.00
Alanine	3.68	3.14	3.51	3.48	3.35	3.13
Valine	6.14	5.51	5.48	6.73	5.75	6.02
Isoleucine	5.25	5.70	6.39	6.25	5.86	5.50
Leucine	9.38	9.79	9.58	9.67	9.61	8.71
Tyrosine	2.10	1.92	1.54	Trace	3.18 (4.97)[d]	5.39
Phenylalanine	7.20	6.18	6.89	7.25	6.92	7.02

HT 2 consists of 2 kinds of immunologically distinct molecules[4] which cannot be separated by the electrophoretic method of Davis[11].
[a] Calculated from the analytical data of hydrolysis for 72 h and reported as g/100 g protein. [b] Average values of 4 hydrolysates. [c] Calculated from data for *Limulus* from Ghiretti-Magaldi et al.[9] and expressed as percents of 15 kinds of amino acids for comparison with *Tachypleus*. [d] A value from hydrolysis for 24 h.

hemocyanin obtained by DEAE-Sephadex chromatography from the horseshoe crab, *Limulus polyphemus*. As far as the photograph of the electrophoretic pattern presented by them is concerned, only the peak IV they obtained seems to indicate a single monomer. In our experiment, the amino acid compositions of the monomers of *Tachypleus tridentatus* hemocyanin are compared with each other.

Materials and methods. The Japanese horseshoe crab, *Tachypleus tridenatus*, was collected at Kasaoka, Japan. Whole hemocyanin from *Tachypleus* serum was prepared and purified by ultracentrifugation at 150,000 × g for 1 h repeated three times. The hemocyanin monomers were purified by preparative polyacrylamide gel electrophoresis; their purity was shown in the previous study[4]. The amino acid composition of each monomer was analyzed by a JEOL analyzer, model JLC 5AH.

Results and discussion. The amino acid composition shown in the table was obtained by hydrolysis for 72 h in 6 N HCl at 105 °C. The value of the tyrosine content, shown in a bracket, was quoted from the data obtained by hydrolysis for 24 h because it decreased during the hydrolysis time. Values of *Limulus* hemocyanin are calculated from the data for *Limulus polyphemus* presented by Ghiretti-Magaldi et al.[9] and expressed as a percent age of 15 kinds of amino acids shown in the table, to compare them with those of *Tachypleus tridentatus*. From the results of these amino acid analyses, it can be noted, firstly, that the hemocyanins from these 2 horseshoe crabs are very similar. Secondly, the amino acid compositions of the 4 kinds of monomers from *Tachypleus* hemocyanin are similar in the contents of most amino acids, and all monomers have a high content of acidic amino acids. However, significant differences are observed in the contents of histidine and arginine and further, we know each monomer was clearly distinct in both immunological and electrophoretic characters[4]. It seems that differences between the monomers are apparent from the amino acid compositions as shown in the table.

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Proline synthesis from glutamate in the mitochondria isolated from a blowfly, *Aldrichina grahami*

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Summary. Proline biosynthesis from glutamate was demonstrated in a cell free system prepared from blowfly abdomen. The biosynthetic activity was found mainly in the mitochondrial fraction. The biosynthesis of proline from glutamate required ATP, NADPH and Mg^{++} as cofactors.

Proline is known to play a specific role as the energy reservoir in insect flight muscle[1,2]. In a blowfly, *Aldrichina grahami*, the main pathway supplying proline is different at different developmental stages of the insect[3]. The insect has a potent proline synthetic activity from glutamate at the adult stage, and is a good organism for elucidating the proline biosynthetic pathway from the amino acid.

Materials and methods. Animals. Laboratory bred adult flies, *A. grahami* were maintained as described previously[4].

Preparation of cell free homogenate and subcellular fractions. The abdomens (100–120) of blowflies, 5–6-day-old, were isolated and homogenized in a Potter-Elvehjem type teflon-glass homogenizer at 0 °C with 10 ml of 0.25 M sucrose solution containing 1 mM EDTA, 0.2% bovine serum albumin and 5 mM Tris-HCl buffer, pH 7.4. The homogenate was centrifuged at 700 × g for 10 min. An aliquot of the supernatant was removed and used as a cell-free extract. The remaining portion of the supernatant was centrifuged at 10,800 × g for 10 min, then the precipitate was washed with the homogenizing medium, then resuspended in the original volume of the medium (mitochondrial fraction).

The 10,800 g supernatant was centrifuged at 100,000 × g for 1 h and the precipitate was resuspended in the same volume as the mitochondrial fraction, then the suspension and the supernatant of the 100,000 × g centrifugation were used as the microsomal fraction and cytosol fraction, respectively.

Incubation and extraction. The reaction mixture was hypoosmotic, and was incubated at 30 °C in a small test tube. The reaction was stopped by adding 0.1 ml of 10% trichlo-

Table 1. Subcellular distribution of the proline synthetic activity in the abdomen of a blowfly, *A. grahami*. The incubation system contained 0.3 ml of each fraction, 100 µl each of 10 mM NADPH, 10 mM NADH and 100 mM ATP, and 10 µl each of 1 M $MgCl_2$ and 6.5 µCi/ml U-^{14}C-glutamate in a final volume of 0.62 ml

	Radioactivity incorporated (dpm)	
	Proline	CO_2
Cell free extract	3210 ± 115	674 ± 35
Mitochondrial fraction	1595 ± 85	1126 ± 18
Microsomal fraction	73 ± 13	102 ± 4
Cytosol fraction	65 ± 30	39 ± 2

Values represent the mean ± SE for simultaneous triplicate incubations for 100 min.