STUDIES ON THE ORGANIC PHOSPHATES IN MUSCLE OF AQUATIC ANIMALS—VIII. PREPARATION OF INOSINIC ACID FROM SQUID MUSCLE

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In the muscle of vertebrates, as is known today, inosinic acid (IMP) is principally formed from adenylic acid (AMP) by the action of 5'-adenylate deaminase which is found commonly in vertebrate muscles. In the previous papers^{1),2)} it has been established that in squid muscle adenosine triphosphate (ATP), in the course of autolysis, is dephosphorylated rapidly with a concomitant accumulation of AMP, while scarcely any IMP is found. Furthermore the living muscle of squid was found to contain as high levels of ATP as muscle of mammals. From these results it will be reasonable to consider that the muscle is a good source of ATP, AMP or the products derived from them. The present paper reports a study on the preparation of IMP from AMP of squid muscle by the action of 5'-adenylate deaminase of fish muscle.

Experimental

Materials:

Freshly obtained mantle muscle of squid, *Ommastrephes sloani pacificus* Steenstrup, was used. Fresh muscle of some fishes, mackerel, *Scomber japonicus* Houttuyn, or carp, *Cyprinus carpio* Linné, was used as a source of 5'-adenylate deaminase.

Method of analysis:

Optical densities were measured with a Shimadzu Spectrophotometer, Model QB-50, using 1-cm. cells. Identification of the recrystallized sodium inosinate was carried out by determining the absorbance ratios, D_{260}/D_{280} and D_{280}/D_{260} , of the solution and by determining the phosphate content by the method of Bartlett (modified method of Fiske and Subbarow's³⁾).

Extraction:

One kg. of squid muscle and 50–100 g. of fish muscle were ground finely with 5 volumes of ice-cold water in a mechanical meat grinder. Incubation was carried out in a refrigerator at 0–5°C with occasional stirring for 2–3 hrs. (under these conditions the action of muscle nucleotidase or phosphatase is reduced markedly). To determine the degree of formation of IMP, 10 ml. of the reaction mixture were taken off several

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times at regular intervals. The enzyme activity was stopped by the addition of 10 ml. of 10 per cent perchloric acid and the mixtures were filtered. The ratios of absorption at $250\text{ m}\mu$ per $260\text{ m}\mu$ of these filtrates were calculated. When these values reached to about 1.2 the reaction was stopped by the addition of perchloric acid to make the final concentration of 4 per cent; then after thorough stirring the mixture was left overnight in a cold room. The mixture was filtered through three layers of gauze. The residue was stirred in 4 per cent of perchloric acid solution to extract the residual IMP. The combined extracts were clarified by filtration through filter paper. The procedures for preparation of IMP from these extracts, which are shown in Fig. 1,

Fig. 1. Preparation of sodium inosinate from squid muscle.

are divided into three steps. The first is removal of inorganic phosphate as barium phosphate, the second is precipitation of IMP as mercuric salt and the last is isolation of barium inosinate. The details of these procedures were already described by J. Marmur et al⁽⁴⁾.

Results

The yields of barium inosinate from squid muscle, which are shown in Table 1,

Table 1. Yields of barium inosinate from squid muscle.

Test No.	Muscle (g)		IMP-Ba (g)	IMP-Ba g/kg of Squid Muscle	Purity* (%)
	Squid	Fish			
1	1,180	118 ¹⁾	3,097	2,624	
2	2,500	250 ²⁾	6,604	2,640	84.5
3	770	77 ²⁾	2,050	2,660	86.4
Mean value				2,642	85.5

* Determined by ionexchange chromatography.

1) Carp

2) Marckerel

were better than that from rabbit¹⁾: the former was about 2.6 g. per kg. of muscle while the latter 1-1.5 g. From these results it can be seen that the activity of 5'-adenylate deaminase of the muscle of these fishes was almost the same. This was also ascertained on that of saury and of horse-mackerel. The barium salt, thus obtained, was recrystallized from water. The sodium salt was prepared from the barium salt by satisfactory removal of barium ion by the addition of calculated amount of sodium sulfate. The sodium salt can be purified by recrystallization from dilute aqueous solution with ethanol. The maximum absorption of the recrystallized sodium salt is at 249 m μ in phosphate buffer of pH 7.0. The absorbance ratios, D_{250}/D_{260} and D_{280}/D_{260} , are shown in Table 2. These are the same as the values shown by Kalcker.

Table 2. Absorbance ratios of sodium inosinate.

Nucleotide	D_{250}/D_{260}		D_{280}/D_{260}	
	pH 2.0	pH 6.0	pH 2.0	pH 6.0
Sample ^a	1.58	1.67	0.20	0.24
Standard ^b	1.58	1.68	0.20	0.24

^a Recrystallized Na-inosinate prepared from squid muscle.

^b H. M. Kalckar; J. Biol. Chem. 176, 429 (1947).

The phosphorus content of the anhydrous sodium salt is as follows:

Test number	1	2
Sample	4.94 mg.	6.33 mg.
found	385 γ	494 γ
P (%) found	7.79	7.80
P (%) calculated		7.89

From these results it can be concluded that IMP, by using these methods, may be prepared from squid muscle at fairly good yield as compared with that prepared from other animals.

Summary

By using the 5'-adenylate deaminase of some fish muscles IMP was prepared from squid muscle at fairly good yield.

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