Short Communications and Preliminary Notes

Apyrase action of an actomyosin-like protein from a sea-anemone

According to the work of MEHL[1], a myosin-natured protein is not found in the sea-anemone, *Cribrina xantho-grammica*. However, this apparent absence might be due to the difficulty of cell disintegration. Recently we were able to obtain an actomyosin-like substance having an apyrase action from the sea-anemone, *Anthopleura japonica*.

Two or three animals (8–9 g wet weight) were cooled for 1 hour at 0° C. After the contents of the coelenteron were removed, the whole bodies were washed, minced with scissors as finely as possible, and made up into a suspension drop by drop in the Weber-Edsall solution (total volume: 20 ml; final molarity: 0.6 *M* KCl, 0.04 *M* $NaHCO_3$ and 0.01 *M* Na_2CO_3), using a glass-rod homogenizer. The suspension was placed for about 30 hours in a refrigerator and then an actomyosin-like protein was extracted and purified by the conventional method, as described elsewhere[2].

The three- or four-times precipitated preparation was quite soluble in 0.6 *M* KCl and easily precipitated in 0.03 *M* KCl. This protein thread shrank slightly on addition of adenosine triphosphate (ATP) and the so-called superprecipitation with ATP was also clearly observed to take place, though very slowly, in the presence of 0.1 *M* KCl. The two phenomena are essentially the same as in rabbit actomyosin (see[3]). This actomyosin-like protein catalyzed the hydrolysis of ATP at an appreciable rate. The inorganic phosphate (P) liberated by the enzyme action was measured by the method of LOHMANN AND JENDRÁSSIK[4]. Details in the enzymic assay have been reported previously[2].

The enzyme activity was more greatly enhanced by Mg^{++} than by Ca^{++}, regardless of K^+ concentration between 0.14 and 0.54 *M*. The per cent increase was about 100 and 300 in the presence of from $3.3 \cdot 10^{-4}$ to $3.3 \cdot 10^{-3}$ *M* $CaCl_2$ and of from $1.0 \cdot 10^{-3}$ to $3.3 \cdot 10^{-3}$ *M* $MgCl_2$, respectively. Ca^{++} inhibited considerably the activation by Mg^{++}. These facts are seen in Table I. An optimal activity was found at 37.5° C at pH 7.0 in the presence of either Mg^{++} or Ca^{++}, as illustrated in Fig. 1. This enzyme is not an adenosine triphosphatase in a strict sense, removing the two energy-rich phosphate groups from ATP. A typical time-activity course is presented in Fig. 2. Much concentrated enzyme showed such an apyrase action even in a short time of incubation. The apyrase is not so heat-labile: 30% of the activity was inactivated on standing for 30 minutes at 37.5° C at pH 7.0 without ATP. Q_{10} was about 2 between 9° and 29° C. Q_p was around 200 at 37.5° C and pH 7.0.

TABLE I

EFFECT OF K, Mg AND Ca IONS ON THE SEA-ANEMONE APYRASE ACTIVITY

Conditions: 0.05 *M* histidine buffer; 0.43 mg protein; ATP = 60 μg labile P; Total volume = 3.0 ml. Incubated for 10 minutes at 37.5° C and pH 7.0.

Final concn. (M/l) of			*μg P liberated*
K^+	Mg^{++}	Ca^{++}	
0.14	—	—	5.6
0.14	—	$3.3 \cdot 10^{-3}$	12.0
0.14	$3.3 \cdot 10^{-3}$	—	21.6
0.14	$3.3 \cdot 10^{-3}$	$3.3 \cdot 10^{-3}$	19.5
0.14	$3.3 \cdot 10^{-3}$	$9.9 \cdot 10^{-3}$	13.9
0.34	—	$3.3 \cdot 10^{-3}$	10.5
0.34	$3.3 \cdot 10^{-3}$	—	18.3
0.54	—	$3.3 \cdot 10^{-3}$	9.3
0.54	$3.3 \cdot 10^{-3}$	—	16.5

These apyrase properties of the contractile protein from the sea-anemone are apparently different from those of actomyosins from the other animals so far as reported (see[2]). It remains possible that a myokinase or a water-extractable apyrase might be still contaminated in the present preparations. But purification by the conventional procedure, such as repeated precipitation, could not alter the enzymic characters. This contractile protein may be of a primitive nature.

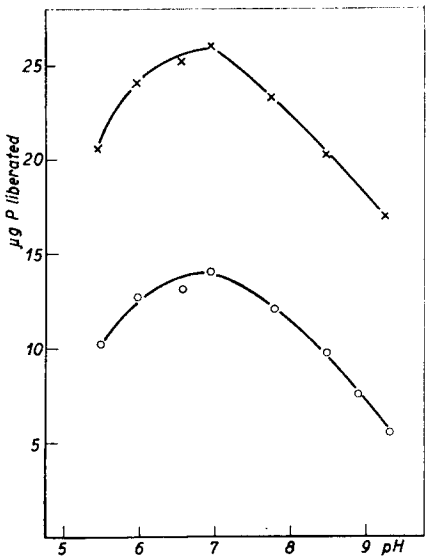

Fig. 1. pH-activity curve of the sea-anemone apyrase activity. $3.3 \cdot 10^{-3}$ *M* $MgCl_2$ (×——×) or $CaCl_2$ (○——○); 0.14 *M* KCl; ATP = 120 µg labile P. Other conditions are the same as in Table I except that pH of the reaction mixture varied.

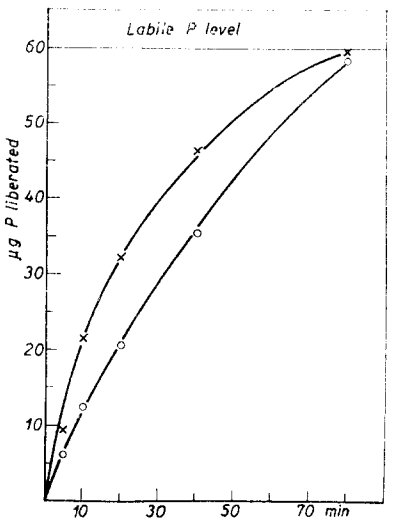

Fig. 2. Time-activity curve of the sea-anemone apyrase activity. $3.3 \cdot 10^{-3}$ *M* $MgCl_2$ (×——×) or $CaCl_2$ (○——○); 0.14 *M* KCl Other conditions are the same as in Table I except that incubation time varied.

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1 J. W. MEHL, *Biol. Bull.*, 79 (1940) 488.
2 K. MARUYAMA, *Enzymologia*, [illegible] (1954) 60.
3 A. SZENT-GYÖRGYI, *Chemistry of Muscular Contraction*, 2nd Ed., Academic Press Inc., New York, 1951.
4 K. LOHMANN AND L. JENDRÁSSIK, *Biochem. Z.*, 178 (1926) 419.

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Formation de sérylarginine pendant l'activation du chymotrypsinogène de boeuf

L'isolement récent d'une chymotrypsine possédant une seule chaîne ouverte[1] nous a incités à schématiser de façon nouvelle[1,2] quelques-unes des réactions de protéolyse conférant au chymotrypsinogène une activité chymotrypsique plus ou moins intense. Le schéma proposé, d'ailleurs approximatif et préliminaire, diffère de celui de GLADNER ET NEURATH[3] par le fait qu'il prévoit la formation de un ou de plusieurs peptides (désignés par le symbole X-Base) aussi bien pendant l'activation "rapide"[4] donnant naissance à l'enzyme-δ que pendant l'activation "lente"[5] engendrant l'enzyme-α.

Afin de vérifier la validité de ce schéma, nous avons traité à 0° et pH = 7.6 des solutions à 2.4 % de chymotrypsinogène 5 fois cristallisé par des quantités de trypsine telles que la concentration finale en enzyme soit 0.06 et 0.02 % (activation rapide) ou $0.056 \cdot 10^{-2}$ % (activation lente). Après des laps de temps variables (10 min–48 h), nous avons mesuré la teneur en azote non-protéique des solutions et leur activité chymotrypsique vis-à-vis de l'acétyl-L-tyrosine éthyl ester. Quelle que