Attempt to Separate ATPase Activity from 5'-AMPdeaminase Activity of Myosin

The actin-free myosin from rabbit muscle, crystallized according to SZENT-GYÖRGYI, is a very active deaminase preparation, with the same order of activity as the deaminase isolated by SCHMIDT and KALCKAR[1].

The separation of 5'-adenylic acid deaminase from myosin by heat treatment was first reported by ENGELHARDT *et al.*[2], LYUBIMOVA and MATLINA[3], and further by LEE[4] and LOCKER[5]. LEE[6] succeeded in crystallizing 5'-adenylic acid deaminase, starting from once-precipitated myosin by means of heat, ethanol, ammonium sulphate, salt at low concentration and calcium phosphate fractionation.

PERRY [quoted by BAILEY, Proteins *2*, 1004 (1954)] demonstrated in washed isolated myofibrils ATPase activity without myokinase and deaminase activity.

We tried to separate ATPase activity from 5'-AMPdeaminase activity of myosin by Ca phosphate gel fractionation.

Myosin was prepared according to SZENT-GYÖRGYI[7] and, after one or two precipitations by dilution, the crystalline myosin was suspended in water and freed from salt by centrifugation and repeated suspension in bidistilled water until a clear and very viscous solution was obtained, from which no myosin could be separated by centrifugation.

Calcium phosphate gel (two or three months old) had been prepared according to the method described by KEILIN and HARTREE[8]. We added to the myosin solution in water one-tenth the volume of gel (17·5 mg dry weight/ml). The suspension was stirred and after 10 min at 2°C was centrifuged. The precipitate was washed twice with one volume of 0·01 *M* sodium phosphate buffer pH 6·8, stirred and centrifuged after 10 min. The suspension was eluted with half the volume of 0·1 *M* NaCl in 0·01 *M* sodium phosphate buffer pH 6·8. The fraction obtained had not much or not at all proteins and enzymes. The elution was repeated with half the volume of 0·2 *M* NaCl in 0·01 *M* sodium phosphate buffer pH 6·8. The fraction obtained had considerable ATPase activity and only traces of deaminase activity. This 0·2 *M* NaCl fraction was also tested for the two myosin properties: (1) the capacity to combine with actin, which is evidenced by means of viscosimetric test, following MOMMAERTS[9]; (2) the superprecipitation as actomyosin after actin addition, following SPICER[10]. The fraction eluted by 0·1 *M* NaCl in 0·01 *M* sodium phosphate buffer pH 6·8, in experiment 4, was found to be homogeneous at the Spinco E ultracentrifuge. The third elution with 1·0 *M* NaCl in 0·01 *M* sodium phosphate buffer pH 6·8 gave the bulk of deaminase activity with the remainder of ATPase activity.

Fractionation of myosin with Calcium phosphate gel: Enzymatic activity per mg of protein and total activity (activity of the total fraction eluted from the starting solution of myosin in water).

Fraction	5'-AMPdeaminase/mg protein	Total activity	ATPase/mg protein	Total activity
Experiment 4				
Myosin in water . .	124	48,000	205	39,000
Eluted in 0·1 *M* NaCl	20	800	312	10,490
Eluted in 0·2 *M* NaCl	30	2,400	232	18,600
Eluted in 1·0 *M* NaCl	1410	48,000	208	7,110
Experiment 6				
Myosin in water . .	208	15,000	82·7	5,900
Eluted in 0·2 *M* NaCl	20	150	122·8	920
Eluted in 1·0 *M* NaCl	1350	15,200	122·8	38,000

The unity of 5'-AMPdeaminase is defined as $\Delta E_{265} = 0{\cdot}001$ in 4×10^{-5} *M* 5'-AMP buffered with 0·01 *M* citric acid-NaOH pH 6·5 at room temperature, when the reaction is of zero order. In experiment 4 ATPase was tested as specified by PERRY[12] and the unity is defined as γ of $P_{inorg.}$ liberated in 5 min at 25°C. In experiment 6 ATPase was essayed as specified by BOWEN and KERWIN[14] and the unity is defined as γ of $P_{inorg.}$ liberated in 5 min at 25°C.

ATPase activity was essayed at 25°C by two procedures: (1) by estimating, by the method of ALLEN[11], the inorganic phosphate, liberated by the enzyme in the presence of Ca^{++} under the conditions indicated by PERRY[12]; (2) by estimating, by the method of FISKE and SUBBAROW[13], the inorganic phosphate, liberated by the enzyme in the presence of EDTA under the conditions specified by BOWEN and KERWIN[14]. This second procedure was used for inhibiting the myokinase action.

5'-AMPdeaminase was tested spectrophotometrically at room temperature in 0·01 *M* citric-acid-NaOH buffer pH 6·5 under the conditions specified by NIKIFORUK and COLOWICK[15].

Proteins were determined spectrophotometrically by the KALCKAR method[16].

Further work is now in progress. This investigation was supported by a grant from the Italian C.N.R.

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Riassunto

Mediante l'adsorbimento su Calcio fosfato gel e la successiva eluzione a pH 6,8 con soluzioni a forza ionica crescente si sono riuscite a separare frazioni aventi diverse attività specifiche 5'-AMPdeaminasica (massima nei campioni eluiti con 1 *M* NaCl) e ATPasica. L'attività ATPasica è associata alla proprietà della miosina di formare actomiosina.

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