The ATP-creatine phosphopherase.

by

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It has been shown by K. LOHMANN[1] that in muscle the easily hydrolysable phosphate of ATP is taken over by creatine. In dialysed muscle extract therefore the reaction taking place can be expressed by the equation:

ATP + 2 creatine = adenylic acid + 2 creatine-phosphoric acid

This reaction was investigated by H. LEHMANN[2] who demonstrated its reversibility, but the equilibrium constant = K calculated for a trimolecular reaction was not constant.

My investigations have proved that the above reaction takes place in two distinct steps and that the presence of two distinct proteins is necessary for this catalysis. The first reaction is:

ATP + creatine = adenosine-diphosphoric acid + creatine-phosphoric acid

The enzyme catalysing the reaction was called the ATP-creatine phosphopherase and was purified to a degree at which no secondary reactions occured. If the kinetics of this reaction were examined at varied concentrations of the reactants, the equilibrium constant calculated for a bimolecular reaction was found to be constant.

The second step of the reaction

Adenosine-diphosphoric acid + creatine = adenylic acid + creatine-phosphoric acid

is catalysed by another protein, which I called ADP-creatine phosphopherase, the isolation of which is in progress.

Experimental:

Test: Unit of the ATP-creatine phosphopherase is the quantity of enzyme which is capable of transferring 0,00213 moles P from ATP to creatine in a period of five minutes in the presence of 0.00475 moles of ATP and 0.0215 moles of creatine in a pH 8.55 veronal-acetate buffer. Half that quantity of enzyme transmits 0.00106 moles and a quarter of it 0.00053 moles of P during the same interval. The resulting creatine-phosphate was determined after FISKE and SUBBAROW.* In this method creatine-phosphate hydrolyses during the five minutes of incubation and appears as inorganic phosphate. There was no preformed inorganic phosphate in my experiments.

The activity of the enzyme is defined as: enzyme units divided by the quantity of the protein dry material, a $= \frac{\text{units}}{\text{mg protein}}$ The protein dry material was determined by placing a given quantity of enzyme in a weighed centrifuge tube and precipitating it with 5% of trichloro-acetic acid. The precipitate was washed twice in a tenfold volume of water, dried and weighed.

The process of isolation: The muscle of a freshly killed rabbit was minced on a Latapie mincer, suspended in 1.5 ml 0.1 *M* KCl per g and was then extracted by stirring it at 0° for 10 min. The muscle was then strained through a cloth and once more extracted in $^1/_3$ of the former volume of 0.1 *M* KCl for a period of ten min. The extracts were united, left alone for 1—2 hours and then centrifuged. The ATP-creatine phosphopherase activity of this extract could not be determined with accuracy because it also contained ADP-creatine phosphopherase. On the whole it was found that 1 g of fresh muscle of the rabbit contained 100—150, that of the pigeon 200—250 units of ATP-creatine phosphopherase.

*Preparation of ATP-creatine phosphopherase:***

First step: To every liter of the above fluid were added 80 ml of 0.5 *M* $NaHCO_3$. Then the fluid was half saturated with ammoniumsulfate and centrifuged once more. The precipitate was discarded. Activity of the fluid = 6.

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* as modified by LOHMANN and JENDRASSIK.

** All operations were carried out at 0°.

Second step: For every liter 30 ml of 0.5 *M* $NaHCO_3$ were added and as much ammoniumsulfate as was needed to bring it up to 0.7 saturation. The precipitate was filtered and dissolved in 250 ml buffer solution of 0.025 *M* borate of pH 8.55. Dry weight 30mg/ml. a=7. No loss of activity.

Third step: To 1 liter of enzyme solution 2.5 litres of alcohol of —20° were added. The precipitate was centrifuged off at 0° and dissolved in 250 ml of 0.02 *M* borate buffer of pH 8.55. The insoluble material was filtered off and the solution

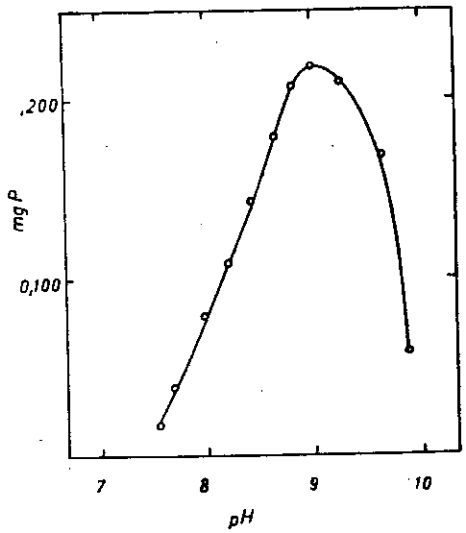

Fig. 1.

was dialysed for a period of five hours against thirty volumes of water. Dry material 7—10 mg/ml; a = 30. Loss 30 %.

Fourth step: Adsorption to γ $Al(OH)_3$. To every liter of dialysed fluid 300 ml of $Al(OH)_3$ suspension was added. This contained 20 mg of $Al(OH)_3$ per ml. After shaking for five minutes the solution was centrifuged. Elution with 250 ml of pH 8.55 borate buffer containing *M*/10 borate and 20 % ammoniumsulfate. Dry material 3—4 mg/ml; a = 95. Loss 45 %.

Fifth step: *M*/50 NH_3 and ammoniumsulfate was added till 0.5 saturation. The resulting precipitate was filtered and dissolved in *M*/10 borate buffer. a=100. Loss 10%.

1 γ of this purest enzyme preparation (a=100) transferred at pH 8.55 in one minute 2 γ P from ATP onto creatine. If the

molecular weight of the enzyme is taken to be 70,000, this would mean, that one molecule of enzyme did react upon 4.400 molecules of the substrate per min.

In the course of this isolation the ADP-creatine phosphopherase disappeared at step 3. together with ADP-isomerase (see page 66.).

With ATP-creatine phosphopherase, thus isolated. I determined how far the activity was dependent on the pH. As can be seen from Fig. 1., in presence of borate buffer the pH optimum was 9.05.

Determining the equilibrium constant at different pH, I found that the value of K depended to a great extent on the pH and reached its maximum at the pH optimum of the reaction. Table I. shows the equilibrium constant $= K$ at varied pH and with constant creatine and ATP concentrations. These investigations were complicated by the fact that the ATP itself acted as a buffer, and thus changed the pH of the original buffer solution. For this reason the pH of the ATP and creatine solutions were colorimetrically brought to the necessary pH and the buffers were used in high concentrations.

Table I.

Experimental technique: 1 ml of borate buffer solution of varying pH, 0.0195 *M* creatine, 0.00890 *M* ATP, 0.1 ml $= 60\,\gamma$ of ATP-phosphopherase. Volume 1.6 ml. Incubation at 38° until the state of equilibrium had been reached.

pH	Measured *M* creatine P	$K = \frac{Cr \,.\, P \,.\, ADP}{ATP \,.\, Cr.}$
7.50	0.00059	0.0023
7.60	0.00085	0.0049
7.80	0.00172	0.0239
8.20	0.00289	0.0925
8.50	0.00383	0.1910
9.05	0.00459	0.3651
9.50	0.00344	0.1382
9.70	0.00094	0.0061

In establishing the pH curve the following experimental technique was employed: 2 ml of buffer solution were mixed with 1 ml (0.01915 *M*) of creatine + 0.5 ml (0.00890 *M*) of ATP + 0.5 ml H_2O. Of this mixture 0.5 ml were removed for the pH determination. 1.5 ml were used to determine the rate of the reaction. 0.05 ml enzyme (30 γ) was added and the mixture was incubated for 5 min. at 38°. Another 1.5 ml in the presence

of enzyme were incubated for 30 min. at 38° in order to determine the equilibrium constant.

Table 2 shows the values of the equilibrium constant (K) at identical pH (veronal acetate buffer of pH 8.55) and with varied ATP and creatine concentrations. As can be seen, the equilibrium constant has the same value throughout. The value is lower in veronal acetate buffer than in borate buffer. This might be attributed to the complex formation of borate and ATP which complex might react faster with creatine than free ATP. To avoid such complications I employed for the estimation of the equilibrium constant a buffer solution of veronal acetate.

Table II.

Experimental technique: 1 ml of veronal-acetate buffer solution of pH 8.55 + 0.1 ml = 60 γ ATP-phosphopherase + varying quantities of ATP and of creatine. Volume: 1.6 ml. Incubation at 38°, until state of equilibrum had been reached.

M creatine added	*M* ATP added	*M* P found	$K = \frac{Cr \,.\, P \,.\, ADP}{ATP \,.\, Cr.}$
0.02390	0.00222	0.00104	0.040
0.02390	0.00444	0.00158	0.042
0.02390	0.00890	0.00232	0.038
0.02390	0.01331	0.00292	0.039
0.01030	0.00236	0.00078	0.040
0.01531	0.00236	0.00090	0.038
0.02040	0.00236	0.00104	0.040
0.02542	0.00236	0.00108	0.038

Further experiments with ATP-creatine phosphopherase showed that the pH stability curve did not coincide with the pH activity curve. After a storage for a longer period, the enzyme had changed its pH optimum.

References.

1. *K. Lohmann.* Biochem Z. **271**, 264, 1934.
2. *H. Lehmann.* Biochem Z. **281**, 271, 1935.