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Received June 9th, 1954

INTERACTION OF CREATINE WITH ADENOSINE PHOSPHATES

by

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Reactions involving the transfer of a phosphate group from creatine phosphate to an adenosine phosphate have been known since 1934, when LOHMANN[1] demonstrated the following reversible reaction in aqueous extracts of skeletal muscle:

$$2 \text{ creatine phosphate} + \text{AMP} \rightleftharpoons 2 \text{ creatine} + \text{ATP} \quad (1)$$

Later, it was suggested by LEHMANN[2] that the reaction takes place in two stages:

$$\text{creatine phosphate} + \text{ADP} \rightleftharpoons \text{creatine} + \text{ATP} \quad (2)$$

$$\text{creatine phosphate} + \text{AMP} \rightleftharpoons \text{creatine} + \text{ADP} \quad (3)$$

BANGA[3], assuming that two enzymes are concerned, endeavoured to isolate them. She failed to obtain an enzyme from mammalian muscle specific for reaction (3) but she isolated an enzyme promoting reaction (2), named ATP-creatine phosphopherase by her, or creatine phosphokinase by later authors. The purification and crystallisation of this enzyme has recently been reported by NODA, KUBY AND LARDY[4].

After the discovery of myokinase by COLOWICK AND KALCKAR[5], it was suggested (COLOWICK[6]) that there is no need to postulate an enzyme catalysing (3), reaction (1) being accounted for by the occurrence of reaction (2) in conjunction with the myokinase reaction:

$$2 \text{ ADP} \rightleftharpoons \text{ATP} + \text{AMP} \quad (4)$$

However, there is as yet no experimental proof of this.

In the present work evidence has been obtained for the view that reactions (2) and (4), rather than (2) and (3) are concerned in the dephosphorylation of creatine phosphate. The method used to follow such reactions employs a modification of KORNBERG'S[7] assay procedure for ATP. In the presence of glucose and a crude preparation of yeast hexokinase[8] any ATP formed gives rise to glucose-6-phosphate. This is oxidised by glucose-6-phosphate dehydrogenase present in the yeast preparation with simultaneous reduction of TPN. The rate of reduction of TPN is measured by observing the rate of change of optical density in the spectrophotometer at 340 mμ. The detection of reaction (3) depends on coupling it with a reaction by which ADP is converted to ATP, either by (2) or by (4). In Fig. 1, curve II shows that there is no reduction of TPN when creatine phosphate is added to the reaction mixture containing highly diluted rat muscle homogenate (final dilution, 1 in 20,000), AMP and the yeast enzyme system. When a small amount of ADP is added (Fig. 1, curve II) there is a rapid reduction of TPN which is mainly due to reaction (2), since a control with no added creatine phosphate shows that the myokinase reaction (4) is suppressed under these conditions (Fig. 1, curve I). If reaction (3) had taken place, the ADP formed would have been available for further phosphorylation to ATP in reaction (2) and reduction of TPN would have resulted. It follows that no phosphorylation of AMP by creatine phosphate was demonstrable.

In a second experiment (Fig. 2), the effect of high concentrations of AMP on reaction (4) was counteracted by enriching the system in myokinase. No reduction of TPN occurred when creatine phosphate was added to the reaction mixture containing AMP, added myokinase, muscle homogenate and the yeast enzyme system. The addition of a small amount of ADP produced reduction of TPN and it is therefore to be concluded that reactions leading to the formation of ATP from ADP can occur under these conditions. Thus ADP arising from reaction (3) would have been detected. This experiment supports the view that there is no phosphorylation of AMP by creatine phosphate.

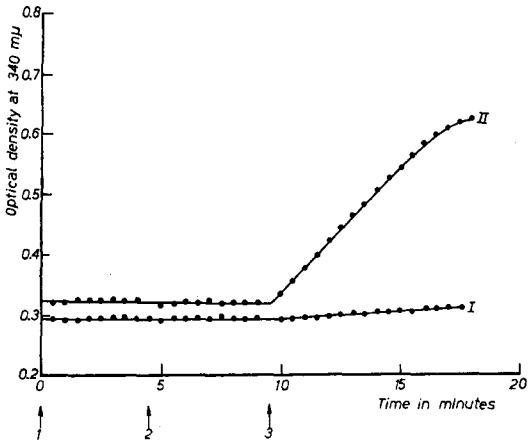

Fig. 1. The reaction mixtures at zero time (I and II) consist of yeast enzyme in 0.05 *M* barbitone buffer pH 8.6, 0.007 *M* $MgCl_2$, 0.07 *M* KCl, 0.005 *M* glucose, 0.01 *M* AMP, 10^{-4} *M* TPN (final concentrations) and had been incubated until no further change in optical density occurred (5 min). At zero time (1) 10 μl of muscle homogenate (1 in 60) in isotonic KCl was added with stirring. At time (2) 0.2 ml of 0.2 *M* creatine phosphate was added to II with mixing, bringing final concentration to 0.01 *M*. At time (3) 0.2 ml of 0.02 *M* ADP added to I and II bringing final concentration to 0.001 *M*. Room temperature 16°.

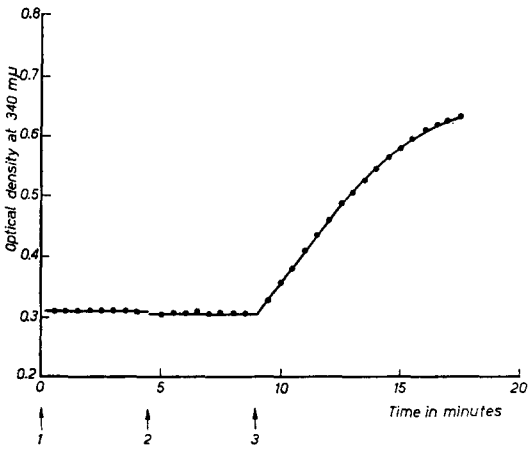

Fig. 2. At zero time reaction mixture consists of yeast enzyme in 0.05 *M* barbitone buffer pH 8.6, 0.007 *M* $MgCl_2$, 0.07 *M* KCl, 0.005 *M* glucose, 0.01 *M* AMP, 10^{-4} *M* TPN (final concentrations) and 0.05 ml of a partially purified preparation of myokinase. Incubated at 16° until no further change in optical density. At zero time (1) 10 μl of a muscle homogenate (1 in 60) in isotonic KCl added with stirring. At time (2) 0.2 ml of 0.2 *M* creatine phosphate added with mixing, bringing final concentration to 0.01 *M*. At time (3) 0.2 ml of 0.02 *M* ADP added bringing final concentration to 0.001 *M*. Room temperature 16°.

Similar results have been obtained with preparations from brain. With this tissue it was found advantageous to use supernatants from centrifuged homogenates in order to overcome the effects of ATP-ase present in the whole homogenates. It was also necessary to use higher tissue concentrations, since the creatine phosphokinase activity of brain is only about one tenth that of muscle.

The method used here can also be applied to the measurement of myokinase activity, provided that ADP is the only phosphate added. When AMP is added at a concentration ten times that of ADP, the rate of reduction of TPN falls by about 80%. This makes it possible to distinguish between the ATP formation due to creatine phosphokinase and myokinase activities and to measure creatine phosphokinase activity in the presence of myokinase. In both brain and muscle, the activity of myokinase, as measured by the rate of ATP formation in the presence of 10^{-3} *M* ADP, and in the absence of added AMP, was found to be about three times that of creatine phosphokinase.

To sum up, a sensitive method has been developed to detect the formation of ATP in enzymic systems by coupling it to the reduction of TPN. With this method it is possible to measure enzymic activities in tissue preparations of 2,000 to 20,000 fold dilution; under these conditions reactions due to endogenous substrates are negligible. Reactions between creatine phosphate and adenosine phosphates in brain and muscle were found to be confined to the phosphorylation of ADP. There is no direct phosphorylation of AMP by creatine phosphate but there is sufficient myokinase to account for an indirect phosphorylation involving the coupling of creatine phosphokinase and myokinase.

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Received June 18th, 1954