The role of DPN in the enzymic phosphorylation of creatine

A soluble enzyme system from rat skeletal muscle can phosphorylate creatine with 1,3 diphosphoglycerate in the absence of added ADP[1]. Using PEP as phosphate donor, ADP is absolutely required, but DPN may replace it. Related results have been reported by others[2,3].

Analytical procedures, substrate and enzyme preparations have been described[1]. The enzyme fraction used was the protein which is precipitated between 35 and 65 % of ammonium sulfate saturation. It was dialysed and treated with charcoal[1].

Creatine phosphorylation by PEP requires a nucleotide. Our rat-muscle preparation contains enough cofactor for this reaction, if not previously treated with charcoal (Table I). Pure CrK[4] plus PyK (Boehringer and Soehne, Mannheim) cannot replace the rat-muscle preparation in the presence of DPN. Apyrase[6] treatment inactivates ADP, but does not affect DPN as a transphosphorylation agent.

TABLE I

PHOSPHOCREATINE SYNTHESIS BY PEP WITH CRUDE AND PURIFIED ENZYMES

0.025 *M* Tris-HCl buffer, pH 7.5; 0.05 *M* creatine; 0.01 *M* $MgCl_2$; 0.005 *M* KCl; 0.0035 *M* PEP; rat-muscle enzyme, 2.4 mg/ml; PyK, 0.1 μg/ml; CrK, 200 μg/ml; volume, 2 ml. After 20 min at 37° reaction was stopped with 5 % trichloroacetic acid and PCr was determined. Apyrase treatment: 0.001 *M* ADP or 0.01 *M* DPN were treated with 5 mg potato apyrase for 10 min at 37°, and deproteinized with 5 % trichloroacetic acid, which was removed with ether. The neutralized aqueous phase was used for PCr synthesis at the indicated concentration.

Enzyme used for PCr synthesis	*Addition*	*Concentration of addition* ($M \times 10^4$)	*PCr synthesis* μ*moles/ml/20 min*
Pyk + Crk	ADP	0.5	2.30
Pyk + Crk	DPN	5.0	0.0
Rat muscle	None	—	0.0
Rat muscle, charcoal treatment omitted	None	—	0.35
Rat muscle	ADP	0.5	1.55
Rat muscle	Apyrase-treated ADP	0.6	0.0
Rat muscle	DPN	5.0	1.60
Rat muscle	Apyrase-treated DPN	5.0	1.55

The concentration of DPN[6] does not change during the experiment. However, the experiments in Fig. 1 suggest that DPN is not directly involved in transphosphorylation. The rat-muscle enzyme was preincubated with ADP or DPN in a system identical to one described in Table I, but lacking PEP. After different periods of preincubation, PEP was added (0.004 *M*) and the complete system was now incubated for another 2 min before determining PCr (Fig. 1 A, B). In another set of experiments (Fig. 1 D, E), ADP or DPN were boiled in 1 *N* HCl for various lengths of time, then neutralized and used for PCr synthesis. It may be seen that ADP and DPN behave differently in both sets of experiments.

Different moieties of the DPN molecule were tested; only ADP-ribose[7] is effective in transphosphorylation (Fig 1 C).

Our data suggest the presence in rat skeletal muscle of a DPN-splitting enzyme.

Abbreviations: ADP, adenosine diphosphate; PEP, phospho(enol)pyruvate; CrK, creatine phosphokinase; Pyk, pyruvic kinase; DPN, diphosphopyridine nucleotide; PCr, phosphocreatine; Tris, Tris(hydroxymethyl)aminomethane.

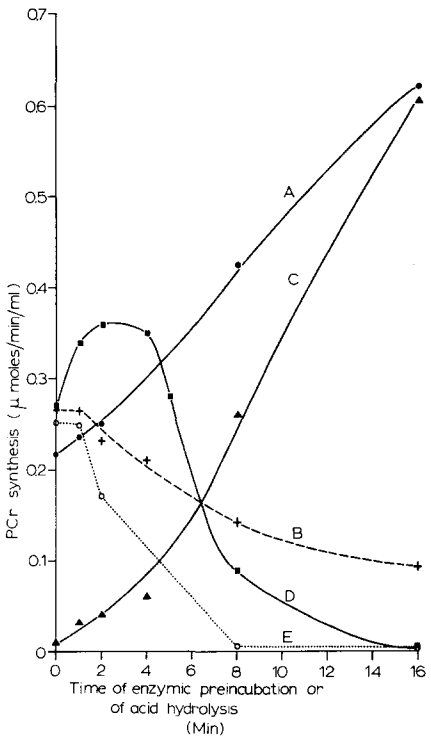

Fig. 1. Phosphocreatine synthesis after pretreatment of the nucleotides. Conditions as in Table I. Incubation time for PCr synthesis, 2 min. Treatment of nucleotides, see text. Nucleotide concentration: A, DPN ($7.5 \cdot 10^{-4}$ *M*), preincubated with muscle enzyme; B, ADP ($5.0 \cdot 10^{-5}$ *M*), preincubated with muscle enzyme; C, ADP-ribose ($1.6 \cdot 10^{-4}$ *M*), preincubated with muscle enzyme; D, HCL-treated DPN ($5 \cdot 10^{-4}$ *M*); E, HCL-treated ADP (10^{-5} *M*).

The split product may be not identical to ADP either in its structure or in the way it is bound by the enzymes involved in phosphate transfer.

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[1] O. CORI, A. TRAVERSO CORI, M. LAGARRIGUE AND F. MARCUS, *Biochem. J.*, 70 (1958) 683.
[2] P. OEHLEMYER AND S. OCHOA, *Biochem. Z.*, 293 (1937) 338.
[3] N. B. CHERNYAK, *Biokhimiya*, 19 (1954) 50.
[4] S. KUBY, L. NODA, AND H. A. LARDY, *J. Biol. Chem.*, 209 (1954) 191.
[5] P. S. KRISHNAN AND W. L. NELSON, *Arch. Biochem.*, 19 (1948) 65.
[6] N. O. KAPLAN, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Academic Press, New York, 1955, p. 660.
[7] S. ROSENBERG AND M. R. BOVARNICK, *J. Biol. Chem.*, 211 (1954) 763.

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