Short Communications

The endogenous formation of phosphoenolpyruvate by rat-liver mitochondria

Phosphoenolpyruvate has been shown to be formed by kidney and liver particulate preparations in the presence, but not in the absence, of added Krebs-cycle acids[1-5]. During the course of a study of phosphorylated components in mitochondria, we have found that [^{32}P]PEP was formed on incubating mitochondria with $H_3{}^{32}PO_4$ in the absence of added substrate. This report concerns the identity of PEP and its formation under various conditions, and the relation of PEP formation to the endogenous activity of mitochondria.

Rat-liver mitochondria were prepared in 0.25 *M* sucrose as described previously[6]. Incubation was carried out aerobically at 30° in a final volume of 1 ml, with final concentrations as follows: mitochondrial N, 1.5 mg/ml; sucrose, 0.125 *M*; Tris (pH 7.4), 0.05 *M*; $MgCl_2$, 0.005 *M*; $H_3{}^{32}PO_4$, 10^{-4} *M*, 100 μC/ml. Incubation time and other substances or changes are noted in the tables. After the period of incubation the suspension was chilled, mitochondria were sedimented at 11,000 $\times$ *g* for 10 min at 3°, and the supernatant was fractionated by anion-exchange chromatography. An 0.8 $\times$ 10 cm Dowex-1-X8 chloride column was used; the elution was performed by two gradient elutions[7]. In the first, the upper or reservoir flask contained 500 ml 0.02 *M* HCl and the lower or mixing flask, water. During this elution an unidentified phosphate ester stable to treatment with 1 *M* HCl for 10 min at 100°, phosphate, and a second unidentified ester of similar stability to the first were successively removed. In the second elution the reservoir contained 500 ml 0.02 *M* HCl and 0.3 *M* NaCl; the mixer contained 500 ml of the final concentration from the first elution, approx. 0.013 *M* HCl. Here, the remainder of the second stable ester mixed with ADP, PEP, and ATP were successively eluted. No other labeled substances were found. Fractions included in peaks of radioactivity were pooled and brought to pH 7. The substances were assayed with myokinase or pyruvic kinase in combination with hexokinase and glucose; the [^{32}P]G-6-P which formed was determined[8] as phosphate ester stable to heating for 30 min at 100° in 1 *M* HCl.

The PEP fraction exhibited lability of the phosphate ester to alkaline iodine and neutral $HgCl_2$, and had a half-life of approx. 10 min in 1 *M* HCl at 100°. The product when recrystallized three times as the silver–barium salt with synthetic PEP[9] as carrier, had a specific activity of 2780, 2700, and 2800 counts/min/mg after the respective recrystallizations. [^{32}P]PEP was synthesized according to OHLMEYER[9] using $^{32}POCl_3$ prepared by a modification of the procedure of KALINSKY AND WEINSTEIN[10]. Synthetic [^{32}P]PEP and the mitochondrial product were eluted from the Dowex-1 column in the same fractions, and had identical R_F's on paper chromatography with

Abbreviations: PEP, phosphoenolpyruvate; Tris, tris(hydroxymethyl)aminomethane; ADP, adenosine diphosphate; ATP, adenosine triphosphate; G-6-P, glucose 6-phosphate; GSH, glutathione; EDTA, ethylenediaminetetraacetate; ITP, GTP, inosine and guanosine triphosphate.

two different solvent systems. It may be noted here that in the phosphate-ester estimation[8], which depends on the extraction of phosphomolybdate with organic solvent, PEP is extracted into the organic phase to the extent of approx. 40 %.

The incorporation of ^{32}P into ATP and PEP under various experimental conditions is depicted in Table I. Of the uncouplers of oxidative phosphorylation, pentachlorophenol[11] and 2,4-dinitrophenol enhanced the ^{32}P incorporation into PEP and depressed that of ATP, while $CaCl_2$ only depressed ATP incorporation. Aging the mitochondria and inclusion of KCN depressed incorporation into both ATP and PEP, while GSH, EDTA, KF, or omission of $MgCl_2$ had little effect. The influence of the duration of incubation is shown in Table II. In the absence of 2,4-dinitrophenol the

TABLE I

INCORPORATION OF ^{32}P INTO ATP AND PEP

Abbreviations: DNP, 2,4-dinitrophenol; PCP, pentachlorophenol.

Expt.	*Experimental condition*	*Incubation time (min)*	*mμmoles ^{32}P incorporated*	
			ATP	*PEP*
1.	Control	30	0.62	0.46
	$2.5 \cdot 10^{-4}$ *M* DNP	30	0.08	1.41
	0.01 *M* KCN	30	0.04	0.21
	0.001 *M* GSH	30	0.59	0.47
	0.001 *M* EDTA	30	0.82	0.39
2.	Control	10	1.09	0.39
	Omit $MgCl_2$	10	1.17	0.46
	$5 \cdot 10^{-5}$ *M* PCP	10	0.18	1.99
	0.02 *M* KF	10	1.94	0.49
	0.002 *M* $CaCl_2$	10	0.23	0.41
3.	Control	30	0.65	0.42
	Mitochondria aged 24 h at 3°	30	0.22	0.07

TABLE II

EFFECT OF INCUBATION TIME ON INCORPORATION OF ^{32}P INTO ATP AND PEP

Incubation time (min)	*mμmoles P incorporated*			
	Control		*$2.5 \cdot 10^{-4}$ M 2,4-dinitrophenol*	
	ATP	*PEP*	*ATP*	*PEP*
0	0.30	0.10	0.06	0.83
5	—	—	0.08	1.86
15	1.13	0.31	0.08	2.31
35	0.43	0.33	0.08	1.55
65	0.12	0.19	0.08	1.06

labeling of ATP reached a peak at 15 min while that of PEP followed at 35 min, indicating that the ^{32}P of PEP was not a precursor of [^{32}P]ATP. In the presence of 2,4-dinitrophenol the labeling of ATP remained negligible and that of PEP reached a peak at 15 min.

One mechanism for PEP formation involves the action of oxaloacetic carboxylase on oxaloacetate and ITP or GTP[5,12]. However, we have found no peaks

corresponding to [^{32}P]ITP or [^{32}P]GTP, which would be expected to occur if these were precursors of [^{32}P]PEP. The stimulation of endogenous formation of PEP by uncouplers is reminiscent of the observation of MUDGE *et al*[3] that in liver mitochondria 2,4-dinitrophenol stimulated PEP formation in the presence of α-ketoglutarate or its immediate precursors but not in the presence of fumarate, malate, or oxaloacetate. The stimulation of PEP formation appears to be more related to the specific action of 2,4-dinitrophenol and pentachlorophenol rather than to the result of uncoupling of endogenous oxidative phosphorylation, since Ca^{++}, which is a potent uncoupler in intact mitochondria, did not exhibit the effect characteristic of the substituted phenols.

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A new approach to the separation and purification of peptides

The isolation of pure peptides is an important step in the study of protein structure. Mixtures of peptides are often difficult to fractionate because they display only small differences in physico-chemical properties. This communication describes a new approach to the chromatographic fractionation of peptides which is based on small differences in the firmness of binding by the free-base form of cellulosic anion exchangers.

The general procedure is simple and rapid. Peptides in a minimal volume of distilled water are added to a cellulosic anion-exchange column[1,2]. The chromatogram is developed with distilled water followed by distilled water saturated with 1 atm. CO_2 (ref. 3). Certain peptides are retarded only slightly and are eluted with the distilled water. Those which are bound more firmly are eluted with the CO_2 solution*.

Abbreviation: DEAE-, diethylaminoethyl-.

* It would be expected that peptides containing dicarboxylic amino acids would be bound very strongly. This was the case with L-Glu-L-Phe, which was not eluted with CO_2 solutions. On the other hand, the tetrapeptide L-Glu-L-His-L-Phe-L-Arg was eluted with CO_2 solutions.