The Presence of a Phosphodeoxyribomutase Activator in Deoxyribose 1-Phosphate Preparations

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The presence of an enzyme in calf thymus converting deoxyribose 1-phosphate to the 5-phosphate was originally described by Manson and Lampen [1]. In studying this reaction, we have prepared the substrate deoxyribose 1-phosphate enzymatically from thymidine and phosphate using horse liver phosphorylase [2]. The sugar phosphate was isolated as the crystalline di*cyclo*hexylamine salt. When chromatographed in *n*-propanol-conc. NH_3 (6:4) and developed with the diphenylamine spray it was homogeneous and had R_F value different from that of deoxyribose 5-phosphate. Paper electrophoresis in phosphate buffer pH 7.5 gave one band only. Its phosphorus/deoxyribose ratio was 1.01.

The phosphodeoxyribomutase used was extracted from calf thymus with 1 % KCl and purified by ammonium sulphate fractionation, phosphate gel adsorption and gradient elution with Tris-hydrochloric acid, pH 7.4, on a DEAE cellulose column. A fifty-fold purification was usually achieved.

The presence of an activator in the substrate was demonstrated in the following way. 3 μmoles of the di*cyclo*hexylamine salt of deoxyribose 1-phosphate were run electrophoretically in phosphate buffer pH 7.5. Ribose 1-phosphate and ribose 1,5-diphosphate were used as reference substances. Deoxyribose 1-phosphate was located by the diphenylamine test whereas the ribose phosphates were detected by the aniline phthalate spray. In one experiment deoxyribose 1-phosphate and ribose 1-phosphate had a mobility of 9.5 cm compared with 12.5 cm for ribose 1,5-diphosphate. Activation was demonstrated with material eluted from an area of the deoxyribose 1-phosphate run corresponding to the mobility of ribose 1,5-diphosphate. The activating material (A) and the deoxyribose 1-phosphate band (purified deoxyribose 1-phosphate) were freeze-dried after elution from the paper.

The enzymatic test system was set up in 0.004 M Tris-hydrochloric acid buffer, pH 7.4, and contained 0.34 μmoles deoxyribose 1-phosphate, 9.6 μg purified enzyme (60 × purification) in a total volume of 0.85 ml. The mixture was incubated for 30 min at 23°C. The amount of deoxyribose 5-phosphate formed was estimated by a novel procedure [3] utilizing the difference in the diphenylamine colour before and after reduction with sodium borohydride.

It is apparent from the table that purification of the substrate by electrophoresis substantially reduces the enzymic activity. The activity is not restored by the addition of 8-hydroxyquinoline whereas the addition of ribose 1,5-diphosphate in the presence of 8-hydroxyquinoline restores the activity. Addition of part of the material present in area A also restores the activity. Furthermore it was shown that the eluate of a similar blank area of the paper did not restore the activity. Since the mobility of the activating substance is equal to that of ribose 1,5-diphosphate, the presence of a sugar diphosphate in the deoxyribose 1-phosphate employed is suggested. Analysis of the material in area A showed that only traces (<0.4 %) of a deoxyribose 1,5-diphosphate could be present.

Deoxyribose 1-phosphate	8-hydroxy quinoline μmoles	Ribose 1.5-diphosphate μmoles	A[a] μl	% Activity [b]
Not purified	0	0	0	100
Purified	0	0	0	25
»	0.1	0	0	27
»	0.1	0.05	0	114
»	0	0.05	0	88
»	0.1	0.01	0	106
»	0.1	0.001	0	116
»	0.1	0.0001	0	64
»	0.1	0	100	109
»	0.1	0	10	41

[a] The material obtained from 3 μmoles of deoxyribose 1-phosphate was dissolved in 1 ml water.
[b] Activity of enzyme in the presence of not purified deoxyribose 1-phosphate: 100 %.

In connection with the present finding it is interesting to note that biosynthetically prepared ribose 1-phosphate contains an activator, for the phosphoribose mutase reaction. This activator is removed by ion exchange chromatography of the substrate [4].

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2. Friedkin, M. and Roberts, D. *J. Biol. Chem.* **207** (1954) 257.
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Enzymic Properties of Human Skeletal Muscle Mitochondria

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Certain enzymic properties of isolated human skeletal muscle mitochondria have been investigated on the basis of knowledge from, and in comparison with, known properties of mitochondria from animal tissues, in the first place skeletal muscle, heart muscle, and liver. Human skeletal muscle mitochondria catalyze a rapid aerobic oxidation of pyruvate (in the presence of malate) and of α-ketoglutarate, whereas other Krebs-cycle metabolites are oxidized at relatively low rates. However, the oxidation of succinate can be greatly enhanced if the accumulation of oxaloacetate is prevented by the addition of either amytal or cysteine sulfinic acid. Glutamate is oxidized usually at high rate, whereas other amino acids as well as fatty acids give rise only to low rates of respiration. Glycerol-1-phosphate was oxidized at low rate under the experimental conditions used.

The respiration with all substrates was accompanied by a phosphate uptake, and the P/O ratios approached the values generally accepted for intact mitochondria, *i. e.* 3 in the case of DPN-linked substrates, and 2 in the case of succinate. The mitochondria exhibited a high extent of respiratory control, as revealed by a severe lowering of the respiratory rate when the terminal phosphate acceptor system, *i. e.* ATP, hexokinase and glucose, was omitted from the incubating medium. Catalytic amounts of ATP enhanced this "resting" respiration, and this enhancement was a function of the concentration of Mg^{++} present in the medium. This function parallelled the resting ATPase activity of the mitochondria, both reaching maximum at a Mg^{++} concentration of 0.5—1 mM. A relatively high Mg^{++}-activated ATPase activity seems to be a common attribute of intact muscle mitochondria from different sources, which, as will be pointed out, must be taken into account when desiring to obtain an adequate estimate of the respiratory control capacity of these mitochondria; addition of catalytic amounts of ATP to the resting respiratory system may obscure the true extent of respiratory control. It is postulated that this ATPase differs in mechanism from that found in structurally damaged rat liver mitochondria in that it does not involve the primary high-energy intermediate(s) of electron transport coupled phosphorylation.

2,4-Dinitrophenol uncouples respiration from phosphorylation in human skeletal muscle mitochondria and gives rise to an increased ATPase activity, both effects reaching maximal extent at a dinitrophenol concentration of 10^{-4} M. On the other hand a concentration of dinitrophenol as low as 10^{-5} M, which affects phosphorylation and ATPase activity only slightly, completely abolishes respiratory control, *i. e.* gives rise to maximal respiration even in the absence of terminal phosphate acceptor. Possible interpretations of this phenomenon will be discussed. 10^{-4} M dinitriphenol also inhibits to some extent the respiration of human skeletal muscle mitochondria when this is supported by glutamate, but not when it is supported by α-ketoglutarate. Bovine serum albumin partially protects against all effects of dinitrophenol, as well as of other uncouplers such as dicoumarol and gramicidine.

The data will be discussed in relation to some earlier findings [1] made with skeletal muscle mitochondria from patients with elevated basal metabolic rates.

This work has been supported by grants from the *Swedish Medical Research Council*, and from *Vera and Carl J. Michaelsens donationsfond*, Hälsingborg.

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