Hydrolytic Activity of Muscle Acyl Phosphatase on 3-Phosphoglyceryl Phosphate[1]

Muscle acyl phosphatase (E.C. 3.6.1.7) catalyzes the hydrolysis of many acyl phosphates[2,3]: the action of this enzyme on physiological acyl phosphates as carbamyl phosphate[4] and 3-phosphoglyceryl phosphate[5,6] is of great interest. Both HARARY[5] and KRIMSKY[6] had, in fact, already carried out research on the hydrolysis of 3-phosphoglyceryl phosphate by acyl phosphatase prepared from muscle. HARARY's measurements were calculated indirectly, by determining the rate of P_i formation by addition of acyl phosphatase purified 600 times. KRIMSKY[6], through a direct method, had shown the catalytic activity of acyl phosphatase on the hydrolysis of 3-phosphoglyceryl phosphate; the enzyme used, prepared according to the method of KOSHLAND[7], had a purification factor of 37.

In this paper a direct determination of 3-phosphoglyceryl phosphate hydrolysis by a highly purified preparation of muscle acyl phosphatase is reported.

Materials and methods. Acyl phosphatase was prepared from horse muscle according to the method of GUERRITORE et al.[8]. The final preparation obtained had a specific activity of 380, expressed as μmoles of split acetyl phosphate/min/mg of protein at 25 °C and pH 5.3, with a 2300-fold purification.

Glyceraldehydephosphate dehydrogenase (E.C. 1.2.1.9), alcohol dehydrogenase (E.C. 1.1.1.1), NAD and $NADH_2$ were purchased from C. F. Boehringer & Söhne G.m.b.H., Mannheim (Germany). 3-Phosphoglyceraldehyde was purchased from SIGMA Chem. Co. All other reagents were obtained from E. Merk AG.

3-Phosphoglyceryl phosphate was prepared according to the method of NEGELEIN and BRÖMEL[9], slightly modified in order to avoid possible impurities of sulphate, that is an inhibitor of muscle acyl phosphatase[6,10]. To 500 μmoles of 3-phosphoglyceraldehyde were added 1 m-mole of phosphate, 24 μmoles of NAD, 0.6 mg of glyceraldehydephosphate dehydrogenase, 3 mg of alcohol dehydrogenase and 0.5 m-moles of acetaldehyde in fractions of 0.05 m-moles. This mixture, kept to pH 7.6, was incubated for 25 min at 18 °C and then acidified at pH 2.6 with perchloric acid. The product was precipitated by 500 ml of cold acetone, acidified with the same quantity of per chloric acid used to acidify the mixture. The precipitate was again washed with cold acidified acetone and dissolved in 3–4 ml of water. This solution was centrifugated to remove the enzymes. The supernatant was then brought to pH 7 by 5 *M* K_2CO_3 and kept at − 20 °C. The enzymes were previously dialyzed against 0.1 *M* triethanolamine hydrochloride-NaOH buffer pH 7.6, containing EDTA 0.01 *M*.

3-Phosphoglyceryl phosphate was measured at 340 nm by $NADH_2$ oxidation in the presence of glyceraldehydephosphate dehydrogenase[11]. Phosphate determination was carried out according to FISKE and SUBBAROW[12] on 3-phosphoglyceryl phosphate. By this procedure the 1-labile phosphate in 3-phosphoglyceryl phosphate was split off and directly determined without previous hydrolysis; in our preparation a maximal P_i impurity of about 20% could be estimated by this way. NAD and

Relative rate of enzymatic hydrolysis and K_m for acetyl phosphate and 3-phosphoglyceryl phosphate as substrates of acyl phosphatase[a]

Substrate	Relative rate of enzymatic hydrolysis	K_m (m*M*)
Acetyl phosphate	1	7.7[b]
3-Phosphoglyceryl phosphate	1.2	1.6

[a] The rate with acetyl phosphate is taken as unity. [b] Datum from [4].

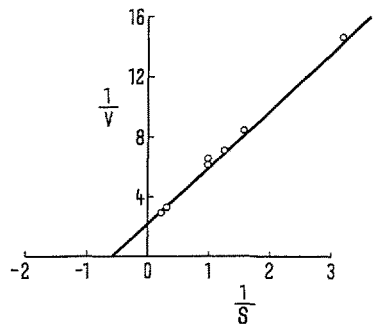

Fig. 1. The effect of 3-phosphoglyceryl phosphate concentration on acyl phosphatase activity. The data are plotted according to LINEWEAVER and BURK[16]. The incubation mixture at 25 °C contained acetate buffer, pH 5.3, 80 μmoles; 3-phosphoglyceryl phosphate at the concentration indicated; acyl phosphatase (diluted before addition), 10 μl; in a total volume of 1 ml. V, μmoles of substrate, hydrolyzed/min. S, substrate concentration (m*M*).

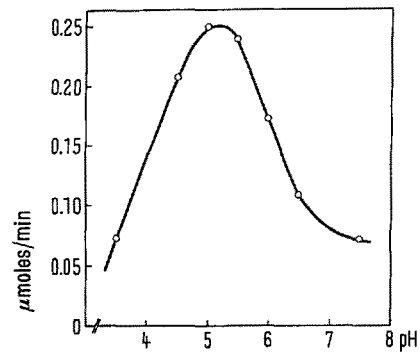

Fig. 2. Effect of pH on the 3-phosphoglyceryl phosphate splitting by acyl phosphatase. The incubation mixture at 25 °C contained β-β'-dimethyl-glutaric acid-NaOH buffer, at the pH values indicated, 80 μmoles; 3-phosphoglyceryl phosphate, 2 μmoles; acylphosphatase diluted before addition), 10 μl; in a total volume of 1 ml.

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3-phosphoglycerate were also present as impurities, but their amounts, determined through optical methods[13], were so low that they did not interfere in our experiments.

The hydrolytic activity of acyl phosphatase on 3-phosphoglyceryl phosphate was measured by the above mentioned optical test, which estimates the residual substrate after suitable periods of time. The enzymatic reaction was stopped by adding ammonium sulphate in final concentration 0.4 *M*: this concentration completely inhibits muscle acyl phosphatase[6,10]. In our conditions, the estimation of enzymatic hydrolysis, by using the method of LOWRY and LOPEZ[14] for the inorganic phosphate, is not suitable due to the catalytic effect of molybdate on the hydrolysis of the 1-radical of 3-phosphoglyceryl phosphate[15].

Results. The Table reports the acyl phosphatase activity on both acetyl phosphate and 3-phosphoglyceryl phosphate, expressed as relative rate of enzymatic hydrolysis.

Figure 1 shows a LINEWEAVER–BURK[16] plot, from which the Michaelis constant for 3-phosphoglyceryl phosphate at pH 5.3 was evaluated: this value is also reported in the Table.

It can be seen (Table) that 3-phosphoglyceryl phosphate is easily hydrolyzed by the enzyme, furthermore the hydrolysis rate is higher and the Michaelis constant is lower than with acetyl phosphate.

The effect of pH on the enzymatic hydrolysis of 3-phosphoglyceryl phosphate is reported in Figure 2. The optimum pH for acyl phosphatase activity on this substrate results in about 5.3, as previously obtained with other acyl phosphates[3,4,17].

These results add some quantitative information about the acyl phosphatase action on 3-phosphoglyceryl phosphate; this hydrolytic activity can explain the increase both of alcoholic fermentation[5] and that of glycolysis[18,19], under particular conditions[20].

Riassunto. Viene studiata l'attività idrolitica dell'acilfosfatasi purificata da muscolo sull'acido 1,3-difosfoglicerico. L'ottimo di pH è uguale a quello ottenuto per altri acilfosfati. Il valore della costante di Michaelis è dello stesso ordine di grandezza di quello trovato per l'acetilfosfato.

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Abnormalities of the Eye Pigments (Pteridins and Ommochromes) Induced in *Drosophila melanogaster* by the Inhibitor of Xanthine Dehydrogenase 4-Hydroxypyrazolo (3,4 d) Pyrimidine

When wild-type strains of *Drosophila melanogaster* are grown on media containing the inhibitor 4-hydroxypyrazolo (3,4 d) pyrimidine (HPP), phenocopies are obtained which mimic the *ma-l* and *ry* mutants. In fact, loss of isoxanthopterin and uric acid with accumulation of the corresponding precursors 2-amino-4-hydroxypteridine and hypoxanthine, as well as diminution of the red pteridins of the eye, have been observed[1,2]. These phenomena do not exclude the possibility that the inhibitor might affect other mutants, with different patterns of eye pigments metabolism.

Strains of the *cl* and *se* mutants were raised, according to KELLER and GLASSMAN[1], on different concentrations of HPP[3]: 0.0; 0.01; 0.02; 0.03; 0.04; 0.06; 0.08 and 0.1 g%. Eye colours were recorded daily when the adults emerged. Flies, 3–5 days old, were placed in boiling water for 1 min, the heads dissected and homogenized in AEA (30% ethanol and HCl q.s. to pH 2[4]), 0.01 ml for each head. Aliquots of 0.01 ml of the homogenates were laid down on Whatman No. 1 filter paper; the chromatograms, developed by ascending chromatography in *n*-propanol and 2% ammonium acetate in water (1:1) and also in 3% NH_4Cl in water[5], were dried and observed in the visible light and under an UV-lamp emitting mainly at 365 nm. The remaining portions of the homogenates were centrifuged (20,000 *g*/30 min); the limpid supernatants, quantitatively collected, were diluted 1:10 and the absorption was measured with a Beckman DU spectrophotometer. The bodies also were treated in the same manner.

In the *cl* mutant, grown on HPP, the eye colour remains dark maroon. Loss of isoxanthopterin and accumulation of 2-amino-4-hydroxypteridin, mainly in the bodies of the male flies, occur at about the same concentrations of HPP as in wild-type strains. Partial loss of the drosopterins is more prominent for neodrosopterin than for iso- and drosopterin; correspondingly accumulation of biopterin, but not of sepiapterins, could be observed.

In *se* strains, grown on HPP (more evidently at the higher concentrations) the eyes are more pale, having a brown-beige colour, and usually smaller as compared with control flies. Loss of isoxanthopterin is in *se*, as in wild-strains, almost complete at the concentrations of HPP higher than 0.04%; accumulation of 2-amino-4-hydroxypteridine seems to be present in somewhat

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