PHOSPHATE-DEPENDENT EFFECTS OF PALMITYL-COA AND STEARYL-COA ON PHOSPHATE-ACTIVATED PIG BRAIN AND PIG KIDNEY GLUTAMINASE

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1. Introduction

Phosphate-activated glutaminase (EC 3.5.1.2), purified from pig kidney [1] and brain [2], has been shown to be activated by low concentrations of Bromothymol Blue (< 0.05 mM) [1,3], and inhibited by higher concentrations. The dye has, thus, a dual and unique effect on the enzyme. Furthermore, the activation is potentiated by phosphate, and phosphate also protects against inhibition by the dye. In a search for physiological compounds with similar properties to Bromothymol Blue, acetyl-CoA has been found to be an activator of glutaminase with K_A about 0.2 mM [4]. As the activation by acetyl-CoA is diminished by phosphate and other anions such as citrate, the mode of action is different from that of Bromothymol Blue.

We show here that palmityl-CoA and stearyl-CoA in principle have similar properties to Bromothymol Blue.

2. Experimental

S-Palmityl-CoA and S-Stearyl-CoA (grade II) were products of Sigma Chemical Co., St. Louis, Mo., USA. Phosphate-activated glutaminase from pig kidney and pig brain was purified and solubilized as described previously [1,2]. The second Tris–HCl solubilized fraction [specific activity, about 40 (μmol of NH_3 formed/min per mg of protein)] was used in this work.

Glutaminase was assayed by measuring the amount of glutamate formed during 5 min at 23°C and at pH 7.0. The reaction mixture (0.5 ml) contained: 40 mM L-glutamine, 3 mM EDTA, 15 mM Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) and 0.01 mg of protein. The enzyme reaction was stopped by the addition of 1 ml of cold (0°C) ethanol. Controls were treated in the same manner for the determination of non-enzymically hydrolysed glutamine except that the enzyme was added after the addition of ethanol. Glutamate was isolated by paper chromatography and measured quantitatively as described previously [4].

3. Results and discussion

The effect of palmityl-CoA on pig brain phosphate-activated glutaminase as compared to that of Bromothymol Blue is shown in fig. 1. Palmityl-CoA has dual effects on glutaminase, both activatory and inhibitory. The activation which occurs at low concentrations ($< 5 \times 10^{-6}$ M) is potentiated by phosphate, and phosphate also protects against the inactivation. The effects of palmityl-CoA are in principle similar to those of Bromothymol Blue, but the dye is a more powerful activator in the presence of low phosphate concentrations, and activates over a wider concentration range than palmityl-CoA, with a maximum at about 5×10^{-5} M. When low phosphate concentrations are present, palmityl-CoA has only inhibitory effects on kidney glutaminase, whereas brain glutaminase is both activated and inhibited. Stearyl-CoA has a somewhat less activatory effect than palmityl-CoA on both brain and kidney glutaminase, but affects the enzymes otherwise in the same manner. The concentration required of either palmityl-CoA or stearyl-CoA to produce 50% inhibition of the activity generally varies in the range from about 5×10^{-6} M – 10^{-5} M when increasing amounts of phosphate in the range 0–25 mM are added. Palmitic acid and stearic acid in concentrations below

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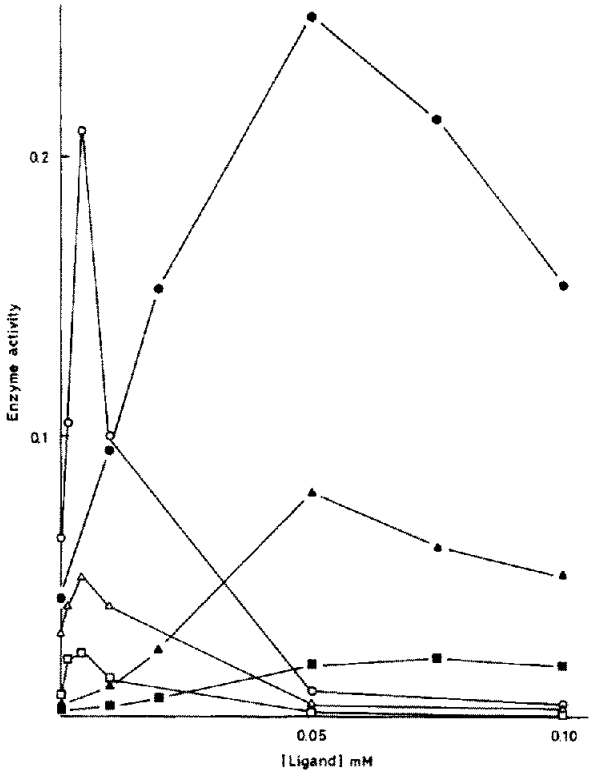

Fig. 1. The effects of palmityl-CoA (open symbols) and Bromothymol Blue (filled symbols) on phosphate-activated pig brain glutaminase. The reaction mixture (0.5 ml) contained (sodium salts), 40 mM L-glutamine, 15 mM Hepes, 3 mM EDTA and 0.01 mg of protein. The glutamate formed was determined by the chromatographic technique after incubation for 5 min at 23°C and pH 7.0. Phosphate added: (○), (■), no addition; (△), (▲), 5 mM; (○), (●), 25 mM.

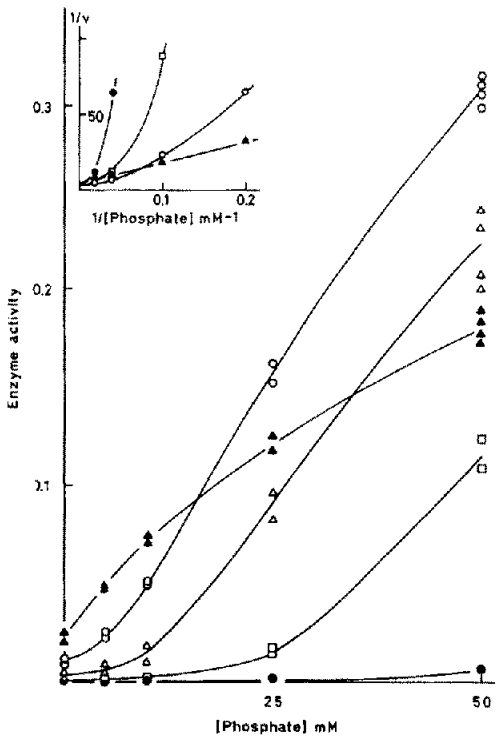

Fig. 2. The effect of stearyl-CoA on phosphate-activated pig kidney glutaminase. The experimental conditions were as in fig. 1. Stearyl-CoA added: (▲), no addition; (○), 0.005 mM; (△), 0.010 mM; (□), 0.020 mM; (●), 0.050 mM. Inset: double reciprocal plots of initial velocity vs phosphate concentration.

10^{-4} M have no effect on either brain or kidney glutaminase.

The addition of palmityl-CoA or stearyl-CoA affects plots of initial velocity versus phosphate concentration so that the sigmoidal shape becomes more pronounced (fig. 2). The positive cooperative effect of phosphate on kidney glutaminase as caused by stearyl-CoA, is clearly seen in the double reciprocal plots (inset fig. 2) which tend to intersect at one point on the ordinate. Palmityl-CoA and stearyl-CoA behave also in this respect in the same manner as Bromothymol Blue, and different from acetyl-CoA which changes the form of the double reciprocal plots to convex-upwards. However, palmityl-CoA and stearyl-CoA increase the negative cooperative interaction of the substrate glutamine at pH 7.0 as shown in fig. 3 for palmityl-CoA, and thus affect the kinetics of substrate saturation similar to acetyl-CoA.

On the basis of kinetic studies it has previously been suggested that phosphate-activated glutaminase has at least two regulatory anionic binding sites, and that the binding of phosphate to one of these sites has a positive cooperative effect on the binding of this ligand to the other site(s) [5]. Bromothymol Blue increases the affinity of the enzyme for phosphate, and phosphate also increases the Bromothymol Blue binding power of glutaminase [3] in a similar way to ligands affecting other allosteric proteins [6]. The activation may be caused by binding of the dye to some allosteric site, whereas the inhibition appears to be due to competition between Bromothymol Blue and phosphate

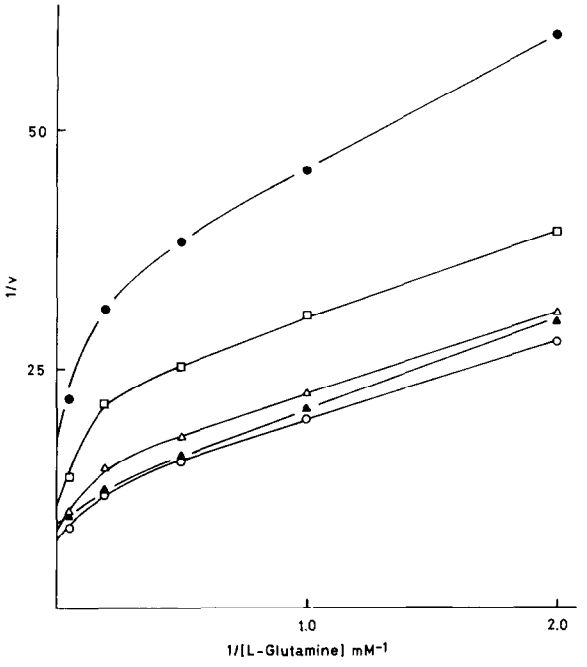

Fig. 3. Activation by the substrate glutamine of pig kidney glutaminase as affected by palmityl-CoA. The experimental conditions were as in fig. 1. Palmityl-CoA added: (▲), no addition; (○), 0.0050 mM; (△), 0.0070 mM; (□), 0.0084 mM; (●), 0.0100 mM.

for the anionic binding. In contrast to acetyl-CoA, palmityl-CoA and stearyl-CoA affect the apparent K_m for phosphate in the same way as Bromothymol Blue, and probably activate by increasing the binding of phosphate to the enzyme, e.g. by increasing the allosteric constant L [7] for phosphate. Since competition between the acyl-CoA's and phosphate is suggested, the inhibition by these compounds may be explained by a similar mechanism as for Bromothymol Blue.

We also investigated the reversibility of the inhibition of brain and kidney glutaminase by palmityl-CoA and stearyl-CoA, because regulatory compounds in general are likely to have reversible effects on enzymes. Reversible inhibition by palmityl-CoA of mitochondrial adenine-nucleotide translocase [8,9], glucose-6-phosphate dehydrogenase [10] and long chain acyl-CoA synthetase [11], has been described whereas other mammalian enzymes, such as glutamic acid dehydrogenase, glucose-6-phosphate dehydrogenase, malic dehydrogenase, α-glycerophosphate dehydrogenase, fumarase and 6-phosphogluconate dehydrogenase are irreversibly inhibited [12]. We thus preincubated brain and kidney glutaminase with inhibitory concentrations of either palmityl-CoA or stearyl-CoA and assayed the enzyme following dilution, so that the activity was determined in the presence of non-inhibitory concentrations of the compounds. The inhibition of both brain and the kidney glutaminase was found to be reversible. This is demonstrated in fig. 4 for kidney glutaminase which was preincubated with inhibitory concentrations of stearyl-CoA.

As far as the physiological importance of the effects of palmityl-CoA and stearyl-CoA on glutaminase are concerned, no conclusions can be drawn from the in vitro experiments alone. The reservations imposed by Morei et al. [13], particularly regarding the high and unspecific acceptance capacity of biological membranes for acyl-CoA's must be considered. Moreover, in vivo there are problems of compartmentalization and local concentrations to be taken into account. However,

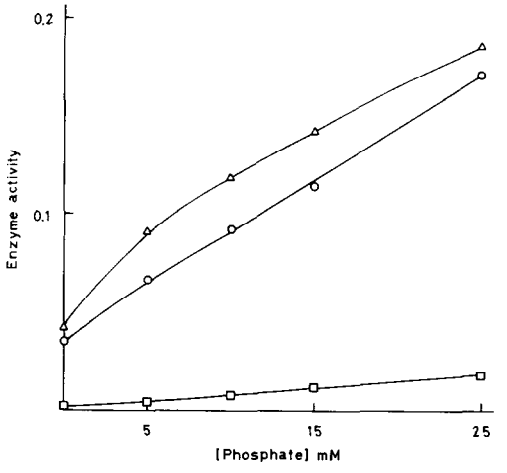

Fig. 4. Reversibility of stearyl-CoA inhibition of pig kidney glutaminase. (○), The enzyme was preincubated with 0.02 mM stearyl-CoA for 5 min at 23°C and pH 7.0, diluted by rapid mixing with the temperature equilibrated assay mixture which was described in fig. 1 and assayed by incubation for 5 min at 23°C and pH 7.0. Final concentration of stearyl-CoA 0.0004 mM. (△), The enzyme was preincubated and assayed as above except that no stearyl-CoA was added. (□), The enzyme was incubated for 5 min at 23°C and pH 7.0 as described in fig. 1, except that 0.02 mM stearyl-CoA was added to the assay mixture.

intramitochondrial palmityl-CoA concentrations in rat liver mitochondria of up to 1 nmol/mg protein have been observed [14]. The concentrations of palmityl-CoA and stearyl-CoA may therefore be well within the effective range if these compounds are assumed to be confined to the mitochondrial matrix where phosphate-activated glutaminase is localized [15]. The strong interaction between the acyl-CoA's and phosphate, which is the most powerful known biological activator of glutaminase, may also indicate a physiological role for palmityl-CoA and stearyl-CoA in the regulation of this enzyme.

Acknowledgements

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