An Abnormal Pattern of Product Inhibition Observed in Phosphate Acetyltransferase Catalysis

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Phosphate acetyltransferase (EC 2.3.1.8) catalyses equilibrium (1) in which certain other acyl phosphates can replace acetyl phosphate:

Hibbert *et al.* (1971) have supplemented previous work (Stadtman, 1957; Marcus & Elliott, 1959; Bergmeyer *et al.*, 1963) on this system with a detailed kinetic analysis of reaction (1a). We used conditions under which inhibition by the products, acetyl-CoA and phosphate, is negligible. We have now undertaken a similar analysis of the reverse reaction (1b), and have also examined, for both reaction (1a) and reaction (1b), the inhibition produced by their respective products. We report here on the very unusual effects observed for inhibition of reaction (1a) by phosphate and of reaction (1b) by acetyl phosphate. Previous work on the inhibition of reaction (1a) is scant and contradictory (Stadtman, 1957; Bergmeyer *et al.*, 1963); reaction (1b) has not been studied separately before.

Our experimental methods followed those previously described (Hibbert *et al.*, 1971), the reactions being monitored by the direct spectroscopic estimation of acetyl-CoA at 235 nm. The equilibrium constant $K = [\text{acetyl-CoA}][\text{phosphate}]/[\text{CoA}][\text{acetyl phosphate}] \approx 80$. The concentration conditions can be arranged so that a reasonable (>50%) conversion into products is obtained at equilibrium starting from either side, both in the presence and in the absence of deliberately added phosphate (or acetyl phosphate) as inhibitor. However, in all the present kinetic experiments initial rates only were measured to avoid any significant contributions from the reverse reactions. Reactions were initiated by the addition of enzyme. The pH (tris buffer) was always 8.3. The effectiveness of the buffer was checked for solutions containing high phosphate concentrations.

Typical results are shown in Fig. 1. As the relevant inhibitor concentration is increased, the initial concentrations of all other species being kept fixed, the initial rate passes successively through a minimum value and a maximum value; exactly similar phenomena are observed in both directions, 1(a) and 1(b). Each curve in Fig. 1 shows two regions of inhibition and one of activation. No other enzyme

has yet been shown to display this type of product inhibition.

Measurements of initial rates at various inhibitor concentrations, for a series of fixed substrate concentrations, permit the construction of Dixon plots (Webb, 1963). We have done this for both regions of inhibition in both directions. A typical set of plots [for the first region of inhibition in reaction (1b)] is shown in Fig. 2. In all cases the inhibition is competitive between phosphate and acetyl phosphate (or

Fig. 1. Typical inhibition curves for phosphate acetyltransferase

k is the initial rate ($\text{mol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$). Curve A, inhibition by acetyl phosphate: [acetyl-CoA], 0.1 mM; [phosphate], 0.40 M; [enzyme], 0.26 μM ; $[\text{NH}_4^+]$, 7.2 mM. Curve B, inhibition by phosphate: [CoA], 0.1 mM; [acetyl phosphate], 2.0 mM; [enzyme], 1.3 μM ; $[\text{NH}_4^+]$, 36 mM.

Fig. 2. Competitive inhibition of phosphate acetyltransferase by acetyl phosphate at low concentrations

k is the initial rate ($\text{mol}\cdot\text{l}^{-1}\cdot\text{min}^{-1}$). Curve A: [phosphate], 76mM. Curve B: [phosphate], 0.15M. Curve C: [phosphate], 0.40M.

vice versa). Average values (in $\text{mol}\cdot\text{l}^{-1}$) of the Michaelis constants derivable from the various Dixon plots are: $K_{AP}=(6.6\pm 1.0)\times 10^{-4}$ and $K_P=(5.0\pm 2.0)\times 10^{-2}$ (for the first region of inhibition); $K_{AP}=(5.0\pm 1.5)\times 10^{-3}$ and $K_P=0.20\pm 0.04$ (for the second region of inhibition); where AP and P represent acetyl phosphate and phosphate respectively. The K_m values for these species determined directly, in the effective absence of inhibition, are: $K_{AP}=(3.0\pm 0.2)\times 10^{-3}$ and $K_P=0.24\pm 0.07$ (Hibbert *et al.*, 1971; S. A. Kyrtopoulos & D. P. N. Satchell, unpublished work).

Our previous work (Hibbert *et al.*, 1971) has shown that the velocity of the reaction between CoA and acyl phosphates on the enzyme surface is very sensitive to the steric bulk of the acyl group. The present results can also be rationalized in terms of important steric effects and of adsorption of phosphate (or acetyl phosphate) at three independent sites, A, B and C, all spatially adjacent to the site that carries the CoA species. The product-forming process is considered to

involve interaction between the adsorbed CoA species and acetyl phosphate (or phosphate) located on either site A or site C. The changing occupation of site B provides a steric acceleration (Ingold, 1969). Thus, for reaction (1a), as the concentration of the inhibitor (phosphate) is increased, the observed initial rate of formation of acetyl-CoA will at first fall, owing to competition between acetyl phosphate and phosphate at site A, the site of strongest adsorption of phosphate species generally. This gives rise to the first region of inhibition. However, if the occupation of site B, assumed to be the site of next-strongest adsorption, by phosphate permits the acyl group to be transferred from site C to the adsorbed CoA much more readily (owing to a decrease in steric crowding around CoA) than when acetyl phosphate occupies site B, then a temporary recovery in the rate of acetyl-CoA formation will occur as phosphate is added. When sufficient phosphate is present to compete significantly with the adsorption of acetyl phosphate at site C, the site of weakest general adsorption of phosphate species, then the rate of acetyl-CoA formation will fall again and will not recover, thus providing the second region of inhibition. An exactly complementary argument can be applied to reaction (1b). Here the product-forming step involves the transfer of the acyl group from acetyl-CoA to phosphate. When acetyl phosphate rather than phosphate occupies site B, steric crowding will lead to a more rapid loss of the acyl group from the adsorbed acetyl-CoA. In this way the unusual shapes of the curves in Fig. 1 can qualitatively be understood. It is not easy to devise a self-consistent alternative rationalization. If our explanation is correct, phosphate acetyltransferase must be considered a multi-site enzyme. The directly determined K_m values for acetyl phosphate and phosphate should therefore (Reiner, 1969) lie somewhere between those given by the present inhibition studies for sites A and C. Our results appear to be just compatible with this requirement.

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