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AROMATIC COMPOUNDS AS ALLOSTERIC INHIBITORS OF GLYCOGEN PHOSPHORYLASE *b*

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Summary

Rabbit muscle glycogen phosphorylase *b* (1,4-α-D-glucan:orthophosphate α-glucosyltransferase, EC 2.4.1.1) was found to be inhibited by a wide variety of soluble aromatic compounds. The effectiveness of the inhibitors appeared to be controlled by steric factors and was not a function of the electron density of the benzene ring or charges per se on the aromatic molecules. In the presence of *p*-nitrophenol the glucose 1-phosphate sites became cooperative and the cooperativity of the AMP sites increased, showing that a compound structurally unrelated to the feed-back inhibitors could influence allosteric transitions. The existence of a specific site for aromatic compounds on phosphorylase is shown to explain the contradictory properties of chemically modified enzyme derivatives.

Chemical modification of apparently the same amino acid residues of glycogen phosphorylase *b* (1,4-α-D-glucan:orthophosphate α-glucosyltransferase, EC 2.4.1.1) from rabbit muscle using different reagents has been shown to yield derivatives with distinctly different catalytic and allosteric properties [1—5]. In an earlier communication we have pointed out the possibility of interaction of reagents such as 1-fluoro-2,4-dinitrobenzene and 5,5′-dithiobis(2-nitrobenzoic acid) with phosphorylase *b* prior to covalent bond formation [5]. Desensitization of the cooperativity of sites has been interpreted as being due to possible apolar binding of the incorporated aromatic groups with specific sites on the enzyme [6,7]. The present communication shows that phosphorylase *b* possesses binding sites for aromatic compounds and throws some light on the nature of interaction with them.

Phosphorylase *b* was prepared from rabbit muscle according to the procedure of Fischer and Krebs [8]. Stock solutions of aromatic compounds were prepared by adding a dilute solution of HCl or NaOH and adjusting the pH to 6.8. Further dilutions were made in 0.03 M cysteine—0.04 M sodium β-glycerophosphate buffer, pH 6.8. Suitably diluted enzyme solutions were mixed with equal volumes of solutions of aromatic compounds prior to initial rate measurements. Initial rates were measured in the direction of glycogen synthesis by estimating the liberated inorganic phosphate by the method of Fiske and SubbaRow [9].

TABLE I

INHIBITION OF PHOSPHORYLASE *b* BY VARIOUS AROMATIC COMPOUNDS

Solutions of the aromatic compounds were prepared and the enzyme assayed at 30 °C as mentioned in the text. The concentration of the inhibitor in the assay mixture in each case was 5 mM. The assay concentrations of glucose 1-phosphate, AMP and glycogen were 16 mM, 1 mM and 1% respectively. The values given were calculated from initial rates and expressed as relative to the uninhibited reaction.

Compound	pK_a	Inhibition (%)
Phenol	9.95	45
p-Nitrophenol	7.14	56
m-Nitrophenol	8.35	83
2,4-Dinitrophenol	4.01	67
3,5-Dinitrobenzoic acid	2.83	47
p-Cresol	10.19	67
o-Cresol	10.28	67
Aniline	4.62	50
m-Aminophenol	6.5 (NH_2)	20

All aromatic compounds tested were found to inhibit phosphorylase *b* and the inhibition was not time dependent. A few selected examples are presented in Table I. As can be seen in the table the effectiveness of the various compounds was not dependent on the electron density of the benzene ring or on the state of ionization of the aromatic compound. Of all the compounds tested, *m*-nitrophenol was the most potent inhibitor. It appears that steric factors determine the effectiveness of these inhibitors. Cyclohexanol and cyclohexylamine, the two alicyclic compounds tested, were not inhibitory at 5 mM concentrations.

p-Nitrophenol was used as a representative compound for detailed investigations. In the presence of *p*-nitrophenol the substrate saturation curves became sigmoidal and double reciprocal plots curved. The results are given in Fig. 1 along with Hill plots. The homotropic cooperativity of the AMP sites was also increased in the presence of *p*-nitrophenol (Fig. 2). In all these respects, *p*-nitrophenol behaved as a typical allosteric inhibitor like glucose, glucose 6-phosphate or ATP. The effect of *m*-nitrophenol was found to be similar to that of *p*-nitrophenol except that the former was a stronger inhibitor. Thus, compounds structurally dissimilar to the metabolic feed-back inhibitors can also influence allosteric transitions. Such an effect by an aromatic compound has not been reported earlier.

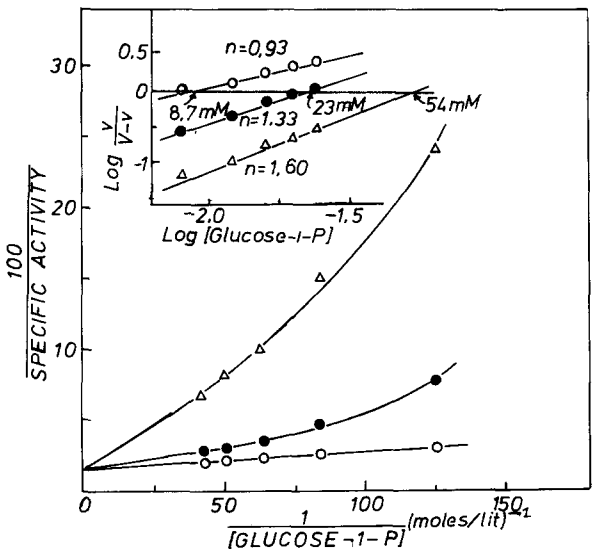

Fig.1. Lineweaver—Burk plot for glucose 1-phosphate in the presence and absence of *p*-nitrophenol for phosphorylase *b*. The enzyme solution was first mixed with *p*-nitrophenol and the reaction started by adding an equal volume of the substrate containing AMP, glycogen and varying concentrations of glucose 1-phosphate at 30 °C. Concentrations of AMP and glycogen in the assay mixtures were 1 mM and 1% respectively and that of the enzyme was $8.1 \cdot 10^{-8}$ M. ○, no inhibitor; ●, with 5 mM *p*-nitrophenol; △, with 10 mM *p*-nitrophenol. Inset, Hill plots for the same data.

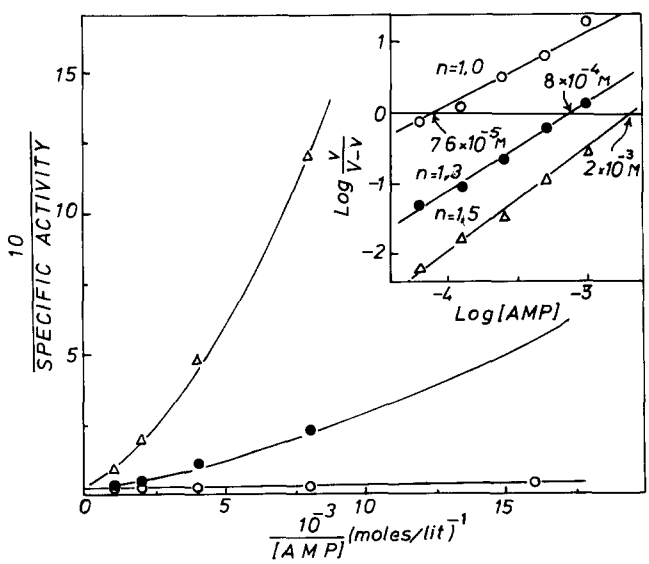

Fig.2. Lineweaver—Burk plot for AMP in the presence and absence of *p*-nitrophenol for phosphorylase *b*. Concentration of glucose 1-phosphate was 16 mM. Other details were as in Fig. 1. ○, no inhibitor; ●, with 5 mM *p*-nitrophenol; △, with 10 mM *p*-nitrophenol. Inset, Hill plots for the same data.

Phosphorylase *b* (4.1 mg/ml) in cysteine—glycerophosphate buffer, pH 6.8, was found to sediment as a single peak in the presence of 15 mM *p*-nitrophenol with the same $s_{20,w}$ at 23.5 °C as that of the enzyme in the absence of the nitrophenol showing that under these conditions the dimeric enzyme did not dissociate or aggregate into a tetramer. Thus, the observed kinetic behavior in the presence of *p*-nitrophenol is unlikely to be due to the inhibitor affecting the aggregate state of phosphorylase *b*.

In the presence of *p*-nitrophenol, inhibition by glucose, glucose 6-phosphate and ATP was found to be enhanced, indicating that the influence of these inhibitors and the nitrophenol was not antagonistic. It is likely that binding of the aromatic compound was attended by a conformational change and that the conformationally altered enzyme is more sensitive to the metabolic inhibitors. The observed effect of the nitrophenol on the cooperativity of the substrate and activator sites (Figs 1 and 2) lends support to its involvement in affecting the conformation of the enzyme.

The observation that any aromatic compound can inhibit phosphorylase is significant. This explains why modification of 2—3 amino acid residues of phosphorylase *b* with 5,5′-dithiobis(2-nitrobenzoic acid) leads to partial desensitization [1,7] where as modification of even more cysteinyl residues with iodoacetamide has no effect on the allosteric property of the enzyme [2].

The biological significance, if any, of the existence of the binding site for aromatic compounds in phosphorylase is not known. Work in this direction would be interesting.

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