A KINETIC STUDY OF MUSCLE PHOSPHORYLASE b VARIANTS OF THE PIG: HEART-SPECIFIC ISOENZYME AND SKELETAL MUSCLE ENZYME.

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Introduction and Summary

Glycogen phosphorylase b (α-1,4-glucan: orthophosphate glucosyltransferase, EC 2. 4. 1. 1) is an enzyme exemplifying the way in which catalytic activity relates to molecular properties. Three different forms of the enzyme were found by Yunis et al. (1) to exist in heart muscle: two parental enzyme species and their hybrid. One of the enzyme variants of heart muscle, isoenzyme 3, is probably identical with the phosphorylase of skeletal muscle (2). Little information exists on the kinetic properties and the physiological role of isoenzyme 1, the heart muscle-specific type of phosphorylase b.

The present paper provides evidence that the phosphorylases b of heart and skeletal muscle of the pig are kinetically heterogeneous. While the kinetic parameters of the phosphorylase variants differ only quantitatively with respect to the substrates glucose-1-phosphate (G1P), orthophosphate (OP), and glycogen, qualitative differences were found with respect to the negative effector glucose-6-phosphate (G6P). As regards activation by adenosine-5'-mono-

phosphate (AMP), pig muscle phosphorylases b falls into the mixed V and K class enzyme system (3).

Methods

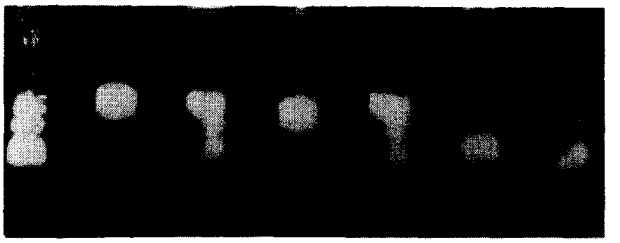
Phosphorylase b was prepared from pig skeletal muscle according to Hanabusa et al. (4). The phosphorylase b isoenzymes of pig heart muscle were purified and separated on DEAE cellulose as described by Davies et al. (2). Isoenzyme 1 was further purified (5) by adsorption on starch and subsequent elution at pH 6,8 with a solution 40 mM in ß-glycerophosphate, 1 mM in EDTA, and 15 mM in mercaptoethanol. Phosphorylase activity was determined as stated in Table I. Disc electrophoresis was performed according to Davies et al. (2), the product of phosphorylase activity being visualized on the gel as $Ca_3(PO_4)_2$. Protein was determined by the method of Lowry et al. (6).

AMP, ATP, G1P, G6P, and ß-glycerophosphate were obtained from Boehringer Mannheim GmbH. Glycogen, obtained from E. Merck AG, Darmstadt, was purified by ion exchange (7).

Results and Discussion

Disc electrophoresis yielded three activity bands for the heart muscle extract and one band each for the phosphorylase from skeletal muscle and the purified heart isoenzymes 1 and 3 (Figure 1). Phosphorylase from skeletal muscle and isoenzyme 3 are electrophoretically identical with the slowest moving band in the heart muscle extract. Isoenzyme 1 is the fastest migrating heart isophosphorylase. These findings suggest that, in analogy to what has been shown in rabbit muscles (2), the phosphorylase of pig skeletal muscle is identical with one of the parental isoenzymes in

the pig heart, namely, isoenzyme 3. For this reason phosphorylase b of pig skeletal muscle and the purified isoenzyme 1 of the pig heart were selected for comparative kinetic investigation.



1 2 3 4 5 6 7

Fig. 1 Electrophoresis of phosphorylase on acrylamide gel. For separate runs of two samples in one column a divider was placed on top of columns 3,5 and 7. 1: I; 2: II; 3: left to right, II/I; 4: isoenzyme 3; 5: isoenzyme 3/I; 6: isoenzyme 1; 7: isoenzyme 1/I. I : heart muscle extract; II : skeletal muscle phosphorylase.

Table I Michaelis constants of phosphorylase b from heart and skeletal muscles of the pig.

	K_m(glycogen)[a] %	K_m(G1P)[b] mM	K_m(OP)[c] mM
Isophosphorylase 1 from heart muscle	0.060	3.5	7.6
Phosphorylase from skeletal muscle	0.039	6.0	4.6

a) Determined according to ref. 8. Glycogen: 0.0025 to 0.5 %; G1P: 18 mM; AMP: 1 mM. b) Determined according to ref. 8. G1P: 1.2 to 56 mM; glycogen: 0.5 %; AMP: 1 mM. c) Determined according to ref. 9. OP: 0.5 to 50 mM; glycogen: 0.1 %; AMP: 0.5 mM. The Lineweaver-Burk plots for G1P and OP yielded curves with slightly upward bents. K_m values were therefore calculated by the method of Silanova et al. (10).

Table I lists the Michaelis constants of the two enzymes for glycogen, G1P, and OP. A comparison of the K_m values shows that the enzymes do not differ greatly in their affinity for their substrates.

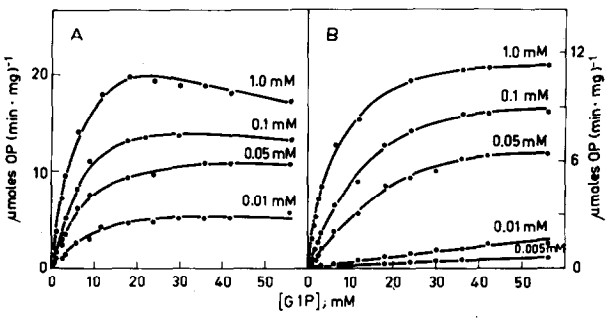


Fig. 2 Activity of phosphorylase b as a function of the AMP concentration. Experimental conditions were those specified in ref. 8. The figures in the diagrams denote the concentrations of AMP. A: Isophosphorylase 1 from heart muscle. B: Phosphorylase from skeletal muscle.

Fig. 2 shows the influence of the concentration of AMP upon the G1P saturation of the two enzymes. It is evident from the pattern of the curves that the enzyme affinity for G1P depends on the concentration of the effector, as would be expected from a K class enzyme on the basis of the "concerted transition" theory of Monod et al. (3, 11). But the curves show, moreover, that also the maximum velocity, V_{max}, depends on the effector concentration. The linear function found for $\sqrt{\frac{1}{V_{max}}}$ vs $\frac{1}{AMP}$ (Fig. 3) may be explained by postulating that in the catalytic breakdown of the enzyme-substrate complexes (G1P bound to one or both of the phosphorylase protomers) both effector binding sites should be occupied by AMP.

Extending the Monod model of the dependence of the velocity, v, on the substrate and effector concentrations, we obtain:

$$\frac{v}{V_{max,max}} = \frac{\alpha(1+\alpha) + Lc\alpha d(1+c\alpha)}{(1+\alpha)^2 + L(1+c\alpha)^2(\frac{1+d\beta}{1+\beta})^2} \times (\frac{\beta}{1+\beta})^2 \qquad (1)$$

$\alpha,\beta \longrightarrow \infty$

where $L = \frac{T}{R}$ represents the allosteric constant,

$\alpha = \frac{G1P}{K_R^{G1P}}$ and $\beta = \frac{AMP}{K_R^{AMP}}$ the normalized ligand concentrations, and $c = \frac{K_R^{G1P}}{K_T^{G1P}}$ and $d = \frac{K_R^{AMP}}{K_T^{AMP}}$. K_R^{G1P}, K_T^{G1P}, K_R^{AMP}, K_T^{AMP} are the dissociation constants of the enzyme-ligand complexes for the two conformations R and T, respectively. At maximum G1P concentrations the first factor in equation (1) approaches unity, if $Lc^2d \ll 1$. One then obtains:

$$v_{\substack{max \\ \alpha \to \infty}} = V_{\substack{max,max \\ \alpha,\beta \to \infty}} \left(\frac{AMP}{K_R^{AMP} + AMP}\right)^2 \qquad (2)$$

In accordance with equation (2) the dissociation constants of the enzyme-AMP complexes (enzyme in the R conformation) were calculated for the skeletal muscle phosphorylase and isoenzyme 1 from the intersection points of the straight lines on the abscissa in Fig. 3. The values obtained are 1.8×10^{-5} and 8.9×10^{-6} M, respectively. A complete mathematical evaluation of all the experimental data by means of computer analysis based on equation (1) will be presented in a subsequent publication (12).

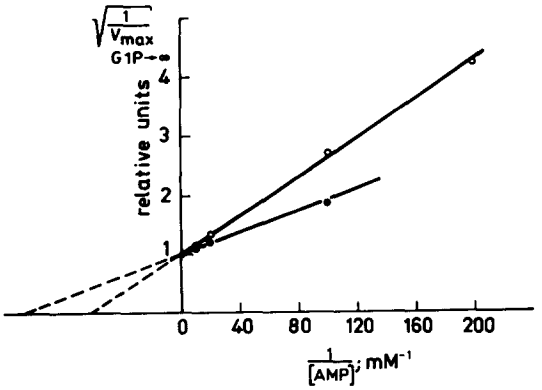


Fig. 3 Effect of increasing the concentration of AMP upon V_{max} (G1P→∞). The V_{max} values were calculated from the curves in Fig. 2 by the method of Silanova et al. (10). ● Heart muscle isoenzyme 1; ○ :skeletal muscle phosphorylase.

Attention may be called at this point to discrepancies in the literature regarding the activation of rabbit skeletal muscle phosphorylase. Black and Wang (13) assumed that the allosteric transitions involved in nucleotide activation of phosphorylase b consist of two stages: a) that of enhancing enzyme affinity towards G1P; and b) that of enhancing catalytic efficiency (V_{max}). Other authors (14,15) supported this assumption, whereas Buc (16) and Kastenschmidt et al. (8) classified phosphorylase b as a K class enzyme. The reason for this discrepancy may lie in differences in experimental conditions or in species differences in the molecular structure of the enzyme.

Experiments dealing with the influence of negative effectors of the two pig muscle phosphorylase variants indicated that the weaker affinity of skeletal muscle phosphorylase for AMP goes along with a stronger affinity for the allosteric inhibitor ATP. Thus isoenzyme 1 is always the more active one for any given ratio of $\frac{AMP}{ATP}$. On the other hand, it is inhibited by G6P, while the activity of the skeletal muscle phosphorylase is not affected by this compound (Fig. 4). The different affinities of isoenzyme 1 for the negative effectors ATP and G6P may be of importance to the regulation of glycogenolysis in aerobic and anaerobic heart muscle (17).

Data comparable to ours on the effect of G6P and on phosphorylase b isoenzyme distribution in pig myocardium are presented in two recent reports (18,19). The differences in sensitivity toward G6P may be of use in the analysis of the phosphorylase b isozymes (12). Thus we find that phosphorylase b activity in pig heart muscle extract may be decreased

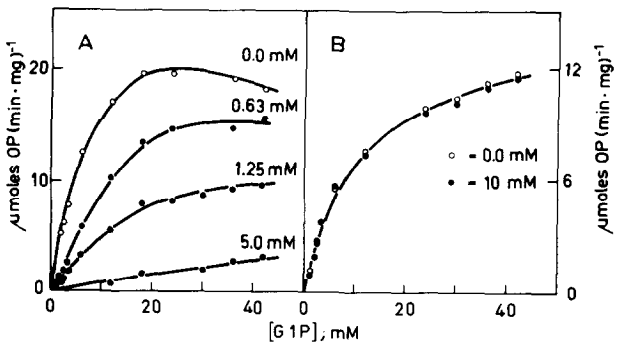


Fig. 4 Dependence of phosphorylase activity upon the concentration of G6P. Except for AMP (1 mM) and glycogen (0.5 per cent) the experimental conditions were those given in ref. 8. The figures on the top of the curves denote the concentration of G6P. A: Isoenzyme 1; B: Phosphorylase from skeletal muscle.

by as much as 70 per cent on addition of G6P, whereas the enzyme activity in skeletal muscle extracts remains unchanged. These responses towards G6P constitute additional evidence for the existence of kinetically differing isoenzymes of phosphorylase b in cardiac muscle and are compatible with the notion that heart isophosphorylase 3 is identical with the phosphorylase of skeletal muscle.

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