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Effect of glycerol on some kinetic parameters of phosphorylase *b*

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SUMMARY

The effect of different glycerol concentrations upon the activity and allosteric regulation of phosphorylase *b* has been studied. Glycerol increased the apparent K_m of the allosteric regulator and decreased the V of the enzyme. The enzyme activity was reduced by glycerol, according to the temperature, similar to the effect of decreasing the concentration of AMP.

The liquid environment influences the conformations of macromolecules. Since the structure and function of enzymes are interdependent, the cellular environment might be expected to influence the catalytic ability and conformational regulation of enzymes through changing the van der Waals forces or other mainly weak interactions.

Some initial attempts have been made to study the effect of highly viscous media on enzyme activity *in vitro*[1–3]. The present short account deals with the effect of glycerol on the substrate binding and allosteric regulation of phosphorylase *b*.

Glycogen phosphorylase *b* has been prepared from rabbit skeletal muscle and purified as reported earlier[4]. The reaction mixtures contained the usual enzyme and substrate constituents. Since the activity assays were carried out in the direction of glycogen synthesis, glucose 1-phosphate was used as substrate of the reaction, and AMP, the obligatory allosteric activator, was used to test the allosteric regulation. Further details are indicated in the legends of the figures. Except for the control samples, different concentrations of the non-ionic and non-reactive viscous agent, glycerol, were also present.

Fig. 1 demonstrates the effect of three different glycerol concentrations upon the substrate-binding capacity of phosphorylase *b*. The results, obtained by using the Lineweaver–Burk plot, suggested that glycerol did not change the binding of the glucose 1-phosphate substrate, while the overall activity, *i.e.* the V of the enzyme, was decreased. The decrease in V was parallel to the increase in the amount of glycerol. It is interesting to

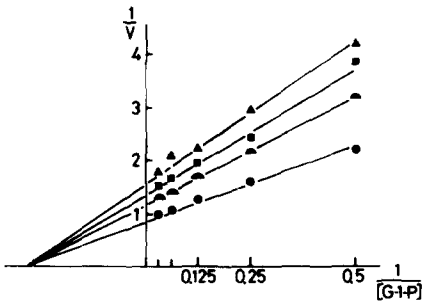

Fig. 1. Lineweaver–Burk plot of phosphorylase *b* activity at activator saturation. The activities were measured with 10^{-7} M phosphorylase *b*, 1 mM AMP, 1% glycogen and various amounts of glucose 1-phosphate (G-1-P) substrate, shown in the figure. The reaction was run in the direction of glycogen synthesis, and P_i liberated at 30 °C in 10 min was determined according to Taussky and Shorr[5]. The ordinate of the figure shows the inverse values of the initial enzyme velocity. $1/v$ means the reciprocal of $A^{1\,cm}_{720\,nm}$ values obtained by the above method where 1.000 $A^{1\,cm}_{720\,nm}$ was equal to 60 μg P_i liberated by the enzyme in the reaction mixture. The symbols represent the control (●—●), and three different reaction mixtures containing 13.3% (—), 16.6% (■—■) and 20% (▲—▲) glycerol, besides the above-mentioned constituents. All experiments were carried out in Tris–HCl buffer at pH 6.8.

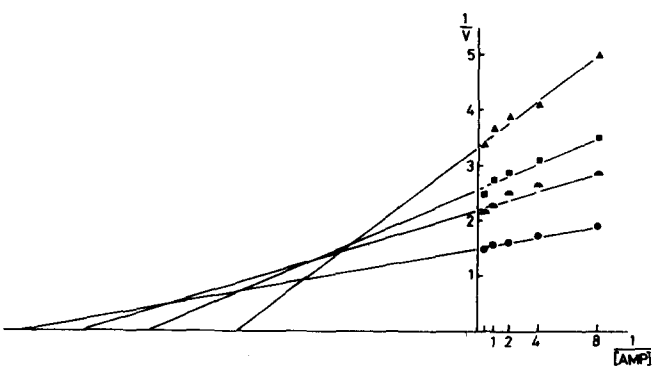

Fig. 2. Lineweaver–Burk plot of phosphorylase *b* activity at saturation level of the substrate, glucose 1-phosphate. Glucose 1-phosphate concentration was 32 mM. Five different concentrations of AMP are shown on the abscissa. All other constituents were as described in the legend to Fig. 1.

note that dextran solutions with the same viscosity values as those of glycerol do not influence either the K_m for glucose 1-phosphate or the V_{max} of the enzyme.

Fig. 2 shows the effect of glycerol on the apparent K_m of the allosteric activator, AMP. Phosphorylase *b* retains only 0.5% of its total activity in the absence of AMP. Thus it is possible to determine the apparent K_m for AMP from the Lineweaver–Burk plot by decreasing the concentration on the allosteric activator. According to Fig. 1, glycerol had no effect upon glucose 1-phosphate binding of the enzyme, saturated with AMP. This

suggested that results shown in Fig. 2 were due to the effect of glycerol on the activation of phosphorylase by AMP. Dextran, similar to its lack of influence on the catalytic ability of the enzyme, had also no effect on the allosteric regulation. The higher the glycerol concentration used, the greater was the increase in the K_m of AMP obtained. As the apparent K_m values had reciprocal relationship to the binding of AMP, the results suggested that glycerol impaired the AMP-binding ability of phosphorylase *b*. These findings were in good agreement with the decrease of the V caused by glycerol.

Fig. 3 illustrates the temperature dependence of enzyme activity, determined at

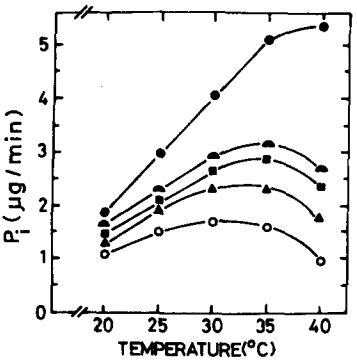

Fig. 3. Effect of four different glycerol concentrations on the phosphorylase *b* activity at various temperatures. The reaction mixture contained 10^{-7} M enzyme, 1 mM AMP, 16 mM glucose 1-phosphate 1% glycogen, and none (●—●), 13.3% (◓—◓), 16.6% (■—■), 20.0% (▲—▲) and 33.3% (○—○) glycerol. The ordinate shows P_i liberated in 10 min in a total volume of 0.6 ml, at 30 °C and pH 6.8 in Tris–HCl buffer.

substrate and activator saturation. The five curves represent five different glycerol concentrations in the incubation mixtures. The concentrations of the mixtures are referred to in the legend of Fig. 3. The results shown in Fig. 3 clearly support those shown in Fig. 2. The increase in amount of glycerol causes a decrease in the enzyme activity and sensitivity to incubation temperature, which is the same as that caused by decrease in AMP concentration. In an earlier study it was found that on examining the enzyme activity at various temperatures and at different activator concentrations, the curves obtained showed a temperature optimum for each activator concentration. Whenever the activator concentration was increased, these maxima of enzyme activity appeared at higher temperatures[4]. Fig. 3 shows that, though no decrease in the activator concentration occurs, nonetheless a similar effect can be produced by means of different glycerol concentrations in the incubation mixtures.

We suggest that glycerol impairs the enzymic activity of phosphorylase *b* through decreasing its AMP-binding capacity. Kinetic investigations failed to support competitive relations between glycerol and AMP. Dextran, the other viscous agent tested, did not

influence the enzyme activity at the same viscosity level. So it seems unlikely that viscosity alone can alter the enzyme activity or regulation. Presumably, the changes in the structure of water induced by glycerol and dextran are sufficiently different to explain the observed different results. It is also suggested that glycerol either stabilized or promoted such a conformational change of protein which was not favourable for AMP binding. Further experimental results on the effect of glycerol and of other viscous agents upon the enzyme activity and regulation will be published later.

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