THE EFFECT OF ANIONS ON THE ACTIVITY OF PHOSPHORYLASE b

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Phosphorylase b has always been considered to possess an absolute dependence on 5' AMP for activity. However, Buc (1967) presented results suggesting that in the presence of high concentrations of P_i (120 mM) the enzyme loses its requirement for AMP. While attempting to repeat these results, we found that P_i at concentrations over 50 mM inhibited severely the conventional coupled auxilliary enzyme assay system (Helmreich and Cori, 1964). This inhibition is in accord with that reported by Glaser and Brown (1955) for glucose-6-phosphate dehydrogenase, one of the components of the phosphorylase assay system.

Several anions have previously been shown to affect the activity of various phosphorylase preparations. Sulfate was found to activate the phosphorylases from lobster muscle (Cowgill 1959), beef adrenal (Riley and Haynes, 1963) and liver (Appleman et al., 1966). Sealock and Graves (1967) reported that phosphorylases a and b from rabbit muscle were activated by fluoride and inhibited by perchlorate. In view of these observed ion effects on phosphorylase, we have reinvestigated the effect of P_i and other anions on the activity of phosphorylase b in the presence and absence of AMP, using a modified phosphorolysis assay system. The effect of P_i on the activity of the enzyme

compares with the results obtained for other anions high in the Hofmeister series (1888), and is most likely non-specific in nature. Moreover, the activity observed in the absence of AMP is more limited than that reported by Buc (1967).

EXPERIMENTAL. Phosphorylase b was prepared from rabbit muscle by the method of Fisher and Krebs (1958) and recrystallized three times. It was fractionated on a 2.5 x 77 cm Sephadex G-200 column to remove any possible contamination by phosphorylase a, recrystallized, and passed through a Sephadex G-25 column to remove Mg^{++} and AMP. Most chemicals and the other enzymes were purchased from Sigma. Possible contamination by AMP was removed from the rabbit liver glycogen by a Dowex-1-chloride column, from glucose-1-P by a charcoal-celite column, and from the bovine serum albumin Fraction V (Mann) by a Sephadex G-25 column. The salts tested were purchased from the Fisher Scientific Co. and used without further purification.

Phosphorylase activity was assayed in the direction of glycogen phosphorolysis in 0.2 ml. of reaction mixture containing the desired concentration of ion(s) and P_i, 10 mM sodium glycerophosphate, 1.5 mM mercaptoethanol, 0.25 mM EDTA, 1% glycogen, 1 mM AMP (if present), 0.04 mg of bovine serum albumin and 8-10 µg of enzyme, all at pH 6.8 and 30°. Reaction times were adjusted to ensure that the release of product with time was linear. The reaction was terminated by immersion in boiling water for 1 minute and the glucose-1-P formed was determined in a 0.1 ml. aliquot by measuring the total reduction of NADP in an auxilliary enzyme reaction mixture similar to that of Helmreich and Cori (1964). This assay system is independent of the possible effect of any added substance on the rate of action of the auxilliary enzymes.

Activity in the direction of glycogen synthesis was determined

by measuring the P_i released from glucose-1-P (Fiske and Subbarow, 1925) or the radioactivity incorporated into glycogen from ^{14}C-glucose 1-P.

RESULTS AND DISCUSSION. The effect of increasing concentrations of P_i on the activity of phosphorylase b is shown in Fig. 1. The maximum activity obtained in the absence of AMP was approximately 30% of the V_{max} with AMP present, and was observed at a concentration of 575 mM P_i. These results are analogous to those obtained with Na_2SO_4 by Appleman et al. (1966) for the activation of inactive liver phosphorylase and the inhibition of active liver phosphorylase, although in their system AMP was present in both cases. Results obtained for high concentrations of glucose-1-P, minus AMP, are also shown in Fig. 1. It is evident that there is very little activity with this substrate in the absence of AMP, and that which is observed (approximately 10% of V_{max} at

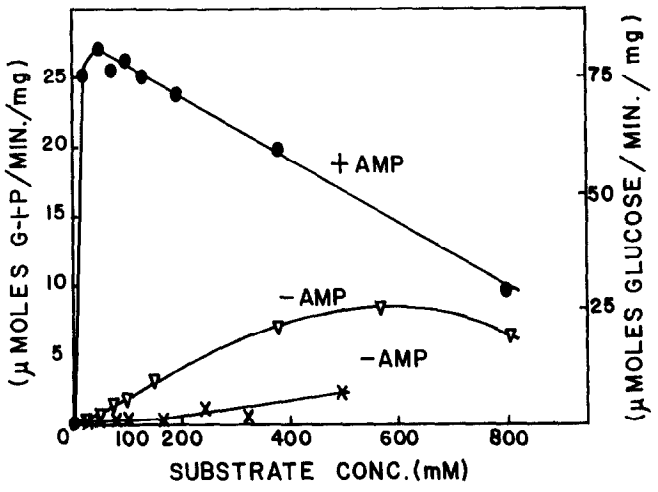

Figure 1. The effect of high concentrations of P_i and glucose-1-P, in the presence and absence of AMP, on the activity of phosphorylase b. i. Left hand scale: phosphorolysis with P_i as substrate; ● P_i plus AMP; ▽ , P_i minus AMP.
ii. Right hand scale: glycogen synthesis with glucose-1-P as substrate; X, glucose-1-P in the absence of AMP. V_{max} for glucose-1-P in the presence of AMP was 80 μMoles/min/mg.

540 mM glucose-1-P) is most likely due to an anionic effect of the very high organic phosphate concentration.

The effect of anions high on the Hofmeister or lyotropic series on the activity of phosphorylase b in the absence of AMP is shown in Fig. 2. The control value was that activity obtained with 24 mM P_i alone and amounted to 1.8% of that for 24 mM P_i plus AMP. A marked similarity exists between the results for these activating anions and the activation observed for P_i.

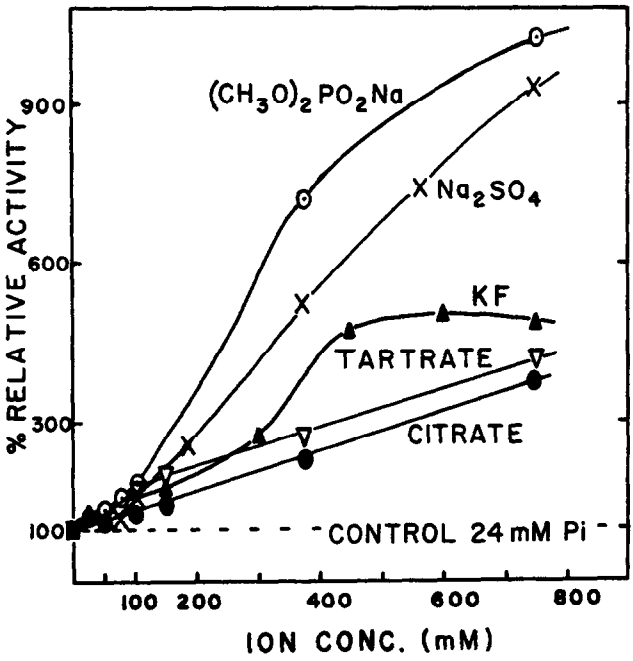

Figure 2. The effect of anions high in the Hofmeister series on the activity of phosphorylase b in the absence of AMP.

Fig. 3 shows that those anions low on the Hofmeister series produce the expected inhibitions. It is evident from the results with the halides that the nature of the effect is due primarily to the anion.

These effects of anions in the Hofmeister series on the activity of phosphorylase b, as studied in the absence of AMP,

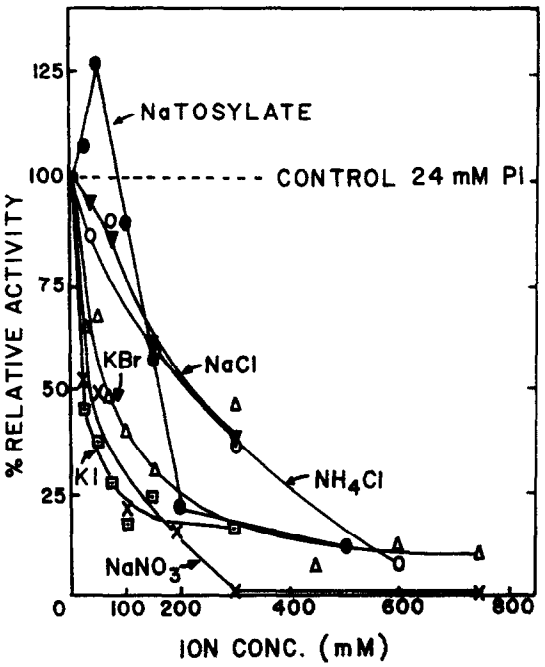

Figure 3. The effect of anions low in the Hofmeister series on the activity of phosphorylase b in the absence of AMP.

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are in direct qualitative agreement with those effects observed by Robinson and Jencks (1965) on the solubility of acetyltetraglycine ethyl ester, a synthetic peptide. As shown in the above data for phosphorylase b, those anions that activate are high on the series, while those that inhibit are low on the series, ie; those ions which tend to salt out proteins activate, while those tending to salt in proteins inhibit.

Studies published by several laboratories suggest that the model for allosteric enzymes proposed by Monod et al. (1965) may be applied to phosphorylase b. Of a few discrepancies so far noted, one of the most serious is the negligible activity in the absence of AMP (Madsen and Shechosky, 1967). The interesting observation by Buc (1967) that very high concentrations of P_i will activate the enzyme in the absence of AMP now

appears to be related to a non-specific phenomenon. That glucose-1-P, the substrate in the reverse direction, provides only a limited activation also supports our contention. As suggested by Avramovic and Madsen (1968), conformational states in addition to the R and T states originally proposed may have to be invoked. It is possible that the activating effect of the anions is mediated by causing a conformational change in the enzyme similar to that induced by AMP and that a detailed study of these interactions may aid in explaining the role of conformational changes as related to the allosteric control of enzymic activity.

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REFERENCES

Appleman, M. M., Krebs, E. G. and Fisher, E. H., Biochemistry 5, 2101, (1966).
Avramovic, O. and Madsen, N. B., J. Biol. Chem. 243, 1656, (1968).
Buc, H., Biochem. Biophys. Res. Comm. 28, 59 (1967).
Cowgill, R. W., J. Biol. Chem. 234, 3146, (1959).
Fisher, E. H. and Krebs, E. G., in Methods in Enzymology, Vol. 5, edited by S. P. Colowick and N. O. Kaplan, Academic Press, New York, p. 369 (1962).
Fiske, C. H. and Subbarow, Y., J. Biol. Chem. 66, 375 (1925).
Glaser, L. and Brown, D. H., J. Biol. Chem. 216, 67 (1955).
Helmreich, E. and Cori, C. F., Proc. Natl. Acad. Sci. U. S. 51, 131 (1964).
Hofmeister, F., Arch. Exptl. Pathol. Pharmakol. 24, 247 (1888).
Madsen, N. B. and Shechosky, S., J. Biol. Chem. 243, 3301 (1967).
Monod, J., Wyman, J. and Changeux, J. P., J. Mol. Biol. 12, 88 (1965).
Riley, G. A. and Haynes, R. C., J. Biol. Chem. 238, 1563, (1963).
Robinson, D. R. and Jencks, W. P., J. Amer. Chem. Soc. 87, 2470 (1965).
Sealock, R. W. and Graves, D. J., Biochemistry 6, 201 (1967).