THE ROLES OF GLUCOSE AND AMP IN REGULATING THE CONVERSION OF PHOSPHORYLASE *a* INTO PHOSPHORYLASE *b*

J. Martyn Bailey and W.J. Whelan

Department of Biochemistry, The George Washington University Medical Center, Washington, D.C. 20005

and

Department of Biochemistry, University of Miami School of Medicine, Miami, Florida 33152

Received November 12, 1971

SUMMARY: The ability of glucose to assist the action of phosphorylase *a* phosphatase on phosphorylase *a*, appears to be mediated through conformational changes in the phosphorylase. The system is an example of control of enzyme activity by regulation of the conformation of the substrate, and parallels the similar effect of AMP in inhibiting the dephosphorylation.

INTRODUCTION

Phosphorylase *a* phosphatase converts "active" phosphorylase *a* into the "inactive" *b* form by cleavage of a phosphate group from a serine residue. The best studied examples of these enzymes are from rabbit muscle (1). AMP inhibits this dephosphorylation, but does not affect the rate of dephosphorylation of a phosphorylated tetradecapeptide isolated from phosphorylase *a*. It was thereby concluded that the action of AMP is to alter the conformation of the substrate rather than to inhibit the enzyme (2).

Recently it was reported that glucose promotes the *a* → *b* conversion both of muscle (3) and liver (4) phosphorylases *a*. We have investigated whether this is due to an activation of the phosphatase, or rather, like the AMP effect, results from interaction of glucose with the substrate. Circumstantial evidence favored the latter possibility since AMP and glucose are known also to have opposing effects on the state of aggregation of phosphorylase *a*. AMP promotes tetramer formation while glucose favors the dimer (5).

Furthermore, the dimeric form is the exclusive variety at physiological temperature (5), at which temperature glucose appears to have no effect on phosphorylase inactivation (6). The positive effects of glucose were noted at temperatures below 30°. A final piece of evidence implicating the actions of glucose and AMP both being on phosphorylase is the observation that cleavage by trypsin or chymotrypsin is enhanced by glucose (7) and opposed by AMP (8).

This study confirms the inference that the effect of glucose in assisting dephosphorylation is indeed by interaction with phosphorylase. We have learned that in an independent study, Dr. H.G. Hers and his colleagues have come to a similar conclusion (9).

MATERIALS AND METHODS

^{32}P-Labelled phosphorylase *a* was prepared from 2 X crystallized rabbit muscle phosphorylase *a* by treatment with phosphorylase *b* kinase and γ-^{32}P-labelled ATP (10). Phosphorylase phosphatase activity was assayed by incubation of labelled phosphorylase (25µg) with phosphorylase phosphatase (24 mU) in 1 ml of 0.02M tris-glycerophosphate buffer, pH 8.16, containing 3mM magnesium acetate and 10mM mercaptoethanol. Samples (100 µl) were removed at intervals and dried on filter paper strips (1X3 cm). The liberated P_i was removed by washing in 50% aqueous ethanol, and ^{32}P-labelled protein remaining on the dried strips was determined by liquid scintillation counting. 1 mU of phosphatase is defined as that amount of enzyme which will convert 1 mU (approx 25 ng) of phosphorylase *a* into *b* per min.

Phosphorylase *a* phosphatase was purified from rat liver homogenates by NaCl gradient chromatography on DEAE-cellulose. The bulk of the enzyme was eluted in the range 0.28-0.32M salt, and the specific activity was 53 mU per mg protein. The enzyme was essentially free from non-specific phosphatase and protease activity when tested against phosphorylated histone, protamine and casein.

RESULTS

When the phosphatase acted on phosphorylase *a* at 15° the reaction was inhibited 90% by 0.1 mM AMP, while 16.7 mM glucose doubled the rate of

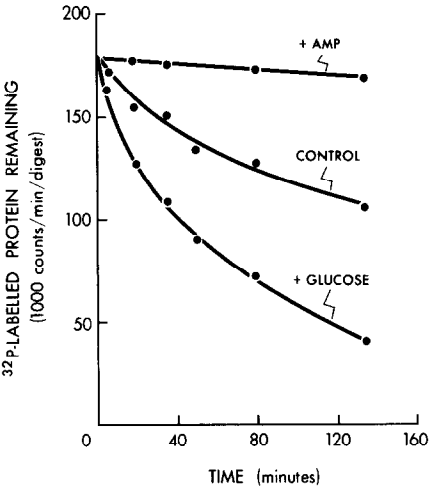

Fig. 1. Influence of glucose and AMP on the action of phosphorylase phosphatase on phosphorylase *a*. ^{32}P-Labelled phosphorylase *a* was incubated at 15° with phosphorylase phosphatase, with 16.7 mM glucose or 0.1 mM AMP added as indicated. Samples were removed at intervals for determination of the ^{32}P-labelled protein remaining (see Materials and Methods).

reaction (Fig. 1). Other sugars had little or no effect.

During the fractionation of phosphatase on DEAE-cellulose, the ratio of its activity in the presence and absence of glucose remained constant across the enzyme profile. It was unlikely therefore that the glucose effect was on a separate phosphatase-activating system.

When the phosphorylase *a* was freed completely from AMP by charcoal treatment, stimulation by glucose was maximal at 15-20° and absent at 37°. AMP still inhibited the enzyme at 37° and a glucose effect at 37° could be demonstrated from the fact that the sugar opposed the AMP inhibition (Fig. 2). Only at levels below 10 μM AMP, however, was glucose able completely to overcome the AMP inhibition.

Paralleling the effects of glucose and AMP on the rate of dephosphorylation were the ultracentrifugal observations that phosphorylase *a* is tetrameric at 15°, wholly dimeric at 35°, and dimer appears at 15° if

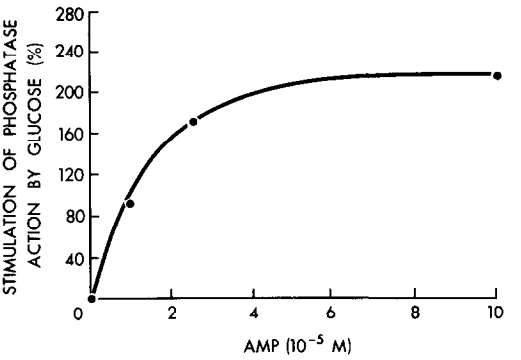

Fig. 2. Restoration of the glucose sensitivity of the dephosphorylation of phosphorylase *a* at 37° by addition of AMP. The activity of the phosphatase was measured in the presence and absence of 28 mM glucose, with increasing amounts of AMP. The ^{32}P-labelled phosphorylase had been treated with charcoal to remove AMP. See Materials and Methods for conditions.

Table 1. Influence of temperature and added glucose on the sedimentation behaviour of phosphorylase *a*

Temperature	Additions	Component(s) observed S_1 (dimer)	S_2 (tetramer)
15°	none	none	15.3
15°	glucose	9.8	14.7
23°	glucose	9.8	13.9
35°	none	9.0	none
35°	glucose	9.1	none
35°	glucose + AMP	9.5	none

The solvent was the phosphatase assay buffer (see Materials and Methods) plus 0.01 M NaCl, with 55 mM glucose and 1 mM AMP as noted. Sedimentation coefficients are corrected to 20° in water.

glucose is added (Fig. 3, Table 1). At 35°, with glucose and AMP present in concentrations at which AMP inhibition of dephosphorylation is manifested, the phosphorylase was wholly dimeric (Table 1).

^{32}P-Labelled peptides were prepared by brief treatment of labelled phosphorylase *a* with chymotrypsin or trypsin. Neither glucose nor

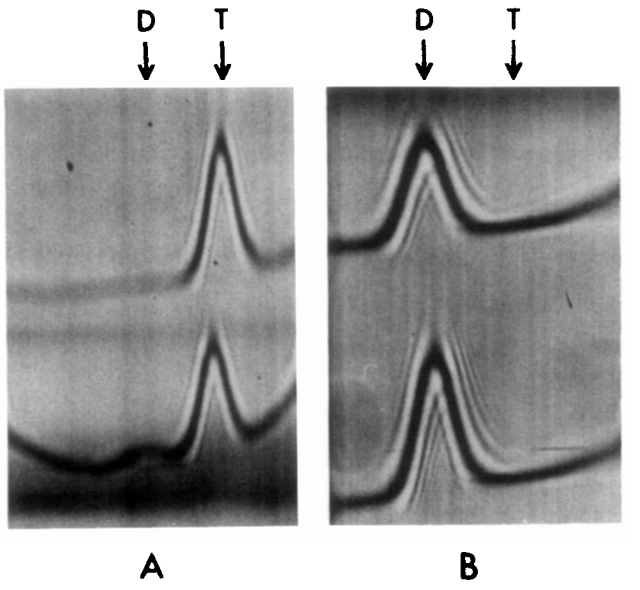

Fig. 3. Influence of temperature and added glucose on the sedimentation of phosphorylase *a* in the ultracentriguge. *A*, sedimentation of phosphorylase *a* at 15°. Upper curve, control. Lower curve, plus 55 mM glucose. Speed 60,000 rev./min. Time 18 min. Movement from left to right. D and T indicate the positions of the dimeric and tetrameric forms of phosphorylasee *a*. *B*, Sedimentation of phosphorylase *a* at 35°. Upper curve, phosphorylase *a*. Lower curve, phosphorylase *b* as reference. Note that phosphorylase *a* behaves as a tetramer at 15° but is completely dissociated to dimer at 35°. Addition of glucose at 15° causes the appearance of dimer (see also Table 1).

AMP affected their rates of dephosphorylation. This lack of effect of AMP is already known (2, 11).

DISCUSSION

The last-mentioned experiment confirmed our original inference that the effect of glucose on the dephosphorylation of phosphorylase, is, like that of AMP (2), on the substrate. Another inference, however, was not sustained. This was that the glucose effect was to produce dimer, a substrate, while the AMP effect was to produce tetramer, not a substrate. While the tetramer may very well not be a substrate, it cannot be the case that dimer *per se* is a substrate. This was shown from the fact that an opposition of the effects of glucose and AMP on dephosphorylation can be seen at 37° (Fig. 2), when no effect of glucose alone is noted, and when the ultracentrifuge shows only the dimeric form (Fig. 2, Table 1). We have to

conclude that there are two forms of the dimer, one resistant to phosphatase, and stabilized by AMP, and one susceptible, stabilized by glucose. At physiological temperature phosphorylase *a* is either all in the susceptible form, or else the equilibrium between the two forms is so rapid that any effect of glucose in promoting the change to the susceptible form is negligible. When, however, AMP is present at 37°, its stabilization of the resistant form permits a glucose effect to be demonstrated (Fig. 2). The opposing effects of these two metabolites point to a significant mechanism for the regulation of the relative levels of phosphorylases *a* and *b*, the regulation depending on conformational changes in the phosphorylase *a* substrate. We noted that at any given AMP level, the concentration of glucose producing half-maximal stimulation of the dephosphorylation reaction is in the physiological range for blood glucose (4-5 mM).

In molecular terms one can envisage glucose as reacting with phosphorylase *a* so as to expose the serine phosphate group, and AMP so as to shield it from phosphatase action. It is significant that this crude model also finds a parallel in explaining the effects of glucose and AMP in respectively enhancing and inhibiting the effects of trypsin, since the early products of trypsinolysis include phosphorylated peptides (8).

ACKNOWLEDGEMENTS

This work was supported by U.S. Public Health Service Grants AM 10082 and AM 12532, and was carried out while J.M. Bailey was on leave of absence at the University of Miami.

REFERENCES

1. Fischer, E.H., Pocker, A. and Saari, J.C., *Essays in Biochemistry* *6*:32 (1970).

2. Nolan, C., Novoa, W.B., Krebs, E.G. and Fischer, E.H., *Biochemistry* *3*:542 (1964).

3. Holmes, P.A. and Mansour, P.E., *Biochim. Biophys. Acta* *156*:266, 275 (1968).

4. Stalmans, W., De Wulf, H., Lederer, B. and Hers, H.G., *Europ. J. Biochem.* *15*:9 (1970).

5. Wang, J.M., Shonka, M.L. and Graves, D.J., *Biochem. Biophys. Res. Commun. 18*:131 (1965).

6. Torres, H.N. and Chelala, C.A., *Biochim. Biophys. Acta 198*:495 (1970).

7. Graves, A.J., Huang, C.Y. and Mann, S.A., in *Control of Glycogen Metabolism*, Ed. by W.J. Whelan, Academic Press, London, 1968, p. 35.

8. Fischer, E.H., Graves, D.J., Crittenden, E.R.S. and Krebs, E.G., *J. Biol. Chem. 234*:1698 (1959).

9. DeBarsy, T., Stalmans, W., Laloux, M., De Wulf, H. and Hers, H.G., *Biochem. Biophys. Res. Commun.*, in the press.

10. Krebs, E.G. in *Methods in Enzymology*, Ed. by S.P. Colowick and N.O. Kaplan, Academic Press, New York, *8*:543 (1966).

11. Martensen, T.M., Brotherton, J.E. and Graves, D.J., *Fed. Proc. 30*:1180 (1971).