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THE ROLE OF PHOSPHORYLASE IN THE INHIBITORY EFFECT OF EDTA AND ATP ON LIVER GLYCOGEN SYNTHASE PHOSPHATASE

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SUMMARY : The work of Gilboe and Nuttall on the inhibition of liver synthase phosphatase activity by EDTA (J. Biol. Chem., *253*, 4078-4081, 1978) and by ATP (Biochim. Biophys. Acta, *338*, 57-67, 1974) has been confirmed and extended. It appears that these inhibitory effects are not specific since they can be elicited by other polyvalent anions and that they are transient since they last only as long as phosphorylase *a* is present. The duration of these inhibitory effects can be shortened by the addition of glucose or caffeine which stimulate phosphorylase phosphatase activity. It is concluded that the inhibitory effects of EDTA and ATP are mediated by phosphorylase *a*.

Gilboe and Nuttall (1) have recently reported that the activity of glycogen synthase phosphatase in a glycogen pellet preparation obtained from the livers of normal fed rats is inhibited by EDTA. This effect was increased after *in vivo* administration of glucagon or cyclic AMP and reduced after glucose injection. From these observations, the authors have suggested the presence of rapidly interconvertible forms of either synthase phosphatase or its substrate synthase *b*, detectable as a change in EDTA inhibitability and subjected to glucose and to glucagon control. These same authors previously described an inhibition of glycogen synthase phosphatase activity in a glycogen pellet by ATP and its reversal by glucose (2).

Work from this laboratory has demonstrated that liver synthase phosphatase is strongly inhibited by phosphorylase *a* (3). This inhibition provides a precise explanation for the sequential inactivation of phosphorylase and activation of glycogen synthase initiated by glucose as observed in a cell-free extract (3), in isolated hepatocytes (4), and

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in vivo (5). The effect of glucose in a liver extract can also be mimicked by caffeine (6). Glucose and caffeine are similar in that they stimulate the activity of phosphorylase phosphatase and, therefore, cause a rapid disappearance of phosphorylase *a* (7) and the secondary activation of glycogen synthase (6). The inhibition of synthase phosphatase activity by phosphorylase is dependent upon the ionic composition of the incubation medium (8,9). Indeed, it was not observed in a glycogen pellet incubated in the presence of imidazole buffer, unless 5 mM-phosphate, sulfate or sulfite or 100 mM-chloride or acetate were added (9). It appears likely, therefore, that the effect of both EDTA and ATP observed by Gilboe and Nuttall (1,2) in a glycogen pellet, would be to provide the anionic charge required for the inhibitory effect of phosphorylase *a* on synthase phosphatase activity. In this view, the *in vivo* effect of glucagon, cyclic AMP and glucose would merely be to modulate the concentration of the inhibitor phosphorylase *a*. The present work was undertaken in order to check this interpretation.

MATERIALS AND METHODS

Particulate glycogen which was obtained from male, normally fed Wistar rats treated with glucagon, was washed and incubated following essentially the procedure of Gilboe and Nuttall (1). Other experimental procedures, including the filtration of a liver extract on Sephadex G-25, as well as the source of chemicals and reagents were those currently used in this laboratory (3-7).

RESULTS

In a Sephadex filtrate (fig. 1) and in a glycogen pellet preparation (fig. 1-3) incubated in the presence of 40 mM-imidazole, glycogen synthase was activated without latency. This activation was greatly inhibited by 5 mM-sulfate (fig. 1), EDTA (fig. 2), or ATP (fig. 3). Fig. 1 shows that the inhibitory effect of sulfate was, however, less complete in a glycogen pellet preparation than in a crude Sephadex filtrate. The inhibitory effect of sulfate or EDTA lasted 20 to 40 min and then was abruptly released. Both glucose and caffeine markedly decreased the duration of the inhibitory period,

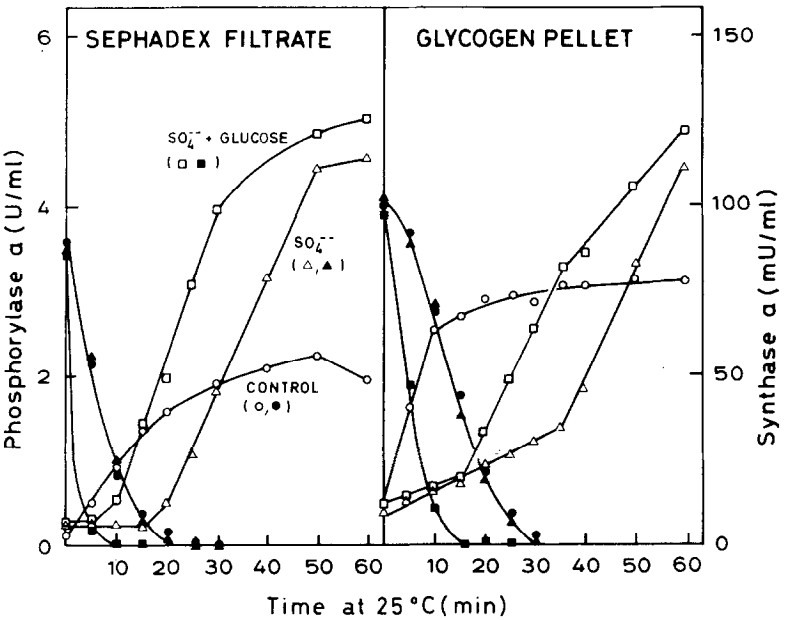

Figure 1. Inactivation of phosphorylase and activation of glycogen synthase in a Sephadex filtrate and in a glycogen pellet. The two preparations were obtained from the same liver. All incubations were performed at 25°C in the presence of 40 mM-imidazole, pH 7, and of various additions, as indicated and at the final concentration shown between parentheses : sulfate (5 mM), glucose (7.5 mM). Closed symbols : phosphorylase *a*; open symbols : synthase *a*.

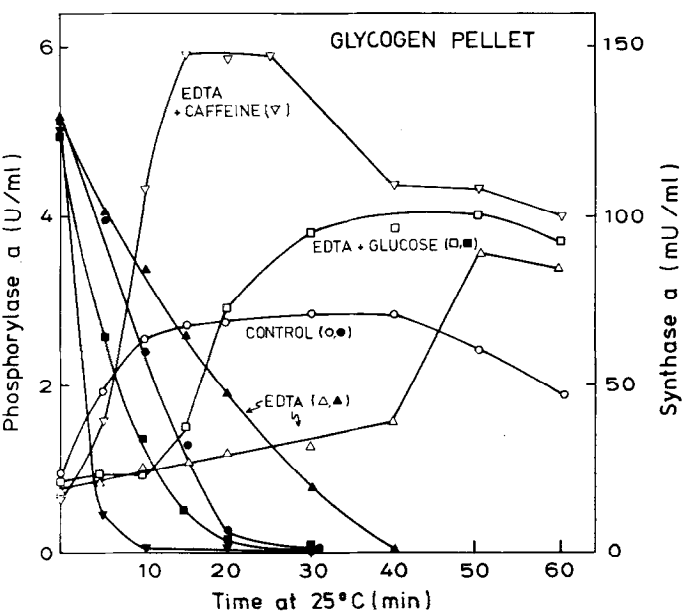

Figure 2. Inactivation of phosphorylase and activation of glycogen synthase in a glycogen pellet. Same procedure and symbols as in fig. 1. Additions are EDTA (5 mM), glucose (10 mM) and caffeine (1 mM).

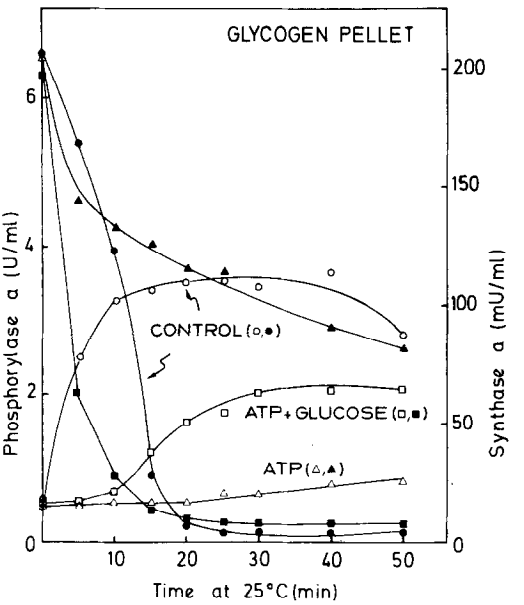

Figure 3. Inactivation of phosphorylase and activation of glycogen synthase in a glycogen pellet. Same procedure and symbols as in fig. 1. Additions are ATP (2.5 mM) and glucose (10 mM).

but they had no effect on the activity of synthase phosphatase itself, when released from inhibition. It is also apparent from fig. 1 and 2 that, when a Sephadex filtrate or a glycogen pellet preparation was incubated in the absence of sulfate or EDTA, the final activation of glycogen synthase was less complete than when the incubation was performed in the presence of these anions. This salt requirement for a complete activation of glycogen synthase has been previously reported and discussed by De Wulf *et al.* (6). Furthermore, synthase *a* was often unstable in the glycogen pellet preparation (fig. 2).

The activity of phosphorylase phosphatase was unaffected by sulfate but inhibited by EDTA; it was greatly stimulated by glucose and caffeine. In all conditions the more rapid disappearance of phosphorylase *a* was associated with a shortening of the latency in synthase activation.

The effect of ATP was similar to that of sulfate and EDTA with, however, some differences. In 5 experiments out of 6, ATP was an inhibitor of phosphorylase phosphatase activity and a complete inactivation of phosphoryl-

ase was obtained only in the presence of glucose (fig. 3) or caffeine (not shown). Furthermore, the activation of synthase was incomplete (fig. 3) even in the presence of sulfate (not shown). The mechanism of this latter effect has not been investigated.

DISCUSSION

The data presented in this paper are in general agreement with those published by Gilboe and Nuttall (1,2). Indeed, EDTA and ATP caused an inhibition of synthase phosphatase activity in a glycogen pellet incubated in the presence of an imidazole buffer. In contrast, however, with the data of Gilboe and Nuttall (1,2), we have observed an inhibition of phosphorylase phosphatase activity by both EDTA and ATP. The reason for this discrepancy is presumably methodological (10).

The important points which we wish to emphasize is that the property of EDTA and ATP to inhibit synthase phosphatase activity is not specific and that it is transient and dependent on the presence of phosphorylase a. The lack of specificity is illustrated by the fact that a similar inhibition is obtained in the presence of inorganic sulfate or phosphate and also of a series of other anions, among them the polyvalent anions being much more efficient than the monovalent ones (8,9). This inhibitory effect appears, therefore, to be related to the anionic charge. The suppression of the effect by magnesium (1), which is known to chelate both EDTA and ATP, is in general agreement with this interpretation. The fact that the inhibition by EDTA is transient was not clearly apparent in the experiments of Gilboe and Nuttall (1) since their experiments lasted only 15 minutes : it is only when glucose was administered to rats before sacrifice that a release of the inhibition could be observed within a few minutes (fig. 1.C in ref. 1). Our data indicate that in the other experimental conditions also, the effect of EDTA, ATP or sulfate is only to introduce a lag in the activation of glycogen synthase. The duration of this lag is related to the presence of phosphoryl-

ase *a* and can be modulated by the addition to the incubation medium of glucose or of caffeine which are known to stimulate the activity of phosphorylase phosphatase (7) and not to affect the activity of synthase phosphatase (6). This type of experiments leaves little doubt concerning the relationship that exists between the inhibitory properties of EDTA and ATP and the presence of phosphorylase *a*. The effect of glucose observed *in vitro* is not specific, since it is similar to that of caffeine; furthermore, it is identical to the one observed *in vivo* (5). It is, indeed, apparent from the work of Gilboe and Nuttall (1) that the effect of the pretreatment with glucose is to bring about an earlier disappearance of phosphorylase *a* (fig. 4 in ref. 1) and to shorten the inhibitory effect of EDTA although not to cancel it. It is finally important to recall that to be able to see the relationship that exists between the presence of phosphorylase *a* and the latency in synthase phosphatase activation, it is necessary to use an assay procedure that allows the determination of phosphorylase *a* without interference from phosphorylase *b* (10).

In conclusion it appears that the sequential inactivation of phosphorylase and activation of glycogen synthase, previously described in the liver *in vivo* (5), in isolated hepatocytes (4) and in a crude liver extract (3), has now been observed in the partially purified system composed of enzymes associated with particulate glycogen. This observation is a further confirmation of the primary role of phosphorylase *a* in the control of synthase phosphatase activity. An extensive description and discussion of this regulatory mechanism has been published elsewhere (11,12).

ACKNOWLEDGEMENTS

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