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THE EFFECT OF DIABETES, INSULIN, MAGNESIUM IONS, AND ATP ON THE MULTIPLE FORMS OF PHOSPHOGLUCOMUTASE FROM RAT ADIPOSE TISSUE

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Summary

Three electrophoretically distinct forms of phosphoglucomutase (α-D-glucose-1,6-diphosphate:α-D-glucose-1-phosphate phosphotransferase, EC 2.7.5.1) were found in rat adipose tissue. The most anodal form, in contrast to the other forms, did not require added Mg^{2+} for maximal activity. All three forms were activated by imidazole. Only those forms which were activated by added Mg^{2+}, were reduced in diabetes and increased by insulin. ATP was a potent inhibitor of all three forms. This inhibition was pH dependent.

Phosphoglucomutase (α-D-glucose-1,6-diphosphate:α-D-glucose-1-phosphate phosphotransferase, EC 2.7.5.1), catalyzes the interconversion of glucose 1-phosphate and glucose 6-phosphate. Glucose 6-phosphate lies at the crossroads of glucose metabolism. Our interest in the regulation of glycolysis [1, 2] and of isoenzymes from the hexose—monophosphate shunt [3—6] has led us to examine the response of the multiple forms of this enzyme from rat adipose tissue, to various regulatory conditions.

Epididymal fat pads from male albino rats (120—150g) were homogenized in cold distilled water (0.1 ml of water per g of pooled fat pads from 4 rats) for 10 s in an ice bath using a Willems-Polytron-Homogenizer (Model PT 10 ST "OD", Kinematica) at a setting of 5. The homogenate was centrifuged at 4°C for 35 min at 15 000 × *g*. Aliquots of 1.25 μl (0.020—0.040 mg protein) of the clear supernatant were placed on cellogel strips using the Beckman microzone electrophoresis cell (Model R-101). Conditions for electrophoresis and color development are given in the figure legends. Controls were run simultaneously in which the substrate, coenzyme or the auxiliary enzyme was omitted. Another part of the clear supernatant solution was diluted with an equal volume

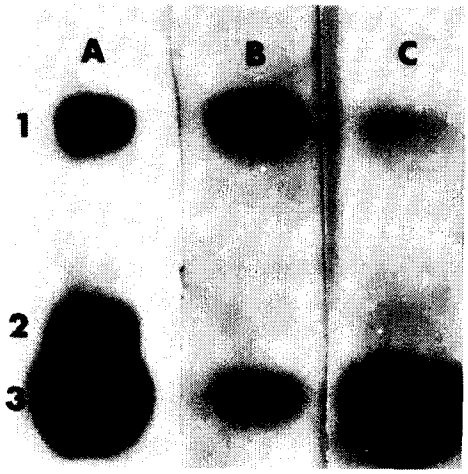

Fig. 1. The effect of Mg^{2+} and imidazole on the electrophoretically distinct phosphoglucomutases from rat adipose tissue. A, with Mg^{2+} and imidazole; B, without Mg^{2+}; C, without imidazole. Electrophoresis on cellogels strips at 250 V for 1 h in $7 \cdot 10^{-4}$ M citric acid and $43 \cdot 10^{-4}$ M Na_2HPO_4 (pH 7.0). After electrophoresis the cellogels were cut into three parts. One part (A) was developed for phosphoglucomutase activity at 37 °C for 30 min in a reaction mixture consisting of 40 mM imidazole—HCl (pH 7.5), 3 mM $MgCl_2$, 1.5 mM EDTA, 50 mM Tris (pH 7.5), 2 mM glucose 1-phosphate, 10 μM glucose 1,6-diphosphate, 3 units $\cdot$ ml^{-1} glucose-6-phosphate dehydrogenase (Sigma), 0.5 mM $NADP^+$, 0.1 mM phenazine methosulfate and 0.4 mM nitro-blue tetrazolium. The other two parts were developed in an identical medium, except for absence of $MgCl_2$ (B), or absence of imidazole (C). 20 experiments were carried out with identical results.

of cold distilled water and 10-μl aliquots (0.075—0.150 mg protein) were used for measurements of total enzymatic activity by the spectrophotometric method used by Dawson and Mitchell [7]. It should be noted that the activity of the auxiliary enzyme (glucose-6-phosphate dehydrogenase), which was added in excess, was not affected by the different conditions investigated in the present study. Protein was estimated by the method of Lowry et al. [8].

Three electrophoretically distinct forms of phosphoglucomutase were found in rat adipose tissue (Fig. 1A). They were designated as 1—3 (1, being the fastest migrating anodal band). The slowest migrating enzyme, was the largest in amount, similarly to the findings in some other tissues [7, 9]. In these experiments Mg^{2+} and imidazole, which are known as activators of the enzyme [10], were present in the reaction mixture. When Mg^{2+} was omitted from the reaction mixture (Fig. 1B), forms 2 and 3 almost completely disappeared, whereas the activity of form 1 was unchanged. The activity of this form could not be due to residual traces of Mg^{2+} in the system, since it did not change even when EDTA was added in concentrations ten to twenty times higher (forms 2 and 3 completely disappeared under these conditions). When imidazole was omitted from the reaction mixture (Fig. 1C), there was a reduction in the activity of all three forms. These experiments reveal that forms 2 and 3 are strongly activated by added Mg^{2+} for maximal activity. All three forms are activated by imidazole.

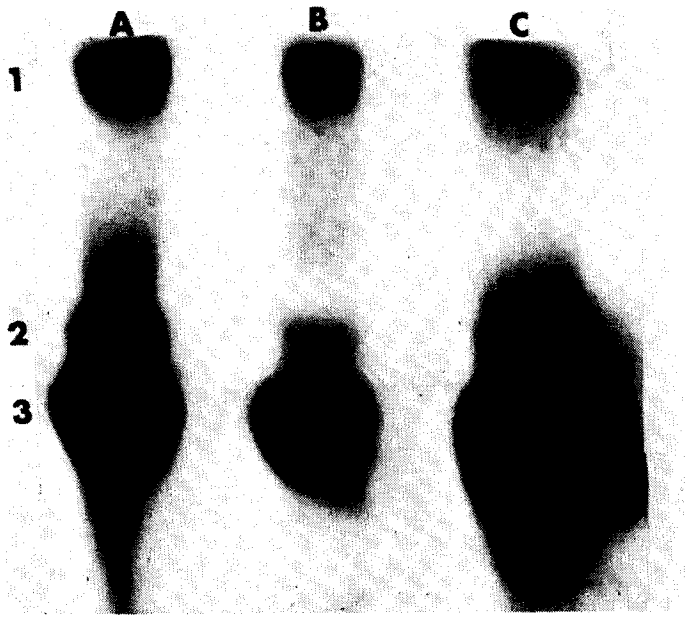

Fig. 2. The effect of alloxan diabetes and insulin on the different forms of phosphoglucomutase from rat adipose tissue. A, adipose tissue from normal rats; B, adipose tissue from diabetic rats. Diabetes was induced in rats starved for 24 h, by injection of alloxan monohydrate (190 $mg \cdot kg^{-1}$ rat, subcutaneously). The animals were allowed unlimited access to food until being killed (after 48 h). C, adipose tissue from diabetic rats treated with insulin (protamine zinc insulin, 4 units $\cdot$ 100 g^{-1} rat $\cdot$ day^{-1}, given subcutaneously in 2 divided doses each day for 48 h). Electrophoresis and color development were conducted as for Fig. 1A. 20 experiments were carried out with identical results.

Cell fractionation, by a method previously described [4] showed that all the three forms of phosphoglucomutase are present exclusively in the cytoplasm.

The activity of phosphoglucomutase in muscle and liver was reported to be increased by administration of insulin [11—13]. Since selective changes in the different enzyme forms cannot be detected in measurements of total enzymatic activity, we have undertaken an investigation of the effect of alloxan diabetes and insulin on the different forms of phosphoglucomutase from the rat adipose tissue. Fig. 2 reveals that the activities of forms 2 and 3 were reduced in diabetes and increased above normal by insulin treatment. The activity of form 1, however, was relatively unaffected by these conditions. Spectrophotometric measurements revealed that the total activity of the adipose tissue phosphoglucomutase declined to 67 ± 3% of the normal values, as a result of diabetes, and increased to 127 ± 6% of the normal values, following insulin treatment (values are means ± S.E. for 11 experiments, $P < 0.001$).

It is interesting that only those forms of phosphoglucomutase which

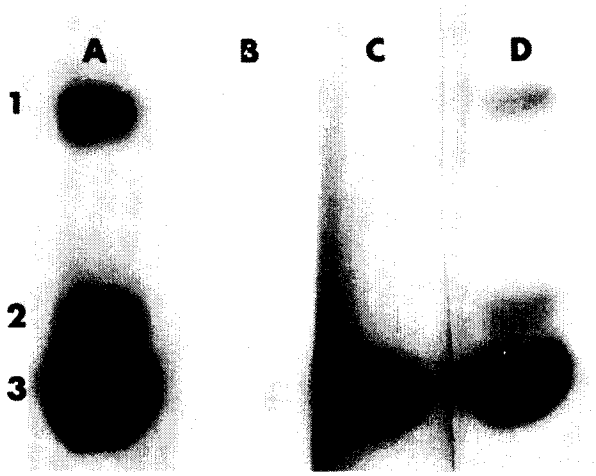

Fig. 3. The effect of ATP on the different forms of phosphoglucomutase from rat adipose tissue. A, without ATP; B, with 10 mM ATP; C, with 5 mM ATP; D, with 1 mM ATP. Electrophoresis was conducted as for Fig. 1 after which the cellogels were cut into four parts Color development of each part was carried out as described in Fig. 1A, in the absence (A) and presence (B—D) of the nucleotide at different concentrations. 20 experiments were carried out with identical results.

require added Mg^{2+} for maximal activity (forms 2 and 3, see Fig. 1), are affected by diabetes and insulin. It is possible that these effects may result from changes in magnesium metabolism [14, 15]. We have observed earlier a similar behaviour of the isoenzymes of glucose-6-phosphate dehydrogenase from the rat adipose tissue; here also only those isoenzymes which were activated by Mg^{2+} [4], were affected by diabetes and insulin [3].

The total activity of phosphoglucomutase from rabbit muscle and *Escherichia coli* was reported to be inhibited by several nucleotides [16]. Considering the important role of adenine nucleotides in the control of carbohydrate metabolism, we have undertaken an investigation of their effects on the different forms of phosphoglucomutase from the rat adipose tissue. As shown in Fig. 3, ATP strongly inhibited the activities of all three phosphoglucomutases. The degree of inhibition was dependent on the concentration of ATP. Other adenine nucleotides, viz. ADP, AMP and adenosine cyclic 3′:5′-monophosphate, had no significant effect when added at physiological concentrations. The inhibition of the enzyme by ATP could be reversed by an increase of the glucose 1,6-diphosphate concentration. We also found that the inhibition by ATP was pH dependent; the enzyme was more sensitive to ATP inhibition at pH 7.0 than at pH 7.5. Therefore phosphoglucomutase behaves similarly to phosphofructokinase. Thus at pH 7.5, 5 mM ATP caused a 55 ± 5% inhibition of the total enzymatic activity, whereas at pH 7.0, 5 mM ATP caused a 89 ± 9% inhibition (the values are means ± S.E. for 10 experiments, $P < 0.001$).

The strong inhibitory effect of ATP, when considered in connection with its concentrations in tissues, suggests that this nucleotide may play a physiological role in controlling the activity of phosphoglucomutase in vivo. Such control may complement phosphofructokinase regulation. The inhibitory effect of ATP on phosphofructokinase of itself may be inadequate since phosphoglucomutase reactions, which are regarded as the principle source of glucose 1,6-diphosphate in cells [17, 18], may provide enough glucose 1,6-diphosphate to reactivate phosphofructokinase. It is known that glucose 1,6-diphosphate is one of the strongest activators and/or deinhibitors of phosphofructokinase [19, 20]. Thus the additional block induced by ATP on the phosphoglucomutase activities, may prevent this reactivation. A similar complementarity by ATP was observed in our earlier studies [21] between aldolase and phosphofructokinase.

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