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Rat Kidney UDPG: α-1,4-glucan α-4-glucosyltransferase *

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The enzyme systems responsible for the biosynthesis of glycogen (UDPG: α-1,4-glucan α-4-glucosyltransferase) can be extracted mainly as a D form (dependent on glucose-6-P for activity). Because of the low glycogen content of this tissue, the enzyme becomes soluble after centrifugation at 100,000 × g for 90-120 minutes. The enzyme can be collected as a particulate fraction if high molecular weight glycogen is added to the soluble fraction, and then centrifuged again for 90 minutes at 100,000 × g.

Both D and the very low I activities increase by preincubation of crude extracts in the presence of mercaptoethanol. D activity rises quickly and stabilizes after the first 15 minutes. I activity increases slowly and during longer periods but a complete D to I transformation is not obtained although the ratio of activities + glucose-6-P/-glucose-6-P decreases to some extent indicating some D to I form transformation.

Increases of both the D and I activities can be prevented by addition of 50 mM NaF to the extracts, indicating that these reactions are brought about by phosphoprotein-phosphatases.

Mg ions do not stimulate the D activity or its increase by preincubation. In fact they prevent this increase and even induce an inactivation of this form during time of preincubation. I activity is slightly stimulated by this cation at 0 time of preincubation but the rate of increase of this activity during preincubation does not seem to be stimulated, or perhaps it is very little.

Addition of ATP an Mg inactivates both I and D activities in a time and concentration of ATP dependent reaction and this reaction reverses with time if the phosphatase is not inhibited by the presence of NaF. The inactivating reactions induced by the addition of ATP and Mg can be stopped by a further addition of EDTA strong enough to trap the necessary Mg^{2+}. Then the recovery of both D and I activities can be observed in those preparations whose phosphatases were not inhibited by the presence of NaF. In those which were inhibited the addition of EDTA only produces the arrest of the inactivating reaction, but the recovery of the D or I activity can no longer be seen.

All these experimental data suggest that the D and I forms of glycogen synthetase extracted from rat kidney can be interconverted in part but also they are able to be transformed into inactive forms, more phosphorylated, that can be reconverted into active by dephosphorylations carried out by phosphatases.

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Species variations have been found on glycogen synthetase (UDPG: α-1,4-glucan α-4-glucosyltransferase; E.C. 2.4.1.11, synthetase or transferase) (11) from muscle and also there are differences in the glycogen synthetase systems of muscle or liver in the rat (8, 13), or polymorphonuclear leucocytes (3), lymphocytes (6) or pletelets (1) of humans.

It is known that the kidney has a low glycogen content and also that its phosphorylase systems have special characteristics (16), therefore it seemed of interest to study the glycogen synthetase systems in this organ well known as a gluconeogenic tissue.

The present paper will show that rat kidney glycogen synthetase, when extracted from this organ, in spite of its low glycogen content, is mainly a D form with a specific activity lower than that of skeletal muscle or cardiac muscle.

There are definite differences with respect to the systems in muscle either skeletal or cardiac. There is very little or no stimulation of Mg ions in the I or D activities. The D to I transformation that can be observed *in vitro* is very slow and is never complete. If the transformation reaction is carried out in the presence of Mg^{2+}, the rate of the D to I transformation is not affected and the rise of D activity by preincubation in mercaptoethanol is prevented by the presence of the cation.

The I to D interconversion by the addition of ATP and Mg can be obtained but a part of the D activity is also lost during the process although with time, part of the D activity can be recovered. The inactivating reaction can be stopped by addition of EDTA and a recovery of activity is immediately observed unless inhibition of phosphoprotein-phosphatases is produced by the presence of NaF.

This data seem to indicate that in kidney also (as in cardiac muscle), inactive forms of glycogen synthetase can be obtained by extraphosphorylation produced by kinases, which can be reconverted into active forms by the action of phosphatases. Some of these data have been already partially reported (7).

Materials and Methods

Enzyme kidney preparations were obtained with a similar procedure to that of muscle preparations. White rats from a local strain, fed ad libitum and weighing between 150 and 200 grams were killed by a blow in the neck. The kidneys from 2 or 3 animals were removed blotted on filter paper, washed in cold saline chilled on ice, and kept on prechilled 50 mM Tris-5 mM EDTA buffer *p*H 8.0 until homogenization.

Kidney homogenates were done in a motor driven, teflon pestle homogenizer chilled with ice in 1-2 minutes intervals with the above-mentioned buffer in relation 1/10, w/v. The homogenates were clarified by discarding the cellular debris by a 10 minutes centrifugation on a clinical refrigerated centrifuge at approximately 3,000 × g. The remaining supernatants were filtered thorough a thick glass-wool layer to eliminate fat and were the enzyme sources in many experiments and will be referred to as crude extracts.

Other extracts were obtained by a second centrifugation of 30 minutes at 8,000-10,000 × g of the above supernatants. Finally to obtain the enzyme in its particulate more purified form it was necessary to centrifuge twice at 100,000 × g during 60-90 minutes. The first time all the enzyme activity remained in the 100,000 × g supernatant. The second time in order to sedimentate the enzyme, high molecular glycogen (5-10 mg/ml) was added to the first 100,000 × g supernatant and then centrifuged again at 100,000 × g for 90 minutes. The glycogen pellet thus obtained had the majority of activity when resuspended in the Tris-EDTA buffer. More details are given in the results.

Glycogen synthetase enzyme activity was measured as radiactivity incorporated into glycogen from C^{14} glucose labeled UDPG as has been described (15). A solid sample gas flow counter (Nuclear Chicago) was utilized (25% erficiency) and UDPG with a specific activity of 6,000-8,000 cpm/μM was used. Test mixtures for the D and I forms of glycogen synthetase were standard as described previously (15). Additions of other chemicals and modifications are described afterwards. Incubations for enzyme activity and preincubations of the extracts were done at 30° C.

C^{14} uniformly labeled glucose UDPG was obtained from New England Nuclear Corp. USA. Cold UDPG, ATP, UTP, 3',5'-AMP, glucose-6-P and glycogen were purchased from Sigma Chemical Company. Other Chemicals were analytical grade from Merck.

Results

Activity of the extracts and its increase by preincubation. The glycogen synthetase activity obtained from rat kidney as described in these methods is mainly in its D form, that is to say almost totally dependent on added glucose-6-P for activity. In these conditions the ratio of activities + glucose 6-P/-glucose-6-P is 6 or more if no other additions are made to the enzyme extracts.

The activity of these extracts is much lower than similar extracts form rat skeletal or cardiac muscles. Activities in the presence of 10 mM glucose-6-P ranged between 23 to 35 μM per hour per gram of wet weight and the specific activity between 1.6 to 2.7 μM/mg. P/hr, values which are 5 to 10 times lower than those of skeletal or cardiac muscles. The low I activity detected is frequently about 1/10 of that described for the total.

If the crude extracts are added with 50 mM mercaptoethanol and preincubated at 30° C during some time there is a double rise in both D (measured with added glucose-6-P) and I (measured without) activities which is quite different in both of them. D activity risses sharply and more quickly than I activity, and afterwards stabilizes, as depicted in figures 1 and 2. The low I activity also rises, although, in general, more slowly and during a longer period than the D and the net result, after 60 or 70 minutes of preincubation, is that the ratio of activities + glucose-6-P/-glucose-6-P had changed from about 10 to 1.6-2 (see also figures 5 and 6).

This different rise of both activities during preincubation can be prevented by the addition to the extracting buffers of 50 mM NaF as figure 2 shows. The ex-

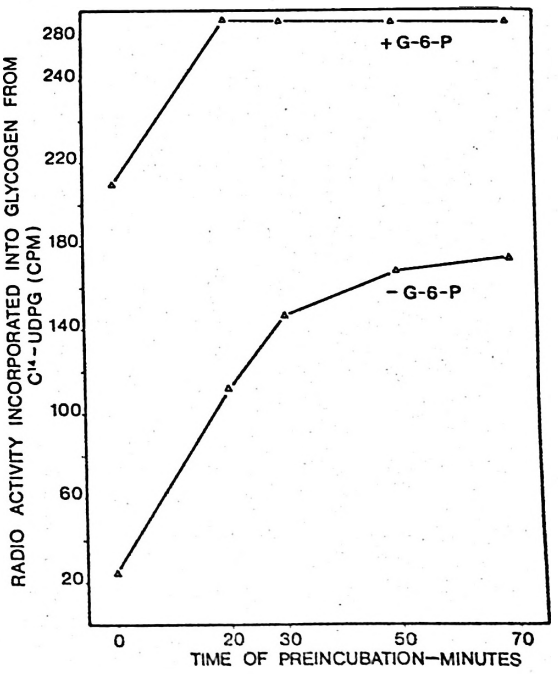

Fig. 1. *Effect of preincubation at 30° C in the presence of mercaptoethanol on the glycogen synthetase activities from rat Kidney.* Crude extracts (1/10, w/v) were done 50 mM with mercapthoethanol and activity measured at 0 time of preincubation and then tested at some intervals in the presence or absence of 10 mM G-6-P.

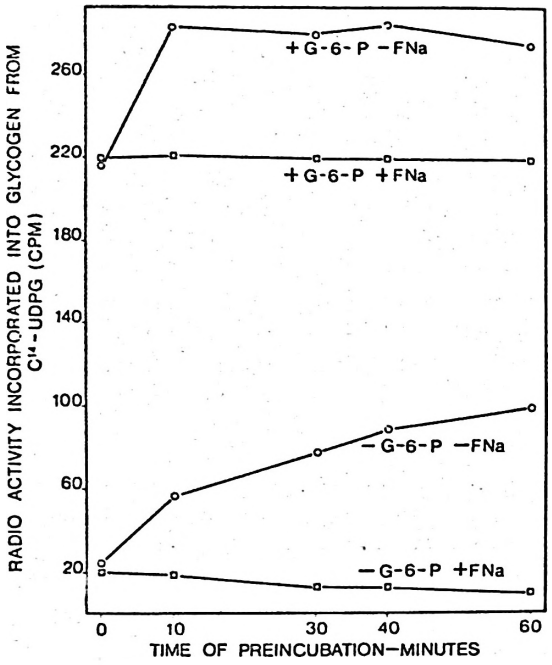

Fig. 2. *Effect of NaF on the increases of the D and I activities during preincubation.* Crude extracts as in figure 1 were done but some aliquot parts had 50 mM NaF. There is no increase in any of the activities measured with or without 10 mM G-6-P, in those extracts with NaF.

tracts that had NaF do not increase activity at all.

The effect of Mg ions on kidney glycogen synthetase activity. Mg ions affect glycogen synthetase activity from different sources in more than one way (2). In rat kidney preparations the effects are different to those found in other enzyme preparations. Figure 3 shows the action of Mg^{2+} on kidney glycogen synthetase activity and stability as well as on the stimulation of activity by preincubation. It can be seen that the D activity is not stimulated at all by any concentration of $MgCl_2$ tested, at 0 time of preincubation, while the I activity is clearly stimulated by the increasing concentrations of the cation. During the preincubation time the presence of Mg^{2+} prevents (depending on its concentration) or even inactivates the total activity, whereas the rate of the rise of I activity is very little or not affected. In the presence of high concentrations of Mg^{2+} then it seems that the conversion D to I can be complete in about 60 minutes, mostly because D activity does not rise at all or even diminishes.

The inactivation in the presence of Mg^{2+} of the D activity is very drastic in the glycogen particulate fraction, as will be seen later. It seems also that it is produced by some kind of binding of Mg^{2+} to the enzy-

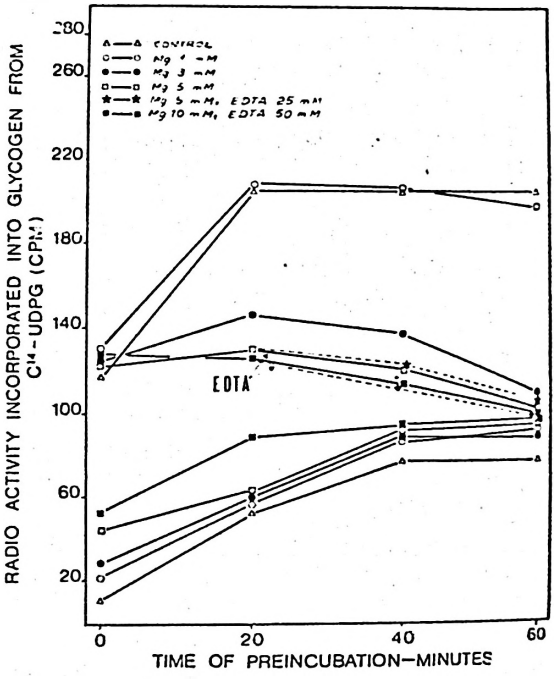

Fig. 3. *Effect of the presence of $MgCl_2$ on the D and I activities during preincubation.* Conditions of extracts as in figure 1 but then different aliquot parts were added of $MgCl_2$ tested at 0 time, preincubated and tested at intervals. Upper lines represent activity measured with 10 mM glucose-6-P and lower lines without the suggar phosphate. Different points mean: △ controls with no $MgCl_2$. All the others had $MgCl_2$: ○, 1 mM; ●, 3 mM; □, 5 mM; ■, 10 mM. Broken lines are activities obtained after addition at 20 minutes of preincubation of ★, 25 mM EDTA and ◆, 50 mM EDTA.

me molecule that may alter its tridimensional structure. The Mg^{2+} must be tightly bound as the addition of strong concentrations of EDTA, once the inactivation begins, is not able to reverse it (Figures 3 and 4).

Fractionation of the enzyme systems and purification. Kidney glycogen synthetase does not sediment ar a 100,000 × g for 90-120 minutes. Figure 4 shows that all the activity measured in the crude extracts can be recovered in the 100,000 × g supernatants after 90 minutes of centrifugation, and that the particulate fraction is devoid of activity.

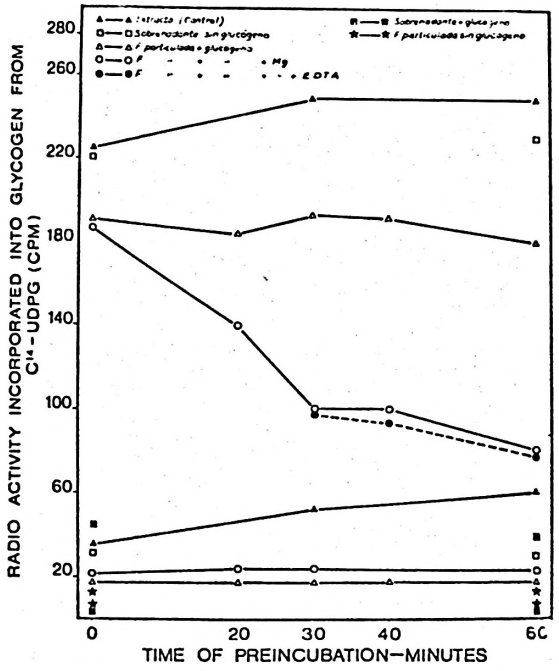

Fig. 4. *Localization of enzyme activities and fractionation by ultracentrifugation.*
The kidney glycogen synthetase activity remained in the 100,000 × g supernatant after 90 minutes of centrifugation □, compared with crude extracts ▲. With added glycogen (1 mg/ml) most of the activity was recovered in the glycogen pellet, △. The same fraction in the presence of 10 mM $MgCl_2$ is inactivated with time, ○. The addition of 25 mM EDTA after some time does not prevent or reverse the inactivation ●. Upper lines indicate activity with 10 mM glucose-6-P, and lower lines without it. ★ indicates the inexistent activity glycogen added.

However the enzyme can be obtained in a particulate form if glycogen is added to the above supernatants and centrifuged again during 90 minutes at 100,000 × g. Figure 4, which was obtained after the addition of 1 mg of glycogen per ml of supernatant, also shows this. As can be seen some activity still remained in the second 100,000 × g supernatant. All the activity could be recovered in the particulate fraction increasing the quantity of glycogen added up to 5-7 mg/ml, or using the same quantity but with glycogen of high molecular weight. It can also be observed that with this treatment the properties of increasing the D or I activities by preincubation in mercapthoethanol have been lost. However they can be recovered if the particulate fraction is resuspended in its own supernatant which has been kept cool at 3-4° C with 50 mM mercaptoethanol, instead of using new Tris-EDTA-mercaptoethanol buffer.

This seems to indicate that the reaction which produces the increases in both activities is enzymatic in nature and that these enzymes remain in the 100,000 × g supernatant. As the reaction is prevented by the presence of NaF, they must be phosphoprotein-phosphatases. As we will see later the inactivating kinases go down with the glycogen particulate fraction.

Figure 4 also shows that the purified glycogen enzyme fraction is very sensitive to the presence of Mg ions. While the very poor I activity is very slightly stimulated and does not show any increase at all by preincubation, the D activity (practically the only one present) is drastically inactivated during the time of preincubation. Again, the addition of EDTA (25 mM), which would be enough to trap the Mg^{2+} present (10 mM), is not able to stop or reverse the inactivation when added some time after this has already

begun. This indicates again that the modification that Mg^{2+} induces on the enzyme protein seems to be irreversible and involves some kind of strong binding of the cation by the enzyme.

The procedure of double centrifugation to obtain the glycogen enzyme fraction gives a fairly good purification. If after the 10 minutes centrifugation at 3,000 per g, we introduce a second centrifugation at 8,000-10,000 × g for 15-20 minutes, and then the two 100,000 × g centrifugations, the enzyme D-form increases its specific activity about 80-100 times with respect to the crude extract. However this glycogen enzyme preparation is contaminated by phosphorylase, the glycogen synthetase kinase, debranching enzyme and some α amylase.

Starting with the first 100,000 × g supernatant (6-10 fold purification) a purified fraction can be obtained, almost without phosphorylase, by DEEA cellulose column chromatography and elution with NaCl 0.32 M after previous elution with 0.20 M. In the two pooled 3 ml peak fractions about 50-fold purification can be obtained but the recovery is poor (14-17 %) whereas by ultracentrifugation the recovery is 74-80 %.

Kinetics of the kidney glycogen synthetase. With the crude extracts and some of the purified preparations some kinetic determinations on the Km for UDPG and Ka for glucose-6-P were done.

Because of the lack of total D to I transformation the kinetics of the I form have not be analized. The Km for UDPG of the D form in the presence of Mg ions is of the range of 2×10^{-4} M. Without Mg^{2+} there is a double saturating curve corresponding to two straight lines in the reciprocal plot. This shape has been seen in other kynetic analyses (1, 11, 13).

The values obtained for the Ka of glucose-6-P have oscillated in the range of $1\text{-}1.8 \times 10^{-4}$ and here Mg^{2+} does not seem to have too much influence.

Action of ATP and Mg on kidney glycogen synthetase. The effects observed after ATP-Mg addition to the crude extracts depend on several circumstances but they are mainly influenced by the concentration of ATP used and the previous preincubation of the extracts with the appearance of I activity.

When the addition is done on extracts preincubated for some time and therefore with some I activity, different kinds of transformation can be seen, depending on the concentration of ATP used. For instance, using concentrations between the range 0.5-1 mM of ATP and Mg^{2+} 10 mM, what normally is observed is an I to D type of transformation although frequently some losses in the D activity are also

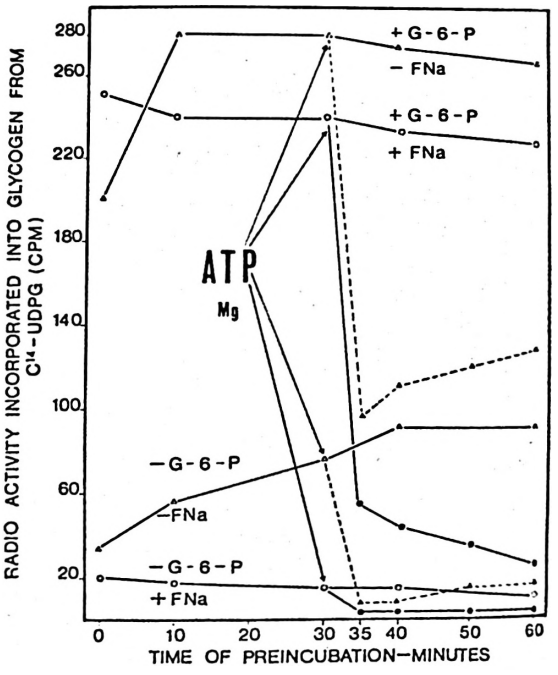

Fig. 5. *Effect of ATP and Mg addition on the activities of rat Kidney glycogen synthetase.* To parallel extracts obtained with or without of the 100,000 × g particulate fraction without 50 mM NaF, ATP-Mg (1-8 mM respectively) were added after 30 minutes of preincubation, and activities of control and treated extracts tested at various intervals of time.

observed. Frequently the I activity also begins to recover after the first 5-10 minutes of the addition.

If concentrations of ATP in the range of 5 mM are used (Mg^{2+} 10 mM) a decrease in D activity much greater than the I (which disappears) can be observed, but a recovery of this activity greater than the I is also observed. This is depicted in figure 5.

Higher concentrations of ATP, such as 10 mM, inactivates the total activity as well as the I (Mg^{2+} 10 mM) without any posterior reactivation.

Figure 5 also shows what happened to an aliquot part of the same extract that had 50 mM NaF previously added. The very low I activity never increased and the addition of similar concentrations of ATP-Mg (5-10 mM respectively) produced an inactivation of the D activity in a time dependent reaction that did not recover at all during 30 minutes.

In these preparations with NaF (practically no I activity) the D activity is inactivated by reactions driven by the addition of ATP and Mg^{2+} which depende both on time and concentration of ATP.

This inactivation of the D activity can equally be obtained if we add the different concentrations of ATP and Mg^{2+} at the beginning of the preincubation time (we have mentioned that the normal extracts have very low I activity).

This inactivation is not produced either by the ATP or Mg^{2+} alone as additions of both separately did not induced the reactions. These reactions can be reproduced in the more purified enzyme obtained as a glycogen particulate fraction after ultracentrifugation at 100,000 × g. In these preparations, as has been mentioned, there is no rise of either the D or I activities by preincubation, therefore this last remains very low. The inactivation of the D form present is time dependent and quantitatively depends on the amount of ATP used.

In none of these preparations the inactivating reaction is too sensitive to the presence of 3′,5′-AMP, although always some stimulation can be seen when used in the range of 10^{-4}-10^{-5} M with low concentrations of ATP.

We used UTP-Mg instead of ATP-Mg and, at concentrations equimolar with ATP, produced a bigger inactivating effect, which was dependent on concentration of UTP, but not time dependent.

Furthermore UTP alone without Mg^{2+} produced similar concentration-dependent inactivations of both activities which did not progress with time. It seems then that

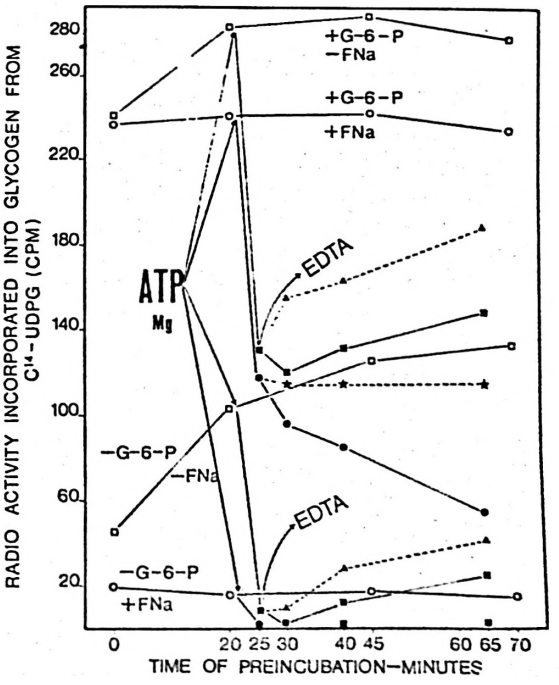

Fig. 6. *The stopping of the inactivating reaction and its reversal by EDTA addition.* Parallel extracts with and without 50 mM NaF were produced and the ATP-Mg (5-10 mM) added after the first 15 minutes of preincubation at 30° C. 5 minutes after such addition EDTA up to 25 mM was added to aliquot parts of the previous preparations and glycogen synthetase activity of controls and treated preparations tested at various intervals of time.

the action of UTP in this organ is not acting upon the kinases, as could be the case in dog muscle (12). On the contrary, the action promoted by the ATP-Mg seems very clearly to be of kinasic nature, the D form being inactivated by phosphorylation.

Reversibility of the ATP-Mg induced inactivation. Figure 5 already showed the normal reactivation obtained *in vitro* after 5-10 minutes of the addition of ATP-Mg in the range of 5 mM ATP. Figure 6 shows now further details which demonstrate that the inactivating reaction can be stopped and the reactivation reaction accelerated.

To two aliquot parts of the same extract of kidney glycogen synthetase, one with 50 mM NaF and the other without, ATP-Mg (5-10 mM respectively) was added after 20 minutes of preincubation. Similar patterns as in figure 5 can be seen and the I and D activities in the extract without NaF begin to recover after the first 10 minutes of the addition. The extract with NaF did not recover as the protein-phosphatases are inhibited.

EDTA 25 mM was added to both extracts after the first 5 minutes of ATP-Mg addition. Two different trends can be observed, depending on the presence of NaF in the extracts. The trapping of Mg^{2+} by EDTA in the extract that had NaF stops the kinase reaction and the activity remains stable at that point during preincubation compared with the controls that continue its decrease. The same trapping action of EDTA in the extract without NaF, not only stops the inactivation driven by the kinases but induces also an immediate reactivation greater than that of the controls which begin 10 minutes later. By comparing this behavior with the one in the preparation that had NaF we are forced to admit that the reactivating reaction is enzymatic in nature and that phosphoprotein-phosphatases are responsible for it.

Discussion

Glycogen synthetase enzyme systems in the kidney of the rat have been studied and the experimental data obtained have provided details on certain aspects of the mechanisms of glycogen biosynthesis which can shed light on these problems.

The enzyme form that is obtained by extraction of this organ in our generalized Tris-EDTA buffer is mainly a D form which requires glucose-6-P for activity. The activity independent of glucose-6-P (I activity) has always been very low and represents 5 to 15 % of the total activity at most findings, in agreement with those obtained by MENDICINO *et al.* (9) in rat kidney. The I activity can be increased *in vitro* by preincubation in the presence of mercaptoethanol, but the total activity also increases at the same time and, during the first 15 minutes of preincubation, generally faster than the I. As this total activity stabilizes later on, with such prolonged periods of preincubation as 60-70 minutes a partial transformation of the D to the I form can be observed. This transformation, however, is very slow compared with similar ones that are produced in cardiac (14) or skeletal muscles (10, 12) and, the independent activity does not reach more than 50 % of the total during this time. A complete transformation *in vitro* can however be obtained in the presence of Mg ions but this transformation is produced not because of stimulation of the phosphatase that converts the D to the I activity as it occurs in other tissues (3, 6, 12, 13, 14) but because they prevent the rise of the D activity during preincubation. Under these conditions, therefore, although the activity is all in an I form, it represents only about 50 % of the total activity initially present.

The increases in both activities produced by preincubation seem to be independent of each other and are prevented altogether by the presence of 50 mM NaF. This fact strongly suggest that they are

of an enzymatic nature and the knowledge from similar systems in other tissues and organs indicates that they may be phosphoprotein-phosphatases. A rise in the D activity which is unrelated to the I (which is not produced) has been recently demonstrated in frog skeletal muscle (2); and this rise was also prevented by the presence of NaF. Furthermore this property was lost in the supernatant after ultracentrifugation at 100,000 × g; also it happens in our kidney preparations obtained in a particulate fraction by addition of glycogen and ultracentrifugation. Therefore the increase of the D activity seems to be produced at the expense of some inactive molecular species present at the time of extraction, which would be converted into the active D-form by a phosphoprotein-phosphatase type of reaction. It would then be a similar mechanism to that found in rat cardiac muscle by SACRISTÁN and ROSELL-PÉREZ (14) and in frog muscle already mentioned (2).

A puzzling contrast is the action of Mg ions in these systems. In practically all the glycogen synthetase systems tested, Mg ions have a positive influence although at multiple levels (2): the D activity is stimulated, the Km and Ka can be more or less modified (13) and the D to I transformation is stimulated also (1, 2, 3, 6, 14). In our kidney preparations either as crude extracts or as more purified glycogen-particulate fractions, the D activity is not stimulated at all. Furthermore its increase by preincubation is prevented and in some concentrations of Mg^{2+} even inactivation is induced, which is very noticeable in the glycogen particulate fractions (Fig. 4).

This inactivation of the D form produced by the presence of Mg^{2+} added to the extracts before the preincubation seems to be irreversible, as once it has been initiated it is not stopped by the addition of strong concentrations of EDTA. It seems that Mg^{2+} gets tightly bound to the enzyme molecules producing some kind of modification which alters their three-dimensional structure and therefore their activity. This action would be of a similar type to that discussed in frog muscle (2) but it is in this case negative.

The possibility of obtaining the enzyme as a particulate fraction by the addition of glycogen and a second centrifugation at 100,000 × g allows a fair purification and also separates some of the enzyme systems that work in the regulation of kidney glycogen synthetase activity. Under this treatment the phosphatases remain soluble and the kinases that interconvert the I to the D form or that inactivate these forms also become particulate.

We find that the *in vitro* available ATP has a definite influence on the type of transformation produced when added together with Mg^{2+}. The I to D transformation can be observed with small quantities of ATP (in the range of 0.5-1 mM) and even so, a decrease of the total activity is also frequently observed although this activity recovers a few minutes later. At concentrations of ATP higher than 1 mM, the inactivation of the D form in addition to the I form is very noticeable and has much greater magnitude than the I form. There is also a recovery some time after the addition, but a considerable part remains inactive. The recovery does not appear in the preparations that have NaF which indicates that this is due to the action of the phosphatases.

MENDICINO *et al.* (9) reported inactivation of the kidney glycogen synthetase by a mitochondrial fraction suplemented by ATP, Mg^{2+} and other cofactors. While the low I activity was arrested, they observed a decrease in the total activity of a greater magnitude. Although our system of inactivation does not seem to be mitochondrial since it remains in the 100,000 × g fraction obtained after previous centrifugation at the same centrifugal force and the addition of glycogen, their data agree with ours in that they show the possibility of inactivation of the

glycogen synthetase by reactions which seem to extraphosphorylate the D form as well as the I.

In our case it is possible to arrest the inactivating reactions by subsequent addition of enough EDTA to trap the necessary Mg^{2+}. Then if the phosphatases are present (in crude extracts) and not inhibited by the presence of NaF, a reactivation or inverse reaction can immediately be seen. By contrast, in preparations in which phosphatases are absent (glycogen particulate fractions) or inhibited by NaF the arrest of the inactivating reaction by EDTA is also obtained, but the inverse reaction, the recovery of activity, can no longer be seen.

All these data seem to indicate that kidney glycogen synthetase can undergo *in vitro* reactions of phosphorylization and dephosphorylization which in addition to intreconverting the D and I forms of the enzyme, may produce quite inactive molecular species by extraphosphorylation. This situation also has been recently demonstrated for the glycogen synthetase systems of rat cardiac muscle (14) and it represents a mechanism which shuts off completely the biosynthetic pathway for glycogen. As these protein kinases seem to be stimulated by the cyclic 3′,5′-AMP, the mechanisms that stimulate glycolysis where there is heavy demand for energy could prevent any kind of futile recycling of glucose-1-P by completely shutting off the pathway to glycogen.

The mechanisms of control proposed for the cardiac muscle systems (14) can equally be postulated here for the systems of rat kidney glycogen synthetase. The various sites of phosphorylation on the enzyme molecule would agree also with a structure of protein sub-units.

Resumen

Los sistemas enzimáticos responsables de la biosíntesis de glucógeno (UDPG: α-1.4-glucan α-4-glucosiltransferasa) pueden extraerse fundamentalmente como forma D (dependiente de la glucosa-6-P para su actividad). Debido al bajo contenido de glucógeno de este tejido, el enzima permanece soluble después de centrifugar a 100.000 × g durante 90-120 minutos. El enzima puede ser recogido en forma de fracción particulada, si se le añade a la fracción soluble glucógeno de alto peso molecular y luego se centrifuga de nuevo a 100.000 × g durante 90 minutos.

Ambas actividades, la D y la I (muy baja inicialmente) aumentan por preincubación de los extractos crudos en presencia de mercaptoetanol. La actividad D se incrementa de manera rápida y se estabiliza con el tiempo después de los 15 primeros minutos generalmente. La actividad I (independiente de la glucosa-6-P) aumenta lentamente y durante períodos más largos pero una transformación D a I completa no se obtiene aunque la relación de actividades + glucosa-6-P/-glucosa-6-P decrece hasta cierto punto indicando cierta transformación D a I.

Los aumentos de ambas actividades por la preincubación pueden evitarse por la adición de NaF 50 mM a los extractos, lo que parece indicar que estas reacciones se llevan a cabo por fosfoprotein-fosfatasas.

Los iones Mg no estimulan la actividad D ni su incremento por preincubación. De hecho impiden este incremento, o todavía más inducen una inactivación de esta forma durante la preincubación. La actividad I es estimulada ligeramente por este catión a tiempo 0 de preincubación, aunque la velocidad de aumento durante la misma parece poco o nada afectada.

La adición de ATP y Mg inactiva a ambas formas de actividad en una reacción dependiente del tiempo y de la concentración de ATP, reacción que es reversible con el tiempo si no hay inhibición de fosfatasas por la presencia de NaF. Las reacciones inactivantes producidas por la adición de ATP y Mg pueden pararse por la adición de una concentración suficientemente fuerte de EDTA para atrapar el Mg^{++} necesario para las kinasas. Entonces se puede observar la recuperación tanto de las actividades D como I en aquellas preparaciones que no tenían inhibidas fosfatasas por la presencia de NaF. En aquéllas con inhibición, la adición de EDTA solamente produce la detención de la reacción inactivante, pero ya no puede observarse la recuperación de las actividades D o I.

Todos estos datos experimentales sugieren que las formas D e I de la glucogenosintetasa extraída del riñón de rata pueden interconvertirse en parte, pero también son capaces de ser transformadas en formas inactivas más fosforiladas que pueden reactivarse por reacciones de defosforilización llevadas a cabo por fosfoprotein-fosfatasas.

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