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Interconversions of Cardiac Muscle Glycogen Synthetase *

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Heart muscle glycogen synthetase can be analyzed as both I and D forms, and the enzyme systems that interconvert them are also active. The percentage of I form that can be analyzed does not always keep an inverse relationship to the amount of glycogen present in muscle in keeping with the findings of others under different experimental conditions.

To explain this (apparently abnormal behavior) as well as other findings we postulated the possibility of the existence of inactive forms of muscle glycogen synthetase which in frog skeletal muscle have been recently demonstrated for the D form.

In this report we present data which demonstrate that the D enzyme form of heart muscle glycogen synthetase can be inactivated by a reaction driven by the addition of ATP and Mg. This inactivation is dependent both on time and the concentration of ATP and is sensitive to the presence of 3',5'-AMP, all these conditions indicating a kinase reaction. The inactivating reaction can be arrested and reversed by the addition of strong concentrations of EDTA to trap the necessary Mg^{2+}.

In a similar manner I activity can also be inactivated by reactions of the same type, and also the D to I form transformation can be prevented by the addition of ATP-Mg. I activity also reappears after arrested the inactivating reaction by the addition of 20 mM EDTA.

All these experimental data lead us to postulate the existence of complete inactive forms of glycogen synthetase from heart muscle which can play a role in the regulation of glycogen metabolism in this organ and can explain some of the observations described by various authors.

SOEVIC *et al.* (20) showed in 1966 that cardiac muscle had both forms of glycogen synthetase (UDPG: α-1,4-glucan α-4-glucosyltransferase. E.C. 2.4.1.11, synthetase or transferase), that could be detected by assaying them in the presence (D-form) or absence (I-form) of glucose-6-P. The systems of transformation between both forms were also present. The percentage of I form present in the perfused hearts oscillated in a manner correlated rather

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more to the ATP content of hearts in different conditions than to the content of heart glycogen.

As the glycogen transferase system of cardiac muscle had not been thoroughly studied at that time, it seemed of interest to know a little more about the behavior of these systems *in vitro* in order to get a better knowledge for the understanding of their behaviour *in vivo*. Recently LARNER *et al.* (11) have published some data about these systems in rat heart.

The present paper will present data on the enzyme systems of glycogen synthetase of rat heart which can shed some light on these problems. It will be seen that both forms of rat heart muscle can be inactivated *in vitro* by reactions induced by the addition of ATP and Mg^{2+}, which are both dependent on time and concentration of ATP, and which are also sensitive to the presence of 3′,5′-AMP. These inactivating reactions can be stopped and reversed by trapping the Mg^{2+} with strong concentrations of EDTA. Some results were already partialy reported (15, 19).

Materials and Methods

Heart muscle enzyme preparations were obtained in a similar manner as in skeletal muscle. White rats, fed ad libitum, from a local strain, weighing between 150 and 200 grams were killed by a blow in the neck, the heart quickly removed by opening the chest, chilled on ice, blotted and kept on prechilled 50 mM Tris-5 mM EDTA buffer *p*H 8.0 until homogenization. Normally 2 to 4 hearts were pooled in this manner to obtain enough material. If frogs were used more animals were killed and hearts removed and pooled in identical form. Up to 20 hearts were pooled from frog brought directly from ponds near the city, which we assumed were fed.

Muscle homogenates were done with a motor driven teflon pestle homogenizer, chilled with ice, in 1 to 3 minutes intervals, in the above mentioned buffer in a relation 1/10, 1/15 or 1/20 w/v. The homogenates were clarified by discarding the cell debris by a short centrifugation on a clinical refrigerated centrifuge at about 3,500 × g for 10 minutes. The supernatants were the enzyme sources in many experiments and will be referred to as crude extracts.

More purified preparations were used in other experiments, obtaining the glycogen particulate fraction, by centrifuging the above mentioned supernatants in a Spinco L-2 ultracentrifuge at 100,000 × g for 90 minutes. The glycogen pellets were suspended in their original volume with the buffer described and then used as enzyme source for different manipulations and tests.

For glycogen determinations hearts were quickly frozen in a Wollemberger type of clamp cooled in liquid O_2. The frozen hearts were crushed in a stainless-steel mortar, and aliquots of the frozen powder weighed, glycogen isolated and determined by the anthrone method of CAROLL *et al.* (4). The ATP was determined by the coupling of the hexokynase reaction and G-6-P dehydrogenase.

In some experiments hearts were excised under ether anaesthesia and installed in a Morgan perfusator, containing Krebs-Ringer bicarbonate buffer with 1 % albumin as was already described (20). Additions of hormones and metabolites were made through the perfusing media, and the hearts frozen as above and determinations of glycogen and enzyme activity performed on the frozen powder.

Glycogen synthetase enzyme activity was measured as radiactivity incorporated into glycogen from C^{14} glucose labeled UDPG as has been described (22). A solid sample gas flow counter (Nuclear Chicago) was used (25 % efficiency) with a specific activity of UDPG of about 6,000 c.p.m./μM. Test mixtures for enzyme activity in the D and I forms were standard

as described previously (22). Additions of other chemicals and manipulations are described afterwads. Incubations for enzyme activity and preincubation of the enzyme systems were done at 30° C. Protein determinations were done by a Biuret method.

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

Results

Levels of enzyme activity and enzyme forms. In the conditions above described there is activity extracted from cardiac muscles of rabbit, rat or frog. In all these species good activity can be detected both in the presence or absence of glucose-6-P. The percentage of «relative intrinsic activity» (2) (activity without/activity with glucose-6-P × 100) oscillates from animal to animal and can vary from 8 to 70 % if no other precautions are taken during the extraction. If NaF up to 100 mM is added to the buffer used for extraction, the activity without glucose-6-P is kept very low, about 5 to 15 % of the total. The activity of total glycogen synthetase (assayed in the presence of 10 mM glucose-6-P) ranged from 60 to 150 μM/g/hr and the specific activity from 13 to 39 μM/mg P/hr.

Glycogen content of hearts and activity of glycogen synthetase. The glycogen content of heart muscle obtained from frozen hearts in open chest preparations varied from 1.8 to 4.6 mg/g wet weight of cardiac tissue in more than 20 determinations and in these preparations the percentage of glycogen synthetase I activity correlated (in general) with the amount of glycogen as proposed by DANFORTH (5) for skeletal muscle, and by HUIJING *et al.* (9) for heart muscle as can be seen in figure 1. However, in perfusion experiments conditions were found in which such correlation was not apparent (20).

Here we tested again, in perfused hearts, some of the above mentioned conditions, perfusing the hearts with 2-DOG or 2-4 DNP in order to deplete the hearts of ATP or glycogen. Depletion of glycogen always coincided with depletion of ATP and so the percentage of I activity

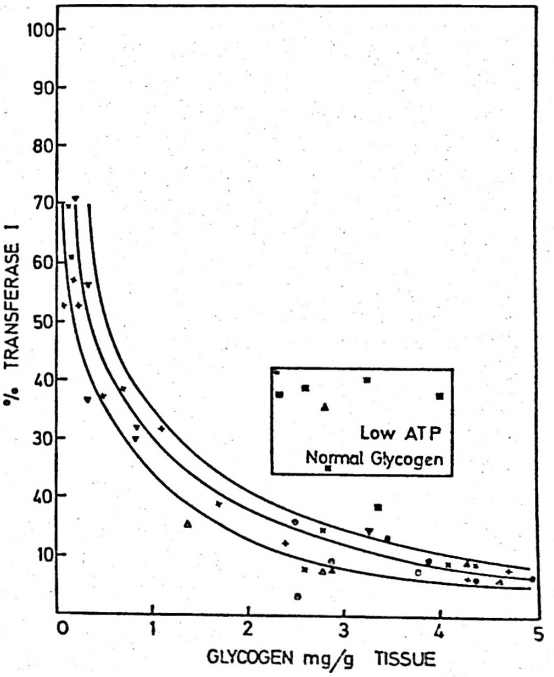


Fig. 1. *Correlation between glycogen content and percentage of I activity in rat heart muscle.*

Hearts were perfused as indicated in the text (Krebs-Ringer bicarbonate buffer) frozen with a Wollemberger type of clamp, powdered in a stainsless-steel mortar and glycogen and synthetase activity measured in the powder. Different conditions were used: △, controls; ▲, controls plus glucose (1.8 mg/ml perfusing medium); ○, with 10 microunits/ml of insulin; ▽, with 12.5 mM 2-4 DNP (low ATP; and low glycogen); ■, with 10 mM 2-deoxyglucose (low ATP, normal glycogen); ×, values obtained from the literature (9).

. Table I. *Glycogen content and transferase activity in normal and insulin treated perfused hearts*
Numbers in brackets indicate the numbres of animals. All values are given as the mean ± standard error of the mean.

Hearts	Protein mg/ml	Glycogen mg/g tissue	Transferase νM/mg P/min		I %
			+ G-6-P	— G-6-P	
Normal (6)	7.22±1.2	3.54±0.32	24.1±3.6	1.91±0.3	7.75±2.3
Insulin * (8)	5.33±1.9	3.12±0.31	27.0±6.82	3.94±1.8	15.0 ±6.02

* 10 microunits of insulin per ml of perfusing medium.

was more or less correlated to the amount of glycogen and fitted in the curve (figure 1). When ATP was low but glycogen was maintained at normal range the percentage of I activity did not correlate (points in the squared area of the curve in figure 1).

Insulin, glycogen and glycogen synthetase activity. In figure 1 some points obtained after perfusion of hearts for 30 minutes with 10 microunits of insulin per ml are depicted.

In Table I there are summarized the results of experiments undertaken to find if insulin, in the perfusing media, could have any influence on glycogen content of the hearts or glycogen synthetase activity. There is no influence at all on glycogen content or total synthetase activity. The increment in I activity and percentage of I activity over total activity has no statistical significance in these experiments.

The systems of interconversion between the D and I forms of cardiac muscle transferase. When normal extracts from rat cardiac muscle are preincubated at 30° C in the presence of 50 mM mercaptoethanol, there is a rise in the I activity (measured without glucose-6-P) during time of preincubation without too much change in the total activity (measured in the presence of 10 mM glucose-6-P). With no mercapthol the rise in I activity is much less noticeable. In other experiments (Fig. 2) there is a rise also in the total activity. In any case the ratio of activities + glucose-6-P/ - glucose-6-P decreases, that is to say, the «intrinsic activity» (2) increases. If we add Mg ions up to 8-10 mM the I activity increases faster and higher and the D to I transformation

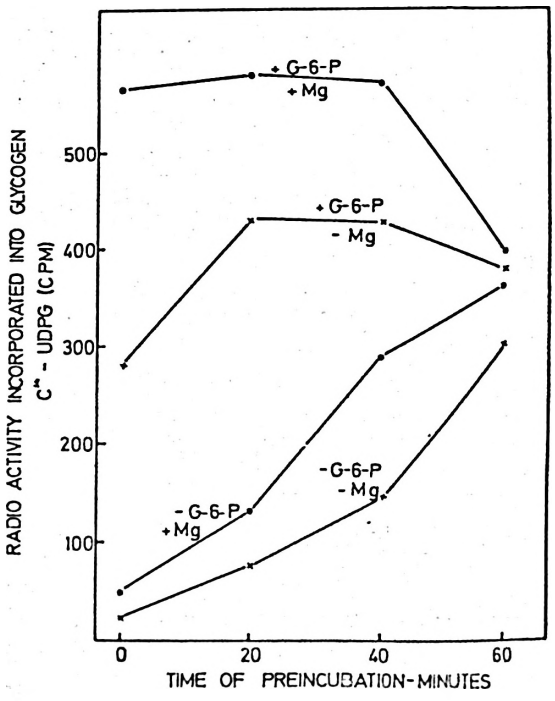


Fig. 2. *Influence of the presence of Mg^{2+} on the activity and the D to I form transformation of heart glycogen synthetase.*
Preincubations of the enzyme were done in the presence or absence of 10 mM $MgCl_2$.

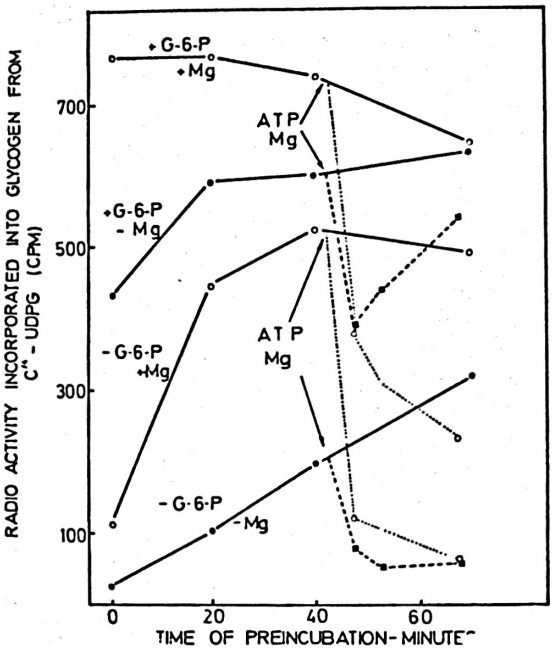


Fig. 3. *Effect of adding ATP-Mg (1-8 mM respectively) to the enzyme extracts after 40 minutes of preincubation.*

$MgCl_2$ 10 mM was present initially in one of the preparations.

is more complete in less time. Total D to I transformation can be reached in 20-30 minutes in some experiments (figures 2 and 3). Too much Mg^{2+}, however (40 mM for instance) can be inhibitory. The total activity in these conditions (8-10 mM Mg^{2+}) is higher at 0 minutes of preincubation when the cation is previously added. During time of preincubation, total activity without Mg^{2+} also rises more or less slowly, to the levels of the total activity with Mg^{2+}.

If ATP-Mg is added (1-8 mM respectively) after some time of the preincubation period, the transformation can be reversed with some characteristic behavior. In figure 3 it is seen that addition of ATP-Mg after 40 minutes of preincubation to the enzyme extract with Mg^{2+} previously added, produces a decrease in I activity with practically an equal decrease in total activity. Addition of ATP-Mg to the enzyme extract with mo Mg^{2+} produced a decrease in I activity and also in total activity, although this last one recovers after the first 5 minutes increasing its activity with time. At the end, practically all that remains is D activity (only active in the presence of glucose-6-P).

Transformation from the D to an inactive more phosphorylated form. Data of a slightly modified experiment are depicted in figure 4. Here we have also enzyme extract with and without Mg^{2+} previously added. The ATP-Mg (5-10 mM) was added after only 3 minutes of preincubation. The effects were a little different from the former type of experiment. The rise of I activity, without initial Mg^{2+} addition, was prevented by the ad-

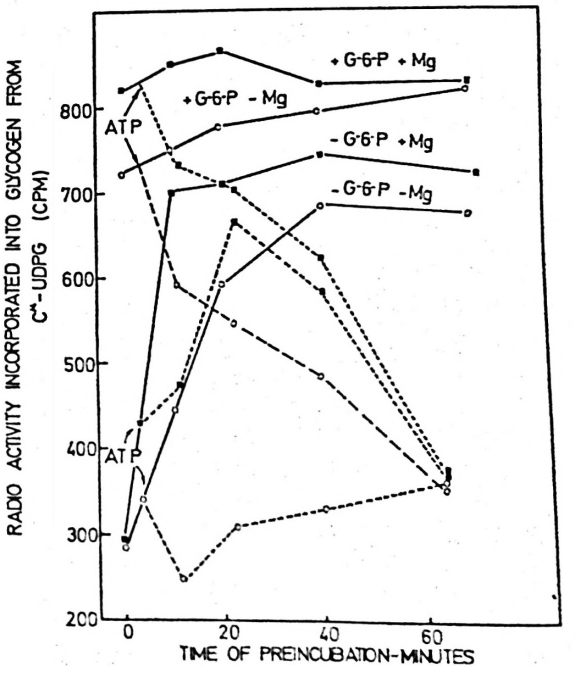


Fig. 4. *Effect of adding ATP-Mg (5-10 mM respectively) after three minutes of preincubation.*

Dotted lines represent enzyme activity after the addition. $MgCl_2$ (10 mM) was present in one of the preparations.

dition of ATP-Mg. The rate of D o I transformation was diminished in the extract with Mg^{2+} but at the same time both total activities began to decrease with time, meanwhile the controls remained stable. The one without initial Mg^{2+} after 65 minutes of preincubation arrived at the level of I activity by an inactivation dependent on time. The other with Mg^{2+} also began to decrease in a parallel pattern and it continued to do so even after being converted into I activity, and then the only activity present decreased to the same level as that without Mg^{2+}. Several types of time dependent transformations were apparently produced: *a*) A reversal of the phosphatase reaction that interconverts D to I activity by the action of a kinase, driven by the ATP-Mg addition to the enzyme extract that had no Mg^{2+}. *b*) A slowing down of this D to I transformation by similar procedure in the enzyme extract that had the cation previously added and *c*) A completely new type of transformation, that is to say, an inactivation or transformation of the D activity into an inactive molecular species, apparently driven also by the addition of Mg^{2+} and ATP. This new type of reaction could mean an extraphosphorylation of this D form that would lead to its inactivation.

New kinds of experiments were designed in order to define a little more these types of reactions. Figure 5 shows an experiment in which the D to I transformation in rat heart extracts was prevented by inhibition of the phosphatase by adding to the extracting buffer, 50 mM NaF. Then at 15 minutes of preincubation Mg^{2+} (10 mM) and different amounts of ATP were added to several aliquots and enzyme activity tested after, 5, 10 and 20 minutes.

It can be seen that in these conditions 1 mM ATP is enough to inactivate the basal I activity, and also that D activity is inactivated in a reaction time and concentration of ATP dependent.

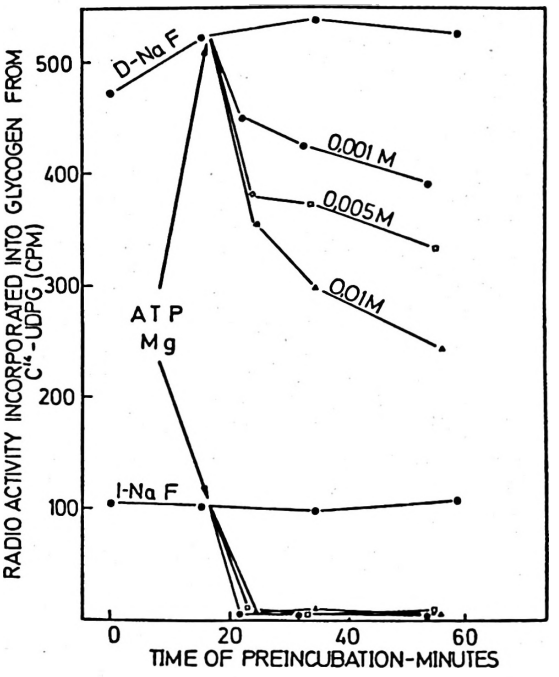


Fig. 5. *Effect of increasing concentrations of ATP on the inactivation of the D-form of glycogen-synthetase.*

The D to I transformation was prevented by the addition of 50 mM NaF to the extracting buffer and after 15 minutes of preincubation 10 mM $MgCl_2$ and different amounts of ATP were added.

In a second experiment depicted in figure 6 it is shown that the effect produced by ATP and Mg^{2+} addition on the D activity *in vitro* is further increased by the addition of 3′,5′-AMP (2×10^{-5} M). Additions were done here after 20 minutes of preincubation and tests for enzyme activity performed 5, 10, 15 and 40 minutes afterwards.

It seems clear from these last two experiments, above described, that some sort of kinase reaction, working on the D form and inactivating it by extraphosphorylation was taking place.

Reversion of the D-form inactivating reaction. Figure 7 shows a type of experiment in part similar to that described

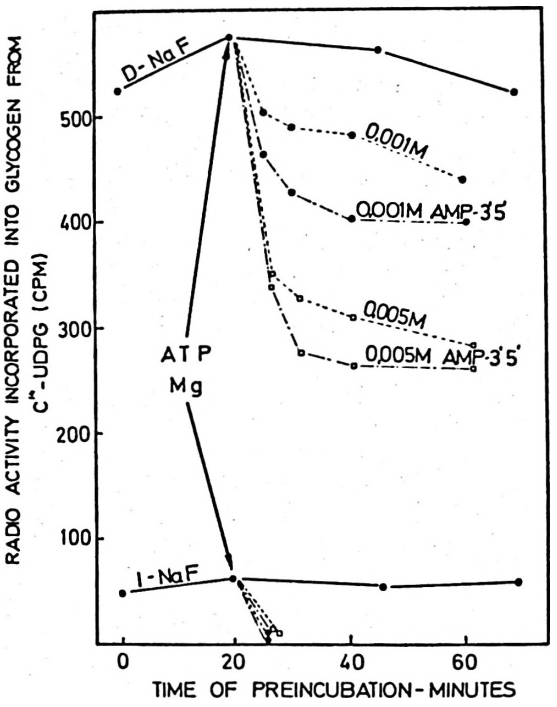


Fig. 6. *Sensitivity of the inactivating reaction of the D-form of glycogen synthetase to the presence of 3',5'-AMP.*

Conditions were similar to those in figure 5, but on some aliquots 3',5'-AMP 2×10^{-3} mM was added. 3',5' mean the presence of the cyclic nucleotide.

in figure 4, although some conditions were slightly different. The I activity with no Mg^{2+} previously added was here more than 70 % of the total at 0 time of preincubation and near 60 % with Mg^{2+} present (very active phosphatase). Total activity with Mg^{2+} was 30 % higher than without. The ATP-Mg (5-10 mM respectively) was added after 3 minutes of preincubation. Here we see the prevention of rise in I activity with no Mg^{2+}. We also see the inhibition, time dependent, of total activity with or without Mg, with a previous rise in this last case due to the Mg^{2+} effect (18).

At 25 minutes of preincubation (22 after ATP-Mg addition) EDTA 20 mM was added to the tubes that had ATP-Mg mixture and activity tested after 6, 10 and 20 minutes of such addition. A complete reversal of the D inactivation produced by ATP-Mg could be observed when the EDTA was added. The total activity reached the levels of the controls after 10 minutes. I inactivation was also reversed but the levels of I activity were far below those of controls. This behaviour has been identically observed in frog heart preparations.

The effects on the D activity produced by ATP-Mg addition cannot be explained by an inhibition of either the cation or the nucleotide as the addition of Mg^{2+} or ATP alone in similar concentrations did not produce such effects. Furthermore, it could be prevented if the mixture of

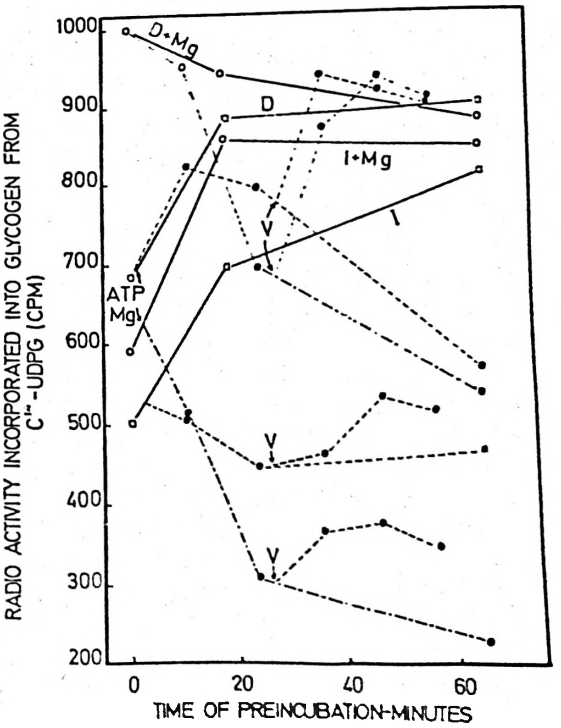


Fig. 7. *Recovery of D and I activities after inactivations by ATP-Mg addition.*

ATP-Mg were added after the first three minutes of preincubation, and 20 minutes latter 20 mM EDTA was added to stop the inactivating reactions. V indicates the addition of EDTA (Versene).

ATP-Mg was added together with a chelating agent as EDTA citrate of fluoride (100 mM).

Addition of shell-fish glycogen up to 8 mg/ml together with the ATP-Mg did not prevent those effects described.

Localization of the phosphatase and kinase systems. The phosphatase system that is to say, the system that interconverts the D into the I form in heart muscle is a soluble one and remains in the supernatant after 90 minutes of centrifugation at 100,000 × g. The glycogen particulate fraction which contains all the glycogen synthetase activity, does not show any D to I form transformation when resuspended in the normal buffer with mercaptoethanol and incubated for 60 minutes at 30° C. The transformation is obtained if this fraction is suspended in the 100,000 × g supernatant or if it is resuspended in a very small volume of the original buffer and then increasing amounts of supernatant are added and the preincubation carried out. The extent of transformation then is proportional to the amount of supernatant added.

The kinase systems on the other hand remain in the particulate fraction and are somehow difficult to separate. The inactivation of the D activity is also seen in these enzyme preparations when they are resuspended in the original buffer and ATP-Mg in correct proportions are added. Here again the action of ATP-Mg is sensitive to the presence of 3′,5′-AMP and the addition of EDTA after some time of the ATP-Mg action produces a recovery of D activity, although this is not as complete as in the crude extract enzyme, preparations, suggesting that this last phosphatase solubilizes, in part, in the 100,000 × g supernatant.

Discussion

From the experiments above described some points emerged that deserve comments and discussion.

The levels of glycogen synthetase activity in rat heart are in agreement with our previous determinations (20) and also with the measurements obtained by other investigators (9, 11) with similar methods of glycogen synthetase assay. Oscillations in per cent of I activity were wide if no precautions were taken and hearts for enzyme extracts were not frozen. In normal frozen hearts or in hearts that were extracted in the presence of 50 mM NaF, the per cent of I activity remained low, which seems an indication that this is the state of the normal working heart.

Glycogen content in normal hearts was also in the range of those levels determinated by several researchers (2, 6, 9, 13, 23) in normal conditions. In order to reduce glycogen content in heart we used the perfusion technique adding 2-4 DNP. Together with the reduction of glycogen, there is also a decrease in ATP in these conditions (20). The per cent of I activity rose in an inverse relationship to the glycogen content as already observed by HUIJING *et al.* (9). If we add instead 2-DG-6-P, a non-metabolizable substrate (and inhibitor of glycolisis) the glycogen content does not decrease but the ATP level does. In these conditions the percentage of I activity also rises and then its values do not fit in the curve of figure 1.

There are a number of observations made by other investigators in which the inverse relationship between the percentage of I activity and glycogen content are reversed or displaced. BELFORD and CUNNINGHAM (2) found that aminophyline and calcium, which increase force of contraction do not increase I activity and aminophyline instead decreased total activity. They concluded that a simple interconversion did not take place and perhaps an inactive species could play a role as previously postulated by ROSELL-PÉREZ and LARNER (16, 17). Some further discussion about this inactive species will follow later on.

In conditions of maximum cardiac work

on a heart-lung preparation MINELLI and MARTINAZI (13) showed that heart glycogen not only did not decrease but, instead, a very significant increase was obtained. If the feed-back regulation by glycogen would have been working the per cent of I activity ought to have diminished.

In other types of experiments the above mentioned authors (3) showed a 40 % decrease in total activity of rat heart on starvation (96 hours) with no comparable decrease in muscle. I activity in heart also decreased but not at a comparable rate and no affect was observed in muscle. Only after 96 hours of starvation did the «intrinsic activity» diminish a little. Although glycogen content in heart under those conditions may increase a little (12) it is known that the glycogen stores in muscle diminish, albeit slowly, in the same circumstances so again there is a lack of correlation between glycogen and per cent of I activity.

WILLIAMS and MAYER (23) with the rat preparation *in situ* showed that increasing doses of epinephrine given intravenously caused an increase of per cent of I activity with no change in glycogen. Infusions of epinephrine (1 μg/kg min^{-1}) did not change glycogen and produced some increase of per cent of I activity during the first 5 minutes. Higher doses of epinephrine caused a significant degree of glycogen breakdown in 10 minutes, and a biphasic change in I activity that rose in the first minute and decreased afterwards below control levels. Here again we found a decrease of per cent of I activity parallel to the decrease of glycogen.

Another significant finding is that of DOW *et al.* (6). They found that adrenalectomy decreased the total transferase activity in rat heart and also the transferase I activity with a resultant decrease in percentage of the I activity. As there is also a very significant decrease in glycogen content in the heart we have again a situation in which the inverse relationship per cent of I activity and glycogen has changed.

The above discussion shows that glycogen levels not always seem to control their own synthesis in heart. The inhibition of synthetase D-phosphatase has been found *in vitro* (9) and exogenous addition of glycogen as high as 12 mg/ml of enzyme extract produces only a 62 % inhibition. We also have found this *in vitro* inhibition, however it seems that the inhibition could be explained as a competition between the exogenous glycogen and the endogenous one. The synthetase D-phosphatase is a soluble enzyme not bound to glycogen (data in this paper) and will need to approach the glycogen macromolecules when the synthetase is bound to it. If exogenous glycogen is added (with no synthetase or degenerated enzyme) the phosphatase molecules will divide between the good and the bad ones and therefore a type of competitive inhibition will be produced. It is not yet clear if this mechanism is functional *in vivo*.

We did not find any significant effect of insulin on hearts perfused *in vitro* neither on glycogen content nor glycogen synthetase I activity. This seems to be a general pattern as this lack of effect has been pointed out also by others (8, 23). As the glycogen content is at normal levels the values fit the curve of figure 1, except one which was also with low ATP content.

The systems of interconversion between the D and I forms of glycogen sinthetase were described previously (20) by SOEVIC *et al.*, who pointed out that the mechanisms ought to be a system of phosphorylation and dephosphorylation as they ocurred in skeletal muscle (17). Recently, LARNER *et al.* (11) have effectively demonstrated this to be so, showing a stoichiometry between the P^{32} incorporated into protein and amount of I form produced and also between the P^{32} released as inorganic P and the amount of I to D

interconversion. They found also that the sites of phosphorylation in the heart muscle enzyme, although similar to those in skeletal muscle, are not identical.

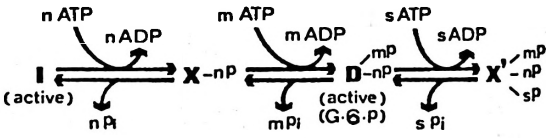
Of great interest here seem to be our findings of the inactivation of glycogen synthetase D form by a time dependent reaction driven by the addition of Mg and ATP. Similar effects were observed in other systems and pointed out in other papers in the human lymphocyte glycogen synthetase systems (8), human diabetic polymorphonuclear leucocytes (7) and frog muscle (1, 7). Recently, in frog muscle, it was demonstrated that this inactivation by ATP-Mg is sensitive to 3′,5′-AMP (1). In skeletal muscle of rat we have been able to find also this kind of inactivation (14) and it will be described at length elsewhere.

The reaction depends both on time and concentration of ATP and is also sensitive to the presence of 3′,5′-AMP. All these are factors that affect protein kinase reactions which strongly support the hypothesis of an inactivation of the glycogen synthetase D due to an extraphosphorylation.

The reaction is easily obtained *in vitro* if the ATP-Mg addition is made at the beginning of preincubation of enzyme extracts, before the glycogen synthetase D-phosphatase had time to initiate its action. It is also easily seen if the phosphatase is inhibited by the presence of NaF. In fact, as seen in figures 4 and 7, the phosphatase reaction can be prevented by the addition of ATP and Mg, not because a direct inhibition is produced but because the delicate equilibrium between the synthetase D-phosphatase and the synthetase I kinase is deviated in favour of the kinase by such addition. Furthermore, in some conditions produced *in vitro*, the I activity is inactivated by the addition of ATP-Mg without conversion into the D activity. A second explanation could be, of course, that the phosphatase itself can be inactivated by an ATP-Mg dependent reaction.

The kinase reactions can be reversed by the addition of chelating agents such as EDTA (Fig. 7) and the inactivating effects are not produced by Mg^{2+} or ATP alone. All these data seem to indicate that in heart glycogen synthetase systems more than one type of phosphorylated molecular species can be produced by the interplay of specific protein phosphatases and protein kynases that interconvert such molecular species through inactive intermediates or that inactivate them completely. This is not difficult to understand as in the case of phosphorylase, an enzyme system very similar to glycogen synthetase, several intermediated phosphorylated forms have been demonstrated by FISCHER *et al.* (10).

A scheme of such interconversions could be the following:



the reactions driven to the right by protein kinases identical or different and towards the left by proteinphosphatases.

Although this is as yet on a hypothetical basis, different degrees of phosphorylation have been found in the metabolic regulation of enzymes. This has been especially substantiated in the case of the conversion of phosphorylase *a* into *b* by HURD *et al.* (10), the intermediate states having different activity.

The physiological effects of the conversions are not clear as yet but they could explain some of the contradictory effects mentioned in this discussion such as those pointed by BELFORD and CUNINGHAM (2, 3) or the effects produced in adrenalectomized rats described by DAW *et al.* (6).

In any case they show still more delicate mechanisms for enzyme metabolic regulation that will modulate the action of a particular enzyme. They could represent a complete shutoff mechanism to the pathway of glycogen biosynthesis in those cases in which the cells have a heavy demand of energy.

The isolation of the inactive intermediates would answer definitely the questions raised by the postulated hypothetical scheme.

Resumen

La glucógenosintetasa de músculo cardíaco puede ser analizada tanto en la forma D como en la I y los sistemas enzimáticos que interconvierten ambas formas son también activos. El porcentaje de forma I que puede ser analizado no guarda siempre una relación inversa con la cantidad de glucógeno presente en el músculo, como también ha sido descrito por otros autores bajo diferentes condiciones experimentales.

Para explicar este comportamiento (anormal en apariencia), así como algún otro hallazgo experimental se postuló la posible existencia de formas inactivas de la glucogenosintetasa de músculo (15, 16), las cuales han sido recientemente demostradas, especialmente en el caso de la forma D de músculo de rana (1).

En este trabajo presentamos datos que demuestran que la forma enzimática D de la glucógeno sintetasa de corazón puede ser inactivada por una reacción originada por la adición de ATP y Mg^{2+}. Esta inactivación depende, a la vez, del tiempo y la concentración de ATP y es sensible a la presencia de 3',5'-AMP, condiciones estas indicadoras de una reacción cinásica. La reacción inactivante puede ser parada e invertida por la adición de concentraciones fuertes de EDTA que atrapan el Mg^{2+} necesario a la kinasa.

De forma similar la actividad I puede ser inactivada también por reacciones de igual condición, y también la transformación de la forma I a la D puede ser evitada por la adición de ATP y Mg^{2+}. La actividad I reaparece después de parar la reacción inactivante mediante la adición de EDTA 20 mM.

Todos estos datos experimentales nos llevan a postular la existencia de formas completamente inactivas de la glucógenosintetasa de músculo cardiaco que pueden tener importancia en la regulación del metabolismo del glucógeno en este órgano y pueden explicar algunas de las observaciones descritas por varios autores.

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