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Glycogen Synthetase Activity in the Sea Mussel (*Mytilus edulis*, L.) *

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The existence of UDPG-dependent glycogen synthetase activity has been determined in the sea mussel. The enzyme, strongly activated by Glucose-6-phosphate and practically dependent on this cofactor for activity, was inhibited by calcium ions. The *Km* value obtained for UDPG was about 3×10^{-4} M, and the *Ka* values determined for the glucose-6-phosphate depended upon the UDPG concentration, showing a cooperative effect between the substrate and the activator; these *Ka* values ranged from 9.2×10^{-5} to 3.6×10^{-4} M with concentrations of UDPG from 0.7 to 3 mM.

The optimum assay conditions were temperature 25° C and *p*H 7.8. The sea mussel's glycogen synthetase was widely distributed in all studied tissues and organs, and its activity was related more to their real metabolic role than to their glycogen content. The enzyme in our homogenate preparations was fairly unstable, showing a rapid time and temperature-dependent irreversible inactivation.

As has been previously indicated (3, 4). the sea mussel has very large amounts of polisaccharidic reserves, which are burned out substantially in the process of gametogenesis. The glycogen accumulation lies in the continuous in-take of food during long periods previous to the production of sexual materials. In order to clarify to some extent the possible mechanisms of this accumulation, some of the known enzyme systems related with the glycogen biosynthetic and degradative pathways were investigated. In this latter aspect a possible and interesting degradative role carried out by amylases had already been identified (1). The primary characters of the biosynthetic pathway are outlined in this work.

Glycogen biosynthesis is carried out by the UDPG: α-glucan glycosyltransferase enzyme system (E.C.2.4.1.11), although it was not possible to find any kind of interconversion between the two well known

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forms of the glycogen synthetase (6, 7), that serve as a control mechanism of the enzyme activity (6, 7). The preliminary data obtained are reported in this paper.

Materials and Methods

The animals used belong to the species *Mytilus edulis*, L. (sea mussel), coming from the NW of Spain, and suitable for commercial human use. All of them were alive as shown by the closing of the shell and the retractions of the foot. During the transport process they probably suffered some degree of anoxia, that produced a rapid partial hydrolisation of the glycogen content of the animal, with the appearance of large amounts of maltooligosaccharides and glucose (3, 4) produced by amylase systems (1).

The different organs of the animal were dissected and homogenized with a teflon-pestle homogenizer in 50 mM tris-HCl 5 mM EDTA buffer, *p*H 7,8. The measure of the glycogen synthetase activity was carried out by the radiactive assay described by THOMAS *et al.* (11) utilizing UDPG * with the glucose uniformely labeled with ^{14}C. The reaction was started by addition of 30 μl of tissue homogenates (1/10 w/v) on 60 μl of standard assay mixture as described by ROSELL-PÉREZ and LARNER (8). After 10-15 minutes of incubation at 25° C the reaction was stopped by drying 75 μl of enzyme mixture suspensions on a square of Watman paper 31-ET that was immersed immediately in cold ethanol. After washing twice in clean ethanol each paper was dryed in acetone and placed in a vial containing toluene and then its radiactivity was measured in a Beckman liquid scintillator.

The chemicals utilized were from «Impex» (UDPG), «Fluka» (G6P and tris), «Merck» (EDTA and inorganic salts) and «Amersham» (radioactive UDPG). The glycogen was a highly purified molecular species obtained in our laboratory (2) from sea mussel's gonadal tissue. The calculations were carried out with personal programs conceived in Fortran IV language and processed in a IBM-360/30 computer.

Results

Conditions of assay of the sea mussel's glycogen synthetase. The optimum *p*H for the glycogen synthetase assay in the sea mussel gonadal eminence was 7.8, and the optimum temperature 25° C.

Figure I shows the relation between activity and temperature; it is important to note that the activity remains relatively

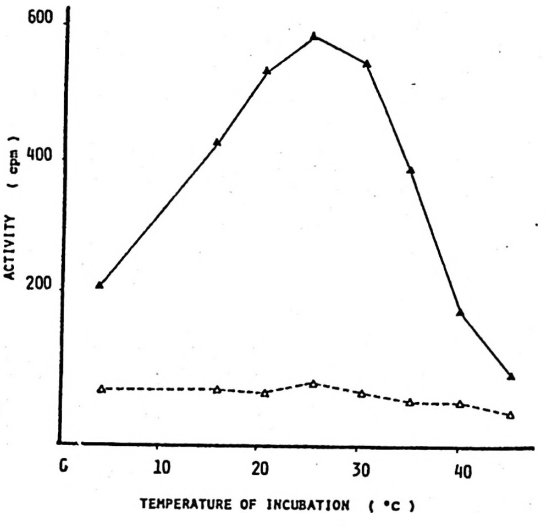

Fig. 1. *Effect of the incubation at different temperatures on the glycogen synthetase activity of the gonadal eminence from sea mussel.*
The meaning of the lines is as follows: (▲) Activity in the presence of added glucose-6-phosphate (6.67 mM). (△) Activity in absence of added glucose-6-P.

* UDPG = uridine - diphosphate - glucose; tris = tris - (hydroxymethyl) - aminomethane; G6P = glucose-6-phosphate: EDTA = etilen-diamino-tetraacetic acid.

high at fairly low temperatures, being easily measurable at temperatures of icewater. All determinations were carried out under the above given conditions.

The crude homogenates showed considerable instability, losing activity with time and temperature more or less depending on conditions of storage. This decrease in activity during time could not be reversed, and it was not possible to obtain any recuperation during preincubation either by the addition of -SH protecting agents such as 2-mercaptoethanol (6, 7) or glucose-6-P and sodium fluoride (10). The loss of enzyme activity in our conditions was very noticeable in 30-60 minutes at 25-30° C (fig. 2), and could be diminished by reducing the temperature to that of ice water. By warming up to 45° C or by freezing and thawing the homogenates, the activity disappeared totally. The only kind of stabilizing agents that showed a protective action to some extent on the inactivation by time-temperature were certain sugars, mainly maltose, a substance also found in large amounts in the studied tissues.

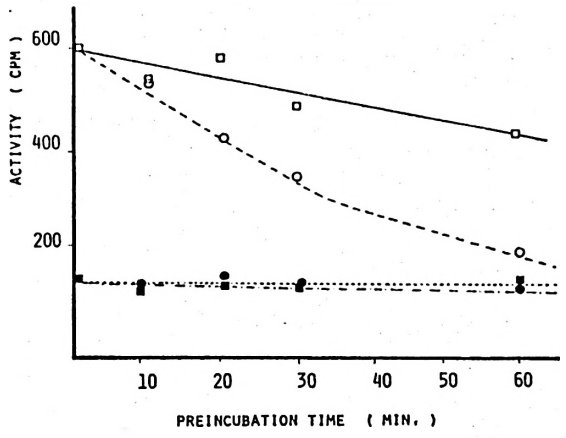

Fig. 2. *Time — and temperature — dependence of the inactivation of the crude extracts of gonadal eminence glycogen synthetase from sea mussel.*
The meaning of the lines is the following: (□) Activity in ice water and in the presence of added Glucose-6-P (6.67 mM). (○) Activity at 25° C and in the presence of added Glucose-6-P (6.67 mM). (■) Activity in ice water and without added Glucose-6-P. (●) Activity at 25° C and without added glucose-6-P.

Glycogen biosynthesis in vitro *and levels of glycogen synthetase activity.* A glycogen synthetase system that utilizes UDPG as substrates was found in the sea mussel. In many instances, this system is highly dependent on glucose-6-P for activity. The levels of enzyme activity in our conditions ranged from 0.06 to 0.42 nmoles of glycosyl groups incorporated into glycogen per minute and mg of fresh tissue. These specific activities varied widely probab'y due to the different degrees of the anoxic states in all of them (table I).

Table I. *Glycogen synthetase specific activity of different organs from the sea mussel.*

Organ	Mean *	S. D.
Gonadal eminence	0.345	0.078
Main abductor muscle	0.333	0.156
Digestive gland	0.203	0.035
Mantle	0.104	0.051
Gill	0.063	0.007

* The specific activities are expressed as nanomoles of glycosyl groups incorporated into glycogen per minute and mg of fresh tissue.

Influence of ions on enzyme activity. Fairly large amounts of magnesium ions (20 mM) inactivated the glycogen synthetase of the sea mussel (50 %), and so did the calcium ions (10-20 mM), but the magnesium ions acted as activators at low concentrations (5 mM). The anions sulphate and phosphate behaved as inhibitors of the glycogen synthetase activity measured in the presence of glucose-6-P. Concentrations of 10 mM of each anion produced an inhibition of 32 % and 44 % respectively with 6.7 mM glucose-6-P being present in the assay.

Kinetic constants. Utilizing computer programs designed by us the kinetic constants of the enzyme from crude extracts were determined; the *Km* values for the UDPG obtained were in the range of 2.6 to 3.8×10^{-4} M for the glucose-6-phosphate-dependent activity, with a *Vmax.* value of about 5 nmoles per minute and mg of fresh tissue (fig 3).

The glucose-6-phosphate-dependence was also determined with another computer program, and the results obtained are summarized in table II. There is a clear interdependence between the substrate concentrations (UDPG) and the activation produced by glucose-6-phosphate, as can be seen in figure 4. The maximum activation was achieved at 1 or 2 mM concentrations of glucose-6-phosphate, depending on the UDPG concentration.

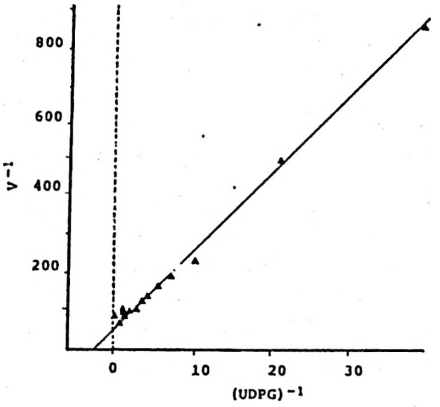

Fig. 3. *Lineweaver-Burk representation of the glycogen synthetase activity from the gonad of the sea mussel.*
The values given are in mM^{-1} for the substrate, and mM^{-1} of glycosyl groups incorporated into glycogen per minute for the velocity. The enzyme source was a crude homogenate as described in the Methods, and the time of assay was 10 minutes for each value.

Table II. *Cooperative effect of UDPG upon the glycogen synthetase system of the sea mussel: variation of the* Ka *for the glucose-6-phosphate depending upon the UDPG concentration.*

UDPG concentration mM/l	Ka value M/l
2.93	3.6×10^{-4}
1.47	1.1×10^{-4}
0.73	9.2×10^{-5}

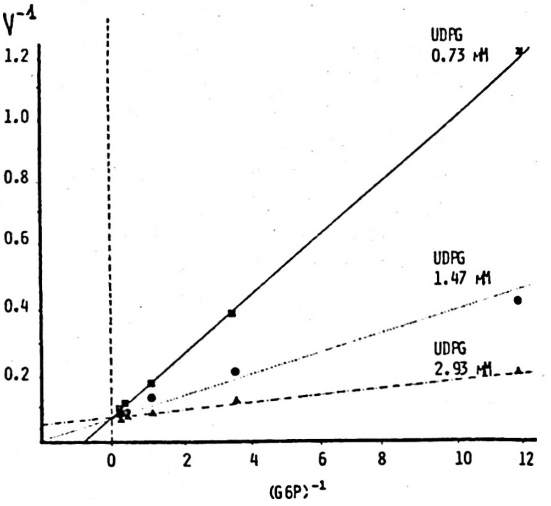

Fig. 4. *Effect of the UDPG on the* Ka *for the glucose-6-phosphate of the gonadal eminence glycogen synthetase from sea mussel.*
The values given are in mM^{-1} for the concentration of glucose-6-phosphate, and in mM^{-1} of glycosyl groups incorporated into glycogen per minute for the velocity. The lines represent the values obtained at the concentrations of UDPG indicated in the graph. The enzyme source was a crude homogenate and the time of assay was 10 minutes for each value.

Discussion

As has been shown, the crude enzyme preparations utilized to carry out the experiments contain very labile glycogen synthetase enzymes, that become inactivated even in ice water. This is probably due to active proteases of endogenous origin released by anoxic conditions, as in the fresh mussels the enzyme is more stable (12). Working with enzymatic preparations under these conditions and never

surpassing the 30 minutes limit — in cold — after the homogenization, the optimum temperature is similar to that for amylases (1), and optimum *p*H was found very close to that described for this enzyme system in other sources. The instability was first attributed to the action of the temperature alone and conssequently an effort was made to stabilize the activity by different procedures without too much success in these conditions. The kinetic constants obtained were also very close to those determined for this enzyme system in other sources (2, 6).

The effect of the calcium ions upon the glycogen synthetase system seems very interesting; it is logical to suppose that the liberation of large amounts of calcium by the anoxic state —as a result of the acidosis, and, consequently, of the shell calcium mobilization — can act as an additional system for stopping the glycogen synthetase activity and, at the same time, for activating the amylase one (1).

The occurrence of relatively high glycogen synthetase activity in the gonadal tissue and muscle is correlated with the metabolic activity of the organs — e.g. the production of spermatozoa or ova and the closing of the shell and their relatively large amounts of stored glycogen. In the case of the mantle — where the glycogen represents quantitatively the main part of the mussel's total glycogen — the glycogen synthetase activity is lower than the gonadal eminence. It can be due to the passive metabolic role of the mantle, primarily concerned with accumulation of sexual products and in interchanges with the shell. In the gills we also found, as other authors in other mollusca (5) some glycogen synthetase activity, which was not activated by glucose-6-phosphate. Due to the occurrence of mucus-producing glands in this structure, we suppose that it could be an artifact, and that really this does not correspond to a real glycogen synthetase activity but to a mucus-producing system.

It is worthwhile to mention that in the case of mussels freshly obtained from the sea —that is, not suffering from anoxia— the conversions between the D and I forms of glycogen synthetase were found (9).

Resumen

Se ha determinado la existencia de una actividad glucógeno sintetasa UDPG-dependiente en el mejillón. El enzima es activado fuertemente por acción de la glucosa-6-fosfato, dependiendo prácticamente toda su actividad de la presencia de dicho cofactor; los iones calcio inhiben potentemente dicha actividad glucógeno sintetasa. El valor de la *Km* obtenido para el UDPG fue de 3×10^{-4} M y los valores de *Ka* para el glucosa-6-fosfato dependen de la concentración de UDPG, mostrando un efecto cooperativo entre el activador y el sustrato; dichos valores de *Ka* oscilaron entre $9{,}2 \times 10^{-5}$ y $3{,}6 \times 10^{-4}$ M con concentraciones de UDPG entre 0,7 y 3 mM.

Las condiciones óptimas para la determinación fueron temperatura 25° C y *p*H 7,8. La glucógeno sintetasa de mejillón se halló ampliamente repartida por todos los órganos y tejidos estudiados, estando sus niveles más relacionados con la actividad metabólica general de los tejidos que con su contenido real de glucógeno. En nuestras condiciones de trabajo, el enzima en los homogenados resultó bastante inestable, mostrando una inactivación irreversible dependiente del tiempo y de la temperatura de incubación.

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