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Biosynthesis of Proteoglycogen: Modulation of Glycogenin Expression in the Developing Chicken

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Glycogenin, the autoglucosyltransferase that primes the biosynthesis of proteoglycogen, is found in the polysaccharide linked proteoglycogen form in mammals and chicken. Glycogenin was released from proteoglycogen and its activity was measured, together with that of glycogen synthase as well as glycogen content, in muscle, liver, and brain during chicken development. The specific activity of glycogenin, expressed per protein, increased with development only in muscle and was higher than the specific activities measured in liver and brain at any time. Concomitant with the rise in activity, an enhanced expression of the protein was observed with Western blot. The specific activity of glycogen synthase increased with development in muscle and liver, while glycogen accumulation was noticeable only in liver. The results indicate that the molar concentration of proteoglycogen is higher in muscle than in liver. The high glycogen content of liver may indicate that the size of the polysaccharide moiety of proteoglycogen is larger in liver than in muscle. This is the first report of developmental modulation of *de novo* biosynthesis of glycogen at the level of the primer that initiates glucose polymerization.

Proteoglycogen, first described in mammals (1), is the end product of the *de novo* biosynthesis of glycogen. The protein moiety of mammalian proteoglycogen is called glycogenin (2). Glycogenin is an autoglucosylating enzyme (3, 4) which initiates glucose polymerization and, under its glucan-linked form, serves as primer for further polymerization by glycogen synthase (5). Furthermore, glycogenin can transglucosylate exogenous acceptors (6-9). Proteoglycogen is also present in microorganisms. The protein moiety of *Neurospora crassa* and *Escherichia coli* proteoglycogen, called M-glycogenin (10), is an autoglucosylating enzyme with similar properties to mammalian glycogenin (11). Recently it has been described that the protein constituent of *Saccharomyces cerevisiae* proteoglycogen is required for glycogen accumulation *in vivo* (12). Thus, the *de novo* biosynthesis pathway of glycogen that ends with the autoglucosylating enzyme trapped in a polysaccharide linked form, appears to occur in all glycogen containing cells, from prokaryotic organisms to mammals. Presently there is not any report about an enzyme activity capable of releasing the entire polysaccharide from its linked protein primer. Furthermore, polysaccharide free glycogenin is detectable neither in mammalian (7, 13, 14) nor in chicken (15) cells. Thus, for mammalian and chicken tissues, the molar concentration of proteoglycogen can be determined measuring its glycogenin content.

The expression of glycogenin and the amount of proteoglycogen in different animal tissues as well as any change that might occur during development, are unknown. Here we report the glycogenin and the glycogen synthase activities and the content of glycogen of muscle, liver and brain in the developing chicken.

MATERIALS AND METHODS

Materials. UDP-[$_{14}$C]glucose (300 mCi/mmol) was purchased from Instituto de Investigaciones Bioquímicas Fundación Campomar (Buenos Aires, Argentina). Leupeptin, pepstatin, TLCK, APMSF, UDP-glucose, Mes, DBM and Tween 20, were from Sigma. Amyloglucosidase from *Aspergillus niger,* α-amylase from *Bacillus subtilis* and D-glucose test kit *(hexokinase / glucose-6-phosphate dehydrogenase containing reagent)* were from Boehringer Mannheim and C_{18} cartridges from Waters. Amyloglucosidase and α-amylase were purified as described before (9).

Preparation of tissue homogenates and amylolysis of proteoglycogen. The muscle, liver and brain homogenates were prepared by

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Abbreviations: TLCK, Nα-p-tosyl-L-lysine chloromethyl ketone; APMSF, 4 amidinophenylmethylsulfonyl fluoride; DBM, n-dodecyl-β-D-maltoside; DBMT, *n*-dodecyl-β-D-maltotrioside.

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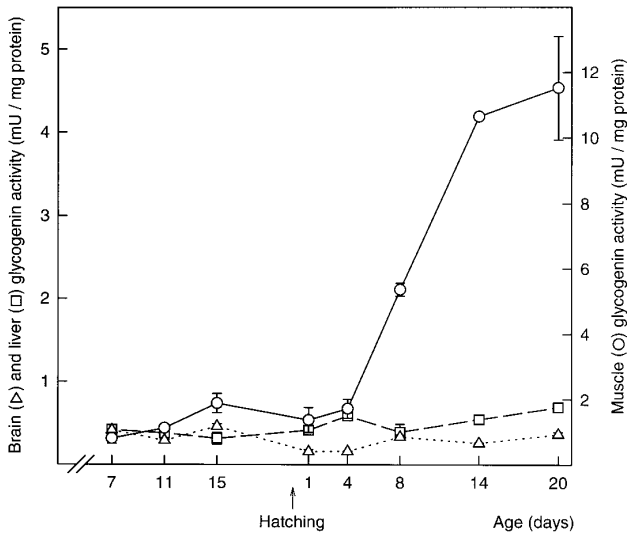

FIG. 1. Glycogenin activity in muscle, liver and brain of developing chicken. Glycogenin transglucosylation activity was measured in amylolyzed homogenates prepared from 3 to 12 embryos and 2 chicks of each age, as indicated in Materials and Methods. Each value is the mean ± S.E. of two independent experiments.

ultrasonic disruption in 5 mM Tris-acetate buffer pH 7.5 containing 8% sucrose, at 1-2°C. The homogenates were prepared from 12, 6 and 3 embryos of seven, eleven and fifteen days, respectively and from 2 chicks of one, four, eight, fourteen and twenty days.

For the assay of glycogenin, the homogenate samples (1.0 mg protein) were subjected to amylolysis for 12 h at 25°C with 0.3 μg of α-amylase, in a final volume of 100 μl containing 0.28 M Mes, 0.3 mM $CaCl_2$, 0.06 % sodium azide, 0.12 mM TLCK and 0.03 mM of each leupeptin, pepstatin and APMSF. For estern blot analysis, the homogenate samples (0.5 mg protein) were amylolyzed in a similar system except that 1 μg of α-amylase was used, amyloglucosidase (1μg) was included, and Mes was omitted.

Assay for glycogenin activity. The incubation mixture contained the following components, in a final volume of 60 μl : UDP-[^{14}C]-glucose 8 μM, 0.1 M Mes pH 7.0, 5 mM $MnSO_4$, 0.2 mM DBM and 50 μl of amylolyzed homogenate (0.5 mg of protein). The incubation was done at 30°C for 7 min, and the reaction was terminated by addition of 16 μl of 0.1 M EDTA, the solution was made 1 mM in glucose and 2 mM in UDP-glucose in a final volume of 200 μl and passed through a C_{18} cartridge. The cartridge was washed with 3.0 ml of water and the [^{14}C]glucosylated DBM eluted with 0.6 ml of methanol and counted after addition of scintillation solution. One unit of activity is defined as 1 nmol of [^{14}C]glucose incorporated into DBM per minute, under the conditions of the assay.

Assay for glycogen synthase activity. The incubation conditions were as described by Thomas et al. (16), in a final volume of 30 μl containing 0.25 mg of homogenate protein, using 6.7 mM UDP-[^{14}C]glucose (1000 cpm/nmol) and where indicated, 6.7 mM glucose-6-phosphate. The reaction was terminated by the addition of 200 μl of 1% KCl containing 0.4 mg of glycogen, followed by 400 μl of ethanol. The incorporation of [^{14}C]glucose into glycogen was measured as indicated before (17). One unit of activity is defined as 1 μmol of [^{14}C]glucose incorporated into glycogen per minute (16).

Other procedures. The polyclonal antibody against chicken muscle glycogenin was raised in rabbit as previously reported (10). Immunoblot analysis was carried out as described before (15) with the following modifications : 12% acrylamide resolving gel was used, the anti-glycogenin serum was diluted 1 :200, PBS was supplemented with 0.12 % Tween 20 for washing after the incubation with the anti-glycogenin serum, and antibodies bound to glycogenin were detected by incubation with 0.25 μCi of [^{125}I]-labeled protein A, followed by radioautography.

Protein was measured according to the Lowry procedure (18) and DNA as described by Labarca and Paigen (19). For the quantification of glycogen the samples (2.2 mg of protein) were subjected to amylolysis with α-amylase (1 μg) and amyloglucosidase (4 μg) in a final volume of 100 μl containing 1mM $CaCl_2$ and 0.2 % sodium azide, at 37°C for 20 h. The released glucose was measured with an hexokinase/glucose-6-phosphate dehydrogenase reagent kit (9).

RESULTS

Glycogenin Activity

The glycogenin activity was determined by measuring transglucosylation of the exogenous acceptor DBM, after releasing the enzyme from proteoglycogen by digestion with α-amylase (9). Liver showed no substantial changes of glycogenin activity through development and at any time the specific activity was lower than in muscle (Fig. 1). On the contrary, in muscle the specific activity doubled between seven-day-old embryos and four-day-old chicks, showing a remarkable rise thereafter. In twenty-day-old chicks the specific activity reached about fourteen times the value determined in seven-day-old embryos. In brain the specific activity was as low as in liver and also showed no substantial changes. The rise of activity in muscle correlated with increase of the glycogenin expression, as revealed by Western blot analysis of amylolyzed homogenates from seven-day-old embryos and four- and twenty-day-old chicks (Fig. 2).

When the specific activity was expressed per DNA (results not shown), both liver and brain showed a moderate increase, that reached in the twenty-day-old chicks about four times the value determined in seven-day-old embryos. In muscle, the activity per DNA increased fifty times during the same period.

Glycogen Synthase Activity and Glycogen Content

The active I form of glycogen synthase increased throughout development about eleven and fourteen

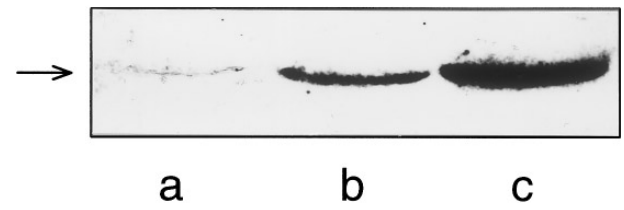

FIG. 2. Expression of glycogenin in chicken muscle. Samples of amylolyzed muscle homogenates from 7-day-old embryos (a) and 4-day- (b) and 20-day-old (c) chicks (125 μg of protein) were analyzed by SDS-PAGE followed by transfer to nitrocellulose and immunolabeling with anti-glycogenin antibodies and [^{125}I]-labeled protein A, followed by radioautography. The arrow points to immunolabeled glycogenin.

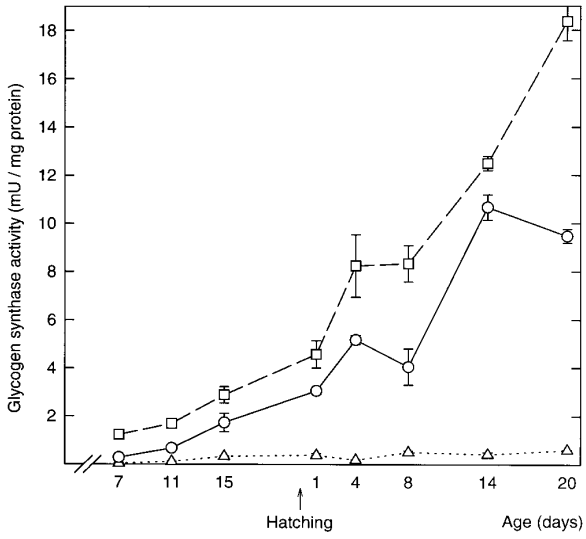

FIG. 3. Glycogen synthase activity in muscle, liver and brain of developing chicken. The independent form of glycogen synthase was measured in muscle (○), liver (□) and brain (△) as indicated in Materials and Methods. Each value is the mean ± S.E. of two independent homogenates prepared as mentioned in Fig. 1.

times in liver and muscle, respectively (Fig. 3). Total glycogen synthase activity was also measured and the active to total enzyme ratio determined. The bulk of the enzyme (80-100 %) was under the active form after hatching except in muscle of chicks at eight to twenty days, where 40-50 % of the enzyme was as the inactive D form (results not shown).

The glycogen concentration in liver increased continuously with development except for the drop observed in the fourteen-day-old chick (Fig. 4). At twenty days posthatching liver showed about eight and ten times higher glycogen content than muscle and brain, respectively. No substantial changes of the glycogen content occurred in muscle and brain during the developmental period studied.

DISCUSSION

Glycogenin is found under the proteoglycogen form in tissues of mammals and chicken (7, 13-15). Since glycogenin is inactive under the proteoglycogen form (9), the determination of glycogenin through its transglucosylation activity required previous release from the glycoconjugate by digestion with α-amylase. We choose to measure the transglucosylation activity toward an exogenous acceptor rather than autoglucosylation, because of the heterogeneous and undetermined structure of both, the self acceptor generated by amylolysis and the reaction product of autoglucosylation. Even more, the product of autoglucosylation would be subjected to degradation by α-amylase during the assay, thus requiring its elimination from the digested tissue preparation or its total inhibition. On the contrary, the transglucosylation of DBM from UDP-glucose results in DBMT as the unique reaction product (9, 20) and both, acceptor and reaction product are resistant to α-amylase (result not shown).

The glycogenin activity increased substantially during development only in muscle (Fig. 1) and was accompanied by an increase in the amount of immunolabeled enzyme (Fig. 2). Thus, the specific activities measured in muscle through development were positive correlated with glycogenin concentrations.

Figs. 1 and 3 show that the increase of glycogenin activity in muscle was accompanied by a similar increase of glycogen synthase activity. In liver also a noticeable increase of glycogen synthase activity was observed with development progression and as glycogenin activity remain constant, the glycogen synthase / glycogenin specific activity ratio increased.

The glycogenin activity in the different organs (Fig. 1) is consistent with the low glycogenin content found in liver compared to that of muscle (21, 22). The levels of glycogen synthase activity (Fig. 3) are in accordance with the higher polysaccharide content in chicken liver than muscle (Fig. 4). Considering that the glycogenin activities measured (Fig. 1) reflect the amount of enzyme (Fig. 2), and taking into account that all the glycogenin is under proteoglycogen form, our results indicate that the molar concentration of proteoglycogen is higher in muscle than in liver. Whether there is glycogenin-free glycogen besides proteoglycogen in chicken tissues can not be ascertained at present. Since there is not known intracellular activity able to originate a

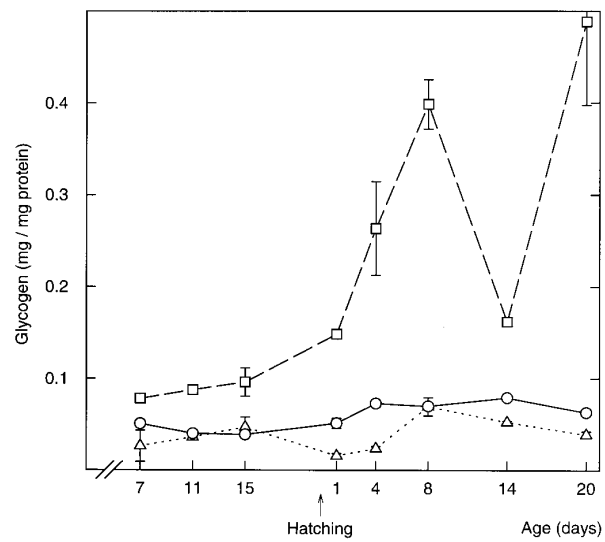

FIG. 4. Glycogen concentration in muscle, liver and brain of developing chicken. Glycogen was determined in muscle (○), liver (□) and brain (△) as indicated in Materials and Methods. Each value is the mean ± S.E. of two independent homogenates prepared as mentioned in Fig. 1.

free reducing end group in glycogen, or release the *de novo* synthesized polysaccharide from its protein moiety, the low molar concentration of proteoglycogen and the high glycogen content of liver would indicate that the size of the polysaccharide moiety of proteoglycogen is larger in chicken liver than in muscle.

The higher number of proteoglycogen molecules in muscle than in liver would be related to the necessity of a high *de novo* glycogen biosynthesis in the former tissue. It has been described that the glycogenin released from muscle proteoglycogen by polysaccharide depletion produced by hormonal or electrical stimulation is able to initiate the synthesis of new proteoglycogen molecules (23). A high concentration of protein primer might be necessary to allow the quick recovery of glycogen after depletion by hard muscle exercise. Such a quick recovery of polysaccharide content might be not necessary in liver where, except under fasted conditions, glycogen is mainly subjected to partial turnover.

In the present work we have described by the first time, that the *de novo* biosynthesis of glycogen is subjected to modulation in muscle at the level of glycogenin expression, during chicken development. Further work will be required to determine the mechanism regulating glycogenin synthesis.

ACKNOWLEDGMENTS

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