

Action Patterns of Phosphorylase and Glycogen Synthetase on Glycogen

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The action patterns of liver and muscle glycogen synthetases and of muscle phosphorylase *b* on glycogen samples of different molecular weight and on β -amylase limit dextrans were studied. For this purpose a method for measuring the number of newly added glucose residues that are at non-reducing ends was developed.

It was found that glucose transfer to the non-reducing ends of glycogen catalyzed by liver glycogen synthetase and muscle phosphorylase followed a Poisson distribution.

The number of outer chains in the glycogen molecules available to both enzymes appeared to be smaller than the actual number of outer chains in the polysaccharide. The number of such available chains diminished as the glycogen was heavier. For the same glycogen sample, the number of available chains to phosphorylase appeared to be equal or smaller than that to glycogen synthetase.

It was found that muscle phosphorylase transferred 1.2 to 1.4 glucose moieties successively per outer chain, independently of the molecular weight of the glycogen.

The number of glucose units added successively to the non-reducing ends of glycogen by liver glycogen synthetase increased from 1.7 to 6.8 with the molecular weight of the polysaccharide.

Both phosphorylase and liver glycogen synthetase transferred more glucose units in a repetitive way to the same outer chain of the β -amylase limit dextrin than to the same outer chain of the undegraded glycogen.

Muscle glycogen synthetase transferred a greater number of glucose units successively per outer chain of glycogen than the liver enzyme.

The outer chains of glycogen can be elongated *in vitro* by the action of phosphorylase or glycogen synthetase. It is generally accepted that the latter enzyme functions during the elongation *in vivo* while the former has only a degradative role.

The term “action pattern” has been employed in connection with oligo and polysaccharide biosynthesis to express the number of glycosyl units that are transferred to a certain acceptor group before the enzyme diffuses to another group. The action pattern of several glycosyl transferring enzymes on oligo and polysaccharides have been reported (for references see Discussion). However, nearly all these studies and especially those carried out on polysaccharides (glycogen, amylopectin and their dextrans) were performed in such a way that the results were only qualitative.

Unusual Abbreviation. Glucose 1-phosphate, Glc-1-P.

Enzymes. Phosphorylase or α -1,4-glucan:orthophosphate glucosyltransferase (EC 2.4.1.1); glycogen synthetase or UDPglucose:glycogen α -4-glucosyltransferase (EC 2.4.1.11); branching enzyme or α -1,4-glucan: α -1,4-glucan 6-glycosyltransferase (EC 2.4.1.18).

The work reported in this paper was undertaken in order to investigate in a more accurate way the action patterns of liver and muscle glycogen synthetase and of muscle phosphorylase *b* on several types of glycogen. For this purpose, a method for measuring the number of newly added glucose residues that are at non-reducing ends was developed.

MATERIALS AND METHODS

Enzymes

Liver and muscle glycogen synthetases with low endogenous acceptor activity were obtained as already described [1,2]. Crystalline muscle phosphorylase *b* was obtained according to Fischer *et al.* [3]. β -Amylase was purchased from Mann & Co. (New York).

Glycogen

Several types of glycogen were used. Rat liver glycogen was obtained according to Laskov and Gross [4] with minor modifications. Three samples

of different molecular weight were obtained from it: the lightest one (A) was obtained by treatment of the whole glycogen with 0.1 N HCl for 15 min at 37°. The other two samples from the rat liver glycogen (B and C) were obtained by large scale separations in sucrose gradients, (C was heavier than B). Rabbit liver glycogen (D) was purchased from Mann & Co. Sample E was obtained by sonication of sample D in a Raytheon sonicator for 90 min in 8 M urea at 10 kHz. This treatment drastically reduces the molecular weight of the glycogen without appreciably affecting the length of the outer chains or the percent of non-reducing ends. A β -amylase limit dextrin (F) was obtained by exhaustive β -amylolysis of glycogen A. Synthetic glycogen (G) was synthesized from Glc-1-P by muscle phosphorylase and rat liver branching enzyme as already described [5]. The molecular weight distributions of all the glycogen samples used are shown in Fig. 1 A–B.

Materials

Glc-1-P and UDPG were obtained from Sigma Chemical Co. (St. Louis, Mo.). Glc-1-P and glucose (both uniformly labeled with ^{14}C) were obtained from the Radiochemical Centre (Amersham). Radioactive UDPG was prepared from $[\text{U-}^{14}\text{C}]$ glucose according to Wright and Robbins [6]. Contaminating uridine diphosphate galactose was separated from UDPG by paper chromatography in morpholinium borate [7]. α -Methyl-glucoside (uniformly labeled with ^{14}C in the glucose moiety) was prepared according to Bollenback [8].

Analytical

The analytical methods used were as follows: glycogen was determined according to Dubois *et al.* [9] and non-reducing ends with periodate as described by Fales [10]. The degree of β -amylolysis was determined by incubating glycogen and a commercial preparation of β -amylase in 0.1 M citrate buffer of pH 6.4. Aliquots were taken at several intervals until the reducing power reached a plateau. The product was characterized as maltose by paper chromatography in butanol–pyridine–water (6:4:3, by vol.) [11]. The reducing power was measured by the Somogyi-Nelson [12, 13] method.

Incubation Mixture and Glycogen Purification

The basic incubation mixture contained: 10.5 mg of glycogen, 0.1 M glycylglycine buffer of pH 8.4, 3.75 mM glucose 6-phosphate, 50 mM 2-mercaptoethanol, 50 mM EDTA (pH 8.6), 2.25 mM adenosine monophosphate and 200 μl of liver glycogen synthetase. The glycogen synthetase preparations were usually contaminated with traces of branching enzyme. However, the latter enzyme was found to

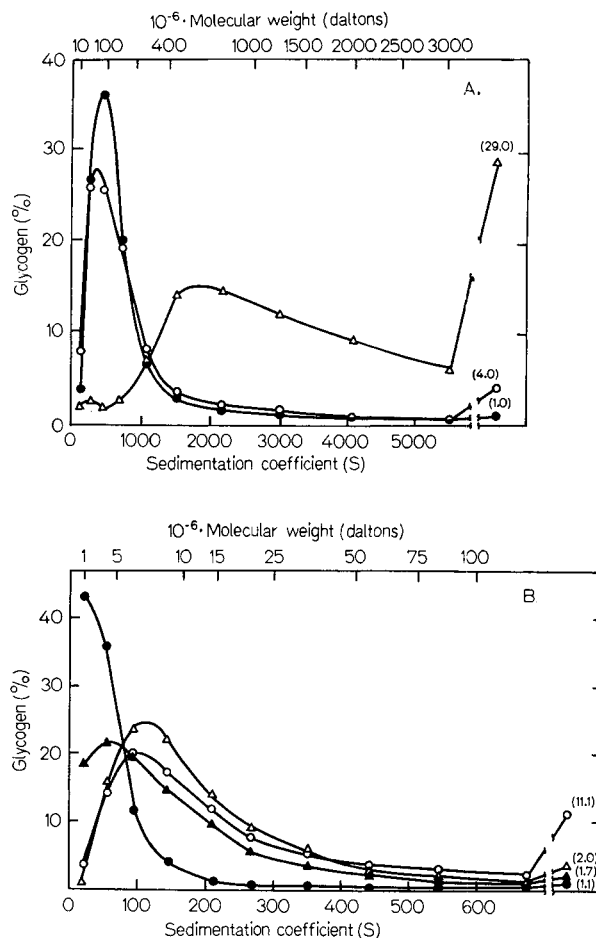


Fig. 1. *Molecular weight distributions of the glycogens.* (A) Glycogens: B (○), C (△), and G (●). (B) Glycogens: A (△), D (○), E (●), and F (▲). The origin of the different glycogen samples is described in Methods. The centrifugation times of the sucrose gradients were 33 and 180 min for A and B, respectively. The numbers in parentheses represent the percents of glycogen in the pellet. This corresponds to glycogen of sedimentation coefficient higher than 5500 (A) or 680 S (B) plus that which sediments along the walls of the tube. Other conditions of the sucrose gradients centrifugations have already been reported [26].

be inactive in 0.1 M glycylglycine pH 8.4, 50 mM EDTA pH 8.6 when assayed as described by Krisman [14].

Radioactive UDPG ($86600 \text{ counts} \times \text{min}^{-1} \times \mu\text{mole}^{-1}$) was added to the basic incubation mixture up to a final concentration of 7.5 mM in order to measure the action pattern of glycogen synthetase. For the determination of the action pattern of muscle phosphorylase 100 μl of crystalline phosphorylase *b* in glycerol and radioactive Glc-1-P ($112000 \text{ counts} \times \text{min}^{-1} \times \mu\text{mole}^{-1}$) up to a final concentration of 15 mM were added to the basic incubation mixture.

The total final volume of the mixture was 2.0 ml. The amount of some of its components was modified where stated. When incubated with the basic incubation mixture, liver glycogen synthetase and phosphorylase incorporated 350 and 450 nmoles of glucose per minute, respectively.

Ten 0.2 ml aliquots were taken from the mixture after different times at 37°. Each aliquot was placed in a tube containing 1 ml of 33% KOH plus unlabelled carrier glycogen so the final amount of polysaccharide was 2 mg. After heating the tubes for 10 min at 100°, glycogen was precipitated by the addition of 1.5 ml of ethanol. The glycogen was redissolved in water and reprecipitated with ethanol twice more.

Determination of Radioactive Non-Reducing Ends

After the third precipitation, glycogen was dissolved in 0.2 ml of water, 0.1 ml of which was used to measure total glucose incorporation. The periodate oxidation conditions were those already described by Fales [10]: 0.1 ml of a 0.2 M sodium metaperiodate solution was added to the remaining 0.1 ml of the glycogen solutions and the tubes were kept for 48 h at 4° after which 10 μ l of ethyleneglycol were added. After 1 h at room temperature, the samples were subjected to paper electrophoresis in order to separate the radioactive formic acid from the rest of the radioactive molecules: 50 μ l of the solutions were placed in a 2.5 \times 22 cm Whatman No 1 paper at 7 cm from one edge. Special care was taken in order to avoid the drying of the papers, so they were immediately imbibed in 0.1 M Tris-Cl buffer of pH 7.8. Electrophoresis was performed at 4° in an apparatus similar to that described by Grassmann and Hannig [15] with a constant current of 2 mAmp per paper for 40 min after which the papers were dried at room temperature. The papers were then cut into strips, placed in toluene scintillation solution and counted. The bulk of the radioactivity remained at the origin or slightly displaced towards the anode while the formic acid appeared as a rather sharp peak at about 7–8 cm from the origin in the same direction.

Each non-reducing terminal glucose residue gives one formic acid molecule so that the specific radioactivity of the latter (expressed as counts \times min⁻¹ \times μ mole⁻¹) is 6-fold smaller than that of the parental glycosyl donor.

In order to test the method used for measuring the radioactive non-reducing ends, α -methyl glucoside (uniformly labeled with ¹⁴C in the glucose moiety) was treated with sodium periodate and the product run in paper electrophoresis under the same conditions used for the glycogen samples. The radioactivity of the peak far from the origin was 16.80

and 16.93% of the total in two experiments. The theoretical value is 16.67%.

A similar method was used by Verhue and Hers [16] for measuring radioactive non-reducing end groups but the formic acid was separated by extraction with ether.

RESULTS

ACTION PATTERNS OF THE ENZYMES—THEORETICAL

Three mechanisms for the elongation of the outer chains of glycogen may be envisaged. They are similar to those that have been described by Bailey and Whelan [17] for the mechanism of β -amylase degradation (see also [18]). These mechanisms are:

- a) Single chain elongation in which glucose residues are added successively to the same chains;
- b) multiple chain elongation in which the glucose residues are added randomly to all chains;
- c) multirepetitive chain elongation which is a combination of the above mentioned mechanisms. The glucose residues are added randomly but in groups of more than one. This mechanism corresponds to that called multiple attack by French and Wild [19].

The Poisson Distribution

If it is assumed that after glucosylation by a random mechanism (mechanism b) the outer chains have the same probability for further glucosylation as the outer chains that have not been enlarged, the process can be calculated theoretically by Poisson's equation [20].

$$C_- = Ca e^{-(T/Ca)}. \quad (1)$$

Where C_- is the number of chains that have not received glucose residues although they are capable of accepting them, Ca is the total number of available chains, which is supposed to remain constant and T is the total number of glucose residues added.

If C_+ is the number of chains which have received one or several glucosyl residues, then $C_+ + C_- = Ca$ and

$$C_+ = Ca(1 - e^{-(T/Ca)}). \quad (2)$$

If more than one residue is transferred successively per chain as in mechanism c (multirepetitive chain elongation) the equation becomes

$$C_+ = Ca(1 - e^{-(T/Ca \cdot t)}) \quad (3)$$

where t is the number of residues transferred successively per chain. This value should be one for random multichain elongation and higher for multirepetitive chain elongation.

Calculation of C_a and t

If only C_+ and T are known, calculation of the constants C_a and t can be performed as follows. The inverse of Equation (3) can be developed in the following way:

$$\frac{1}{C_+} = \frac{1}{C_a(1 - e^{-(T/C_a \cdot t)})} \quad (4)$$

$$e^{-(T/C_a \cdot t)} = 1 - \left(\frac{T}{C_a \cdot t}\right) + \left(\frac{T}{C_a \cdot t}\right)^2 \frac{1}{2!} - \left(\frac{T}{C_a \cdot t}\right)^3 \frac{1}{3!} + \dots \quad (5)$$

For small values of T

$$e^{-T/C_a \cdot t} = 1 - \frac{T}{C_a \cdot t} \quad (6)$$

Then

$$\frac{1}{C_+} = \frac{t}{T} \quad (7)$$

From Equation (4) it follows that a plot of $1/C_+$ vs. $1/T$ should give curves that intercept the ordinates at $1/C_a$ and that asymptotize the straight line described by Equation (7) at high values of $1/T$.

Theoretical values of C_+ were calculated for different values of T , C_a and t . The ranges of those values were those found under our experimental conditions (T : 80–900 nmoles of glucose) or those supposed to occur (C_a : 150, 300 and 500 nmoles of glucose; t : 1, 3 and 5).

It was found that within those ranges, representation of $1/C_+$ vs. $1/T$ gave practically straight lines nearly parallel to those described by Equation (7) (Fig. 2A–C and Table 1). It can be seen that if those straight lines are extrapolated for $1/T = 0$, they

Table 1. Action patterns of the enzymes—Theoretical. The formula used was Equation (3). C_a is the total number of available chains, t is the number of glucose residues that are transferred successively to the same chain, $1/C_a'$ is the value at which the extrapolation of the straight lines of Fig. 2A–B–C intercept the ordinate scale, t' is the same as t but obtained graphically from the slopes of the straight lines of Fig. 2A–B–C

C_a (non-reducing end groups)	t	C_a'	C_a'/C_a	t'
150	1	222.2	1.48	0.93
	3			2.80
	5			4.80
300	1	465	1.55	0.93
	3			2.83
	5			4.88
500	1	800	1.60	0.94
	3			2.98
	5			5.0

intercept the ordinates at a point ($1/C_a'$) different from $1/C_a$ and also from the origin. For a fixed C_a and different t values, extrapolation of all the straight lines intercepted the ordinates at the same $1/C_a'$ point.

It was found graphically that the quotient between C_a' and C_a is approximately equal to 1.50 within a rather wide range of C_a values (Table 1).

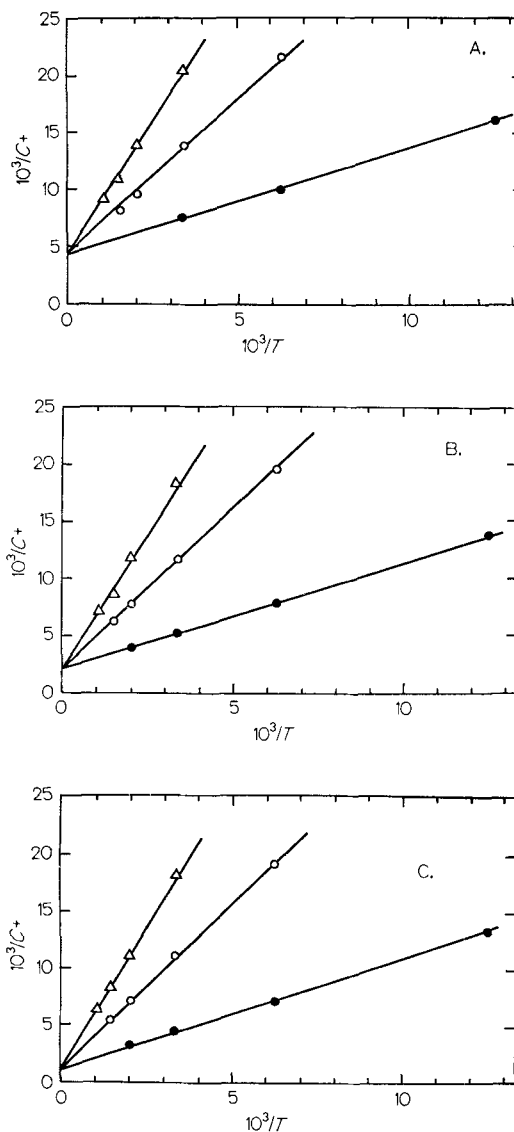
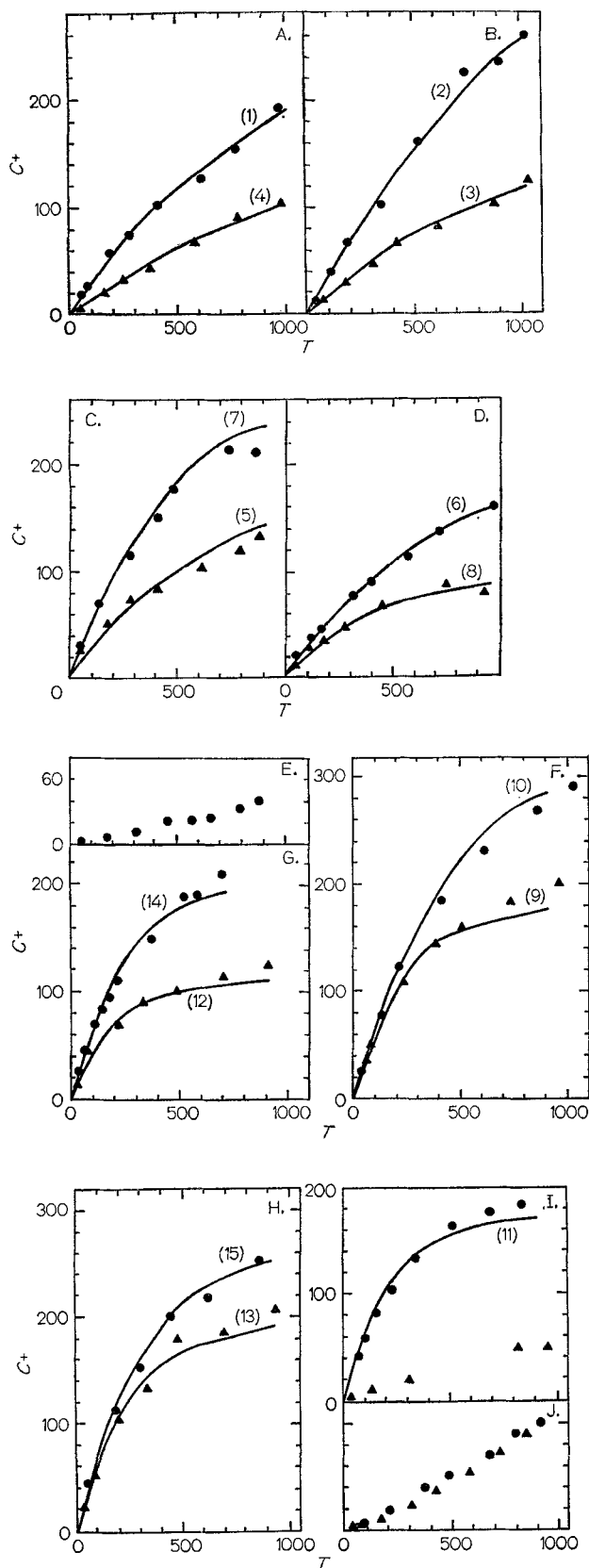


Fig. 2. Action patterns of the enzymes—Theoretical. The formula used was Equation (3) (see text). T is the total number of residues added expressed as nmoles of glucose. C_+ is the number of non-reducing ends (expressed as nmoles of glucose) that have received one or several glucose residues. (A), (B) and (C) correspond to $C_a = 150$, 300 and 500, respectively. The t values were 1 (●), 3 (○) and 5 (△). C_a is the total number of available non-reducing ends expressed as nmoles of glucose and t is the number of glucose residues transferred successively per chain



ACTION PATTERNS OF THE ENZYMES—EXPERIMENTAL

The results of measurements of C_+ and T under different conditions are shown in Fig.3A—J. The plot of the inverse of the experimental values ($1/C_+$ vs. $1/T$) gave straight lines similar to those shown in Fig.2A—C. From the slopes of those lines the t values were obtained. Extrapolation for $1/T = 0$ gave the Ca' values. They were divided by 1.5 in order to obtain the total number of available chains (Ca). The results are shown in Table 2.

The curves obtained by using these values in the Poisson equation [Equation (3)] are also shown in Fig.3. It may be observed that the fit is very good. The χ^2 test [21] showed that all the experimental data followed the Poisson distribution within a security range of 95%. Elongation of maltohexaose by potato phosphorylase also followed the same distribution as shown by Whelan and Bailey [22] by a different detection method.

One question which may be raised is if by adjusting both constants in the Poisson equation any type of results (C_+ and T) could be fitted in and then the values of Ca and t may not represent what is believed they do. However, the value of Ca becomes double when the amount of glycogen is increased 2-fold. This result was obtained both with glycogen synthetase (Expts. 1 and 2, Table 2) and with phosphorylase (Expts. 9 and 10, Table 2). In both cases the values of t remained constant. On the other hand the enzyme concentration did not appreciably change Ca or t (Expts. 5 and 6, 13 and 14, Table 2). It seems therefore that the calculated values of Ca and t are reliable.

Fig.3. Action patterns of the enzymes—Experimental. Full points or triangles correspond to experimental results and the curves to values of C_+ obtained by Equation (3) and the values of t and Ca obtained as described in text (see Table 2). The meaning and units of T , C_+ , Ca and t are in Fig.2 and in text. The numbers in parentheses indicate the number of the experiment as shown in Table 2. (A)—(E) correspond to liver glycogen synthetase, (F)—(I) to muscle phosphorylase *b*, and (J) to muscle glycogen synthetase. (A): Glycogen A (●) and glycogen C (▲); (B): Double glycogen A concentration (●) and glycogen B (▲); (C): Glycogen E (●) and glycogen D (▲); (D): Glycogen D (●) with triple enzyme concentration and glycogen G (▲); (E): Glycogen F (●); (F): Glycogen A (●) and double glycogen A concentration (▲); (G): Glycogen D (●) with triple enzyme concentration and glycogen C (▲); (H): Glycogen E (●) and glycogen D (▲); (I): Glycogen A (●) without glycogen synthetase in the incubation mixture and glycogen F (▲); (J): Glycogen D. Two muscle glycogen synthetases were used. One (●) is capable of high molecular weight glycogen synthesis *in vitro* and the other is not (▲). The origin of the different types of glycogen is explained in Methods. Their molecular weight distributions are shown in Fig.1A—B

Table 2. Action patterns of the enzymes—Experimental

Further explanation of the nature of the different types of glycogen is given in Methods. The values of molecular weight are those of the peaks of the molecular weight distributions shown in Fig. 1A—B. *Ca* and *t* values were obtained as described in text, for their meaning see legend of Table 1. All glycogen samples had approximately the same percentage of non-reducing ends (7.0—7.1%) and also the same outer chain length (48—52% of β -amylolysis). Each aliquot of 0.2 ml from the incubation mixtures had then 455—462 nmoles of total non-reducing glucose residues (except those from Expts. 2 and 10 which had double glycogen concentration). The values given for the percent of available chains (*Ca*) are referred to the total ones. Experiments performed with the β -amylase limit dextrin (Fig. 3E and 3I) and muscle glycogen synthetase (Fig. 3J) were omitted here because the values of *Ca* and *t* obtained were not reliable due to the small values of \bar{C}_+ obtained even at high values of *T*.

Expt. no.	Enzyme		Glycogen type	Mol. wt.	<i>Ca</i>	Available chains	<i>t</i>	
				$\times 10^{-4}$				
				daltons	nmoles Glc	%		
1	Liver	A	Rat liver acid treated	8	221	48.0		2.90
2	glycogen	A ^a	Rat liver acid treated	8	445	48.4		2.70
3	synthetase	B	Rat liver	60	206	44.8		5.55
4		C	Rat liver	700	190	41.3		6.80
5		D	Commercial rabbit liver	7	187	40.6		3.50
6		D ^b	Commercial rabbit liver	7	190	41.3		3.10
7		E	Commercial rabbit liver sonicated	1	276	60.0		1.70
8		G	Synthetic	70	95	20.7		4.20
<hr/>								
9	Muscle	A	Rat liver acid treated	8	170	37.0		1.25
10	phospho- rylase <i>b</i>	A ^a	Rat liver acid treated	8	334	36.3		1.40
11		A ^c	Rat liver acid treated	8	170	37.0		1.20
12		C	Rat liver	700	103	22.4		1.35
13		D	Commercial rabbit liver	7	190	41.3		1.25
14		D ^b	Commercial rabbit liver	7	190	41.3		1.20
15		E	Commercial rabbit liver sonicated	1	276	60.0		1.25

^a The same as the preceding experiment but with double glycogen concentration.

^b The same as the preceding experiment but with triple enzyme concentration.

^c The same as experiment 9 but without glycogen synthetase in the incubation mixture.

Liver Glycogen Synthetase and Muscle Phosphorylase

The liver glycogen synthetase preparations were not as pure as those of muscle phosphorylase *b*. The former enzyme could be contaminated with traces of α -amylase or branching enzyme. Therefore in order to equalize conditions glycogen synthetase was added to the incubation mixtures where the action pattern of phosphorylase was determined. However, Expts. 9 and 11 (Table 2) (Fig. 3F and I) showed that synthesis by phosphorylase was not affected by the presence of glycogen synthetase.

Available Chains

The results show (Table 2) that the number of chains measured with periodate is much higher than the number of end groups available to the enzymes (*Ca*). The latter vary with different types of glycogen. Heavier glycogens had fewer available chains than the lighter ones of the same origin. (Rat liver glycogen; glycogen synthetase: Expts. 1, 3 and 4; phosphorylase: Expts. 9 and 12. Commercial rabbit liver glycogen; glycogen synthetase: Expts. 5 and 7; phosphorylase: Expts. 13 and 15, Table 2).

Glycogen synthesized with phosphorylase and Glc-1-*P* gave the lowest percentage of available groups for glycogen synthetase (20.7%). This value

must be compared with that of Expt. 3 (44.8%) in which a native glycogen of approximately the same molecular weight was used.

For the same glycogen, the number of available chains for phosphorylase appeared to be equal (Expts. 5 and 13, 6 and 14, 7 and 15, Table 2) or smaller (Expts. 1 and 9, 2 and 10, 4 and 12, Table 2) than that for glycogen synthetase. According to Brown *et al.* [23, 24] muscle glycogen synthetase only uses the main chains as acceptor while phosphorylase uses both main and side branches. If this were so, after covering the chains available to glycogen synthetase, addition of phosphorylase and radioactive Glc-1-*P* should give rise to the introduction of more radioactive end groups. To check this point an experiment was carried out as follows: sonicated glycogen was incubated with liver glycogen synthetase and radioactive UDPG until the labelled glucoses at non-reducing ends nearly reached a plateau (Fig. 4). Glycogen synthetase was then inactivated by heating the incubation mixture for 2 min at 100°. After cooling the tube, phosphorylase and labelled Glc-1-*P* were added to it. As shown in the same figure, no more radioactive non-reducing ends were produced as if further glucosylation by phosphorylase occurred only at the same non-reducing ends already labelled by the synthetase.

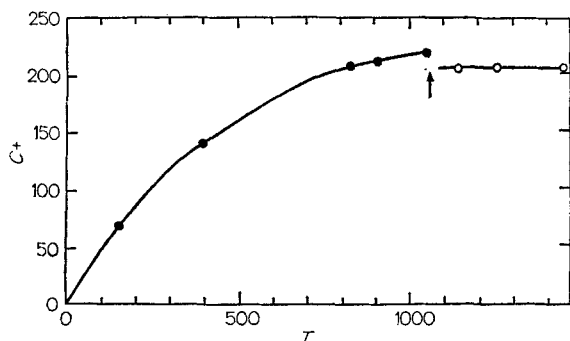


Fig. 4. Glucose transfer by liver glycogen synthetase and muscle phosphorylase b on the same glycogen. Glycogen E was incubated with liver glycogen synthetase and radioactive UDPG (●) as described in Methods until total glucose transfer (T) was 1045 nmoles (arrow). The tube was heated for 2 min at 100°, cooled and further incubated with phosphorylase and radioactive Glc-1- P at 37° (○). A correction for dilution was made. For the meaning and units of T and C_+ see legend on Fig. 2

Action Patterns

Both phosphorylase and liver glycogen synthetase appeared to act by a multirepetitive chain elongation mechanism. However, for the same glycogen, the action pattern of phosphorylase was close to a multiple chain elongation mechanism since the values of t were near to one but much higher for synthetase (compare the t values of Expts. 1 and 9, 2 and 10, 4 and 12, 5 and 13, 6 and 14, 7 and 15, Table 2).

It appears from the t values of Expts. 1, 3 and 4, 5 and 7 (Table 2) that the molecular weight of the glycogen influences the action pattern of glycogen synthetase: as the glycogen is heavier, the values of t increase and may be as high as 6.8. The same is not true for the action pattern of phosphorylase (compare the t values of Expts. 9 and 12, 13 and 15, Table 2).

β -Amylase Dextrin

The elongation of the chains of the β -amylase dextrin seems to occur by a nearly single chain mechanism both with phosphorylase and liver glycogen synthetase: as shown in Fig. 3A and E, F and I, for the same extent of glucose transfer there are far fewer radioactive ends in the β -amylase dextrin than in the original glycogen.

Muscle Glycogen Synthetase

The elongation mechanism of muscle glycogen synthetase appeared to be closer to a single chain one than that of the liver enzyme: for the same total glucose transfer there were less radioactive glucoses at non-reducing ends in the experiments performed

with muscle glycogen synthetase (Fig. 3C and J). Two muscle enzymes were used: an aged preparation that is capable of high molecular weight glycogen synthesis *in vitro* and a fresh one that is not [2]. No difference in their action patterns was found.

DISCUSSION

One of the interesting results of the measurements is the fact that only a part of the end groups measured with periodate is used by the transferring enzymes. Two explanations can be considered. One is that some of the chains are not used as substrates for reasons of enzyme specificity. For instance some outer chains could be too short. This seems to be the case with β -limit dextrans which are dealt with below. The other explanation for the use of only some chains is that some of them are buried in the interior of the glycogen molecule where the enzymes cannot penetrate. If this were the case it seems likely that heavier glycogens should have more buried chains. Glycogen of high molecular weight appears under the electron microscope as big particles (α particles), composed of rosettes or clusters of smaller elements (β particles) [25]. The α particles are decomposed under the action of alkali or mild acid into subparticles of 8 million daltons molecular weight (glycogen A), composed in average by 2.4 β particles [26, 27]. The non-reducing ends not accessible to the transferring enzymes are in part possibly those of the β particles that are in the inner parts of the α particles.

For glycogen C, which is the heaviest, the percent of total ends available for glycogen synthetase and phosphorylase was 41.3 and 22.4, respectively, (Expts. 4 and 12, Table 2). When that glycogen was broken down to molecules of about 8 million daltons by mild acid treatment, the respective values increased up to 48 and 37% (Expts. 1 and 9, Table 2). From these figures it may be deduced that glycogen synthetase can enter more deeply into the glycogen molecules of high molecular weight than phosphorylase. Even the molecules of 8 million daltons seemed to have non-reducing ends hidden to glucose transfer: further breakdown of such molecules by sonication produced lighter glycogen with more outer chains accessible to both enzymes (compare C_a of Expts. 5 and 7, 13 and 15, Table 2).

The lowest percentage of available chains for glycogen synthetase was found in glycogen synthesized from Glc-1- P (20.7%, Expt. 8, Table 2). The value for a native glycogen of approximately the same molecular weight was considerably higher (44.8%, Expt. 3, Table 2). This seems understandable since electron microscopy has shown that the molecules of synthetic glycogen have a more compact aspect with less defined subparticles than native glycogen. Therefore, glucosylation of non-reducing

ends of the β particles that are not in the external surface of the molecules is possibly facilitated in native glycogen by its more loose structure.

The elongation mechanism of phosphorylase resembles more a multichain one than that of glycogen synthetase (compare the t values of Expts. 1, and 9, 2 and 10, 4 and 12, 5 and 13, 6 and 14, 7 and 15, Table 2). These results agree with those of Brown *et al.* [24] with phosphorylase limit dextrin. They found that when such degraded polysaccharide was enlarged to the same extent by muscle glycogen synthetase or by muscle phosphorylase, the product gave more color with iodine in the former case. That is as if transfer from UDPG led to the formation of longer branches.

Yeast glycogen synthetase appears to act like that of muscle [28]. The enzyme was incubated with excess glycogen and UDP-2-deoxyglucose. The polysaccharide was then isolated and subjected to β -amylolysis. The products expected from a multichain transfer would be maltose and the analog containing glucose and 2-deoxymaltose. However, the latter product could not be detected and instead maltose and 2,2'-dideoxymaltose were found as if repetitive transfer occurred in the same branches.

The action pattern of the synthetases on oligosaccharides appears to be different from that on polysaccharides. Thus, the elongation of linear oligosaccharides by potato, waxy maize [29,30] and bean [31] starch synthetases and by muscle glycogen synthetase [32] and of branched oligosaccharides [23] by the latter enzyme have been shown to occur by a multichain mechanism. The general procedure used was to incubate the enzymes with an excess of oligosaccharides and radioactive UDPG or adenosine diphosphate glucose. When the reaction products were identified by paper chromatography it appeared that they were mainly the original oligosaccharides with only another glucose attached to them.

A rather unexpected result was the effect of the molecular weight of the glycogen on the action pattern of glycogen synthetase (compare the t values of Expts. 1, 3 and 4, 5 and 7, Table 2). On the contrary, the size of the acceptor molecules had no influence on the t values of phosphorylase (compare the t values of Expts. 9 and 12, 13 and 15, Table 2). As already mentioned, it can be supposed that glycogen synthetase can enter rather deep within the particles through the spaces between the β particles, while penetration of phosphorylase may be prevented for some unknown reason. Once in the interior of the α particles, the glycogen synthetase would lose mobility and this would explain the higher number of transfers per chain. In accordance with this explanation, the t value appeared to be smaller for the synthetic glycogen than for native glycogen of the same molecular weight (compare t of Expts. 3 and 8, Table 2). The former polysac-

charide had fewer non-reducing ends available for synthetase than the latter. This result was ascribed to the more compact structure of the synthetic glycogen, so that the penetration of synthetase within the glycogen molecules is hindered.

The results obtained with the β -amylase limit dextrin may be explained as follows: the β -amylase dextrin is a bad acceptor for both enzymes because enzymes because the outer chains are short (2.0 glucoses average). When only one or two glucoses are added to a certain non-reducing end, the enlarged outer chain becomes a much better primer than the ends that have not been enlarged. Thus, further glucosylation can be supposed to occur mainly at the outer chains that have been previously enlarged.

Similar results were obtained by Whelan and Bailey [22] when they studied the action pattern of potato phosphorylase on maltotriose and maltotetraose. The former was a bad primer and was enlarged by a single chain mechanism, while the latter, a rather good primer, grew by multichain addition. The method used by Whelan and Bailey in order to distinguish between both mechanisms was to follow the changes in the λ_{\max} of the iodine-polysaccharide complex during synthesis. The λ_{\max} value increases with the size of the amylose molecules. In a multichain mechanism all the acceptor molecules grow randomly so the λ_{\max} should increase as synthesis of amylose proceeds. In a single chain mechanism, synthesis proceeds on the same primer molecules until the polysaccharide loses its acceptor capacity. Thus, all over the synthesis, the λ_{\max} should have the highest value attainable, provided that the number of polysaccharide molecules that are being enlarged at a given moment is negligible. De Souza and Cardini [33] used the same method and found that elongation of amylopectin or its β -amylase limit dextrin by potato phosphorylase occurred by a multiple and single chain mechanism, respectively.

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