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GLYCOGEN OF HIGH MOLECULAR WEIGHT FROM MAMMALIAN MUSCLE

PHILIP C. CALDER AND ROBERT GEDDES
Department of Biochemistry, University of Auckland, Auckland (New Zealand)
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ABSTRACT

Glycogen of high molecular weight has been isolated from mammalian muscle, in contrast to the material of low molecular weight commonly described. The large polysaccharide is similar to liver glycogen in the structure of its individual β-particles and also, partially, in the mode of assembly into the gross α-particles. The large particles may be disrupted by 2-mercaptoethanol, but not to the same extent as their liver counterparts.

INTRODUCTION

The ubiquity of glycogen in mammalian tissues is well established, but fine-structural analyses have largely been confined to glycogen from liver[1–12] and muscle[13–16], with only isolated investigations in other tissues, *e.g.*, brain[17–19]. Liver glycogen is composed of a very heterogeneous population[1,3,4,20], ranging in molecular weight to $>10^9$. Heterogeneity of structure has also been demonstrated; liver glycogen of high molecular weight is constructed on a protein backbone[4,5,21], apparently with a molecular structure similar to that of the mucins[22,23], and thus differs from its low-molecular-weight counterpart[5,7]. Apart from its water-binding characteristics[24], there is no gross discrepancy between the glycogens of low and high molecular weight in liver, but they differ significantly in their metabolism[1,2,7,25]. Despite the close similarity of muscle-glycogen metabolism to that in liver and the similar cellular storage in the systemic disease, glycogenosis type II (Pompe's disease)[26], glycogen from mammalian muscle has only been observed in the low-molecular-weight (single β-particle) form[13–15]. However, the glycogen in insect flight-muscle is polydisperse with a significant component[27] of high molecular weight. Apart from the obvious species difference, the greatest contrast in the preparation of the insect, as compared to the mammalian, glycogen was in the rate and manner by which the tissue was treated to isolate the glycogen. The insects were suddenly frozen in liquid nitrogen, and the thoraces were isolated and maintained at −80° until the glycogen was isolated[27] by an essentially aqueous procedure. By contrast, in electron-microscopic studies of mammalian muscle tissue, a prolonged (up to 3 h) fixation period[14,28] was used or the animals were anesthesized and

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bled[14,15] before excision of the muscle tissue and subsequent extraction of the glycogen. The time involved in the mammalian preparations could permit post-mortem degradation of the type already described for liver[29]. Indeed, it has been reported[30] that soleus muscle, when subjected to anaerobic incubation, underwent a 33% loss of glycogen in 30 min and 83% in 1 h. It therefore seemed important to investigate the effect of more rapid extraction on glycogen from mammalian muscle.

EXPERIMENTAL

New Zealand white rats were used in all experiments. Muscles (predominantly adductor magnus) were quickly removed after cervical dislocation. The tissue was chopped into cubes (~3 mm), obvious fat and connective tissue were discarded, and the cubes were then rapidly frozen in liquid nitrogen (the average time from death to freezing of the tissue was 3.5 min). The frozen tissue was homogenised with 5 vol. of aqueous 3% mercuric chloride in an Ultra-Turrax homogeniser (60 s, maximum speed). After centrifugation (500*g* × 15 min), the supernatant solution was filtered through two layers of cheese-cloth. The pellet was re-extracted with aqueous 3% mercuric chloride, the extract was centrifuged, and the supernatant solution was combined with that from the original extract. From the combined supernatants at 2°, the glycogen was precipitated overnight with 3 vol. of ethanol, isolated by centrifugation (1000*g* × 20 min), resuspended, reprecipitated, and recentrifuged. After a final resuspension, the glycogen was redissolved in water and the solution was freeze-dried.

Glycogen was fractionated on sucrose density-gradients as previously described[1]. Diffusion coefficients for glycogen fractions had been measured previously by laser intensity-fluctuation spectroscopy[3,4], and molecular weights were determined by application of the Svedberg equation[3].

Glycogen concentrations were determined by an iodine–iodide reaction[14], using rat-liver glycogen for calibration (450 nm). Protein concentrations were determined by a modification of the method of Lowry *et al.*[31].

For treatment with 2-mercaptoethanol, freeze-dried glycogen was dissolved (2 mg/mL) in 0.1M Tris buffer (pH 8.5) containing 8M urea. After bubbling oxygen-free nitrogen through the solution for 30 min, the sample was divided into 3 parts. One part received no further treatment (control). The other parts were treated with 2-mercaptoethanol (0.1 mL/mL, 30 min), and one was subsequently treated with iodoacetamide (0.3 g/mL). The 3 parts were then fractionated on sucrose density-gradients[1,5].

RESULTS AND DISCUSSION

As can be seen in Fig. 1, careful, but rapid, extraction of mammalian muscle indicates that this tissue, like the liver[1–5], has a heterogeneous population of glycogen with respect to molecular size. The data in Table I illustrate the apparent

polydispersity of this glycogen as compared to that in other tissues. However, caution must be expressed over this comparison since, even in liver (the most accessible of these tissues), post-mortem changes are exceedingly rapid[29] and, as mentioned above[30], similar changes may be expected in muscle. Nonetheless, it is clear from Table I that, even after our relatively slow time (~3.5 min) from death to immersion in liquid nitrogen, there is a substantial amount of high-molecular-weight material. In Table I, size is described in terms of *apparent* molecular weight, since the sedimentation coefficient–diffusion coefficient relationship has yet to be checked; these experiments will be reported elsewhere[32].

Large amounts of high-molecular-weight glycogen in the form of aggregates or spheres (α-particles) have been observed by electron microscopy of mosquito *(C. tarsalis)* flight-muscle, particularly in the sarcoplasm, while single glycogen spheres (β-particles) have been observed[33] in the M-band. In contrast, previous reports on mammalian muscle glycogen[8,9] have indicated that, practically exclusively, only single β-particles are present. We have now shown this conclusion to be incorrect, and it appears that earlier results could be related to the prolonged time between death and the observation of the glycogen. Particularly with the prolonged fixation-process required for electron microscopy, the danger of artefactual observations is greatly increased[34].

Fig. 2 shows the effect of the glycogen source on the glycogen–iodine spectrum as enhanced by part-saturated calcium chloride[35], and contains a typical spectrum of liver glycogen with absorbance peaks at ~460 and ~410 nm. This pattern is characteristic of undegraded glycogen[9,14]. The spectrum of muscle glycogen also exhibits these maxima, whereas oyster glycogen shows a single peak at ~410 nm.

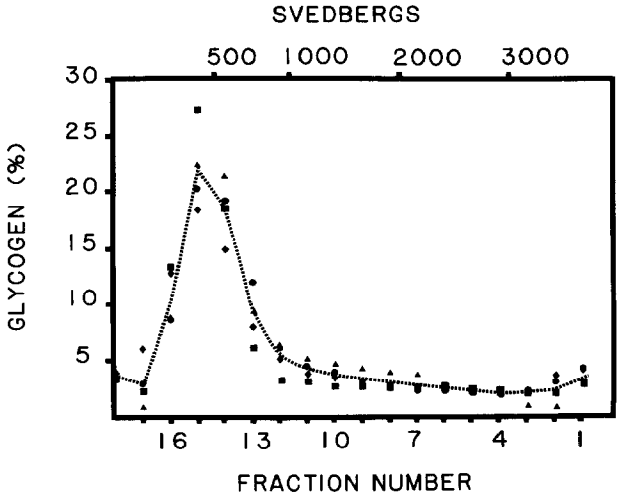

Fig. 1. Concentration distribution of 4 separate preparations of muscle glycogen after centrifugation on sucrose density-gradients. The line is drawn through averaged values. Sedimentation coefficients are displayed at the top of the graph.

TABLE I

CONCENTRATION DISTRIBUTION OF MUSCLE-GLYCOGEN PREPARATIONS IN COMPARISON WITH OTHER GLYCOGENS

Glycogen source	*Glycogen content (%) in various apparent-molecular-weight ranges (× 10^{-6})*				
	0–50	*50–100*	*100–250*	*250–500*	*>500*
Brain[a]	94	6	0	0	0
Liver[b]	24	20	20	18	18
Liver[c]					
KOH-degraded	96	4	0	0	0
Muscle[d] 1	19	30	24	10	18
2	20	36	22	8	15
3	17	28	21	13	21
4	20	14	17	16	34
Normalised average	18	25	19	18	20

[a]Interpolated from results of Chee *et al.*[17] [b]Average of 30 different preparations. Calculated from the results of Geddes *et al.*[3]. [c]Calculated from the results of Bullivant *et al.*[6]. [d]Results of four independent preparations Values averaged from up to four separate centrifugations (sucrose density-gradient).

These spectra indicate that muscle glycogen is closely related to its counterpart in the liver. Absorption in the 350–600 nm region of the glycogen–iodine spectrum is related to the fine structure, most probably the external chain-lengths, of individual β-particles[9,35]. Therefore, it can be concluded that the external surface of muscle-glycogen particles closely resembles that of liver-glycogen particles. Preliminary experiments with beta-amylase confirm this conclusion, in that liver and muscle glycogen are degraded to approximately the same extent[32].

Further to the initial demonstration that liver glycogen could be synthesised on a protein backbone[21], it is known that this backbone has disulphide bridges which, when disrupted, cause a large decrease in molecular size in liver glycogen[4,5]. As can be seen from Table II, a similar phenomenon occurs in muscle glycogen. Treatment solely with 2-mercaptoethanol was reversible by exhaustive dialysis although with some rearrangement in the distribution of molecular size, resulting, in some cases, in an increase in the proportion of high-molecular-weight glycogen. Blocking of the reduced disulphide bonds with iodoacetamide resulted in a permanent change in distribution, with the production of a much higher proportion of low-molecular-weight material compared to the untreated glycogen. Both these effects paralleled those in liver glycogen but, after reducing and blocking, there was distinctly more material of high molecular weight left in the muscle preparations.

This finding suggests that muscle may contain glycogen having a gross hydrodynamic structure distinguishable from that of the glycogen of liver[32], as has already been observed for glycogen isolated from brain[17]. Nevertheless, after the 2-mercaptoethanol/iodoacetamide treatment, ~50% of the glycogen of >2000S

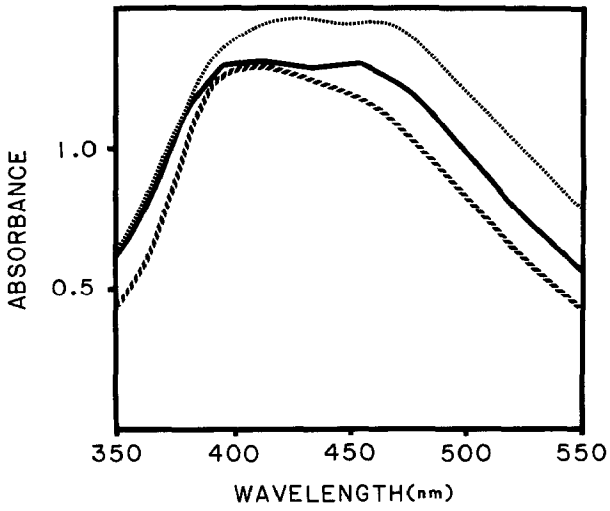

Fig. 2. Calcium chloride-enhanced iodine–iodide spectra of various glycogen solution (~1 mg/mL): ▮▮▮▮▮, average of 4 muscle-glycogen preparations; ▬▬, liver glycogen; ////////, commercial oyster-glycogen (Sigma).

had been reduced to smaller material compared to 80–90% in the case of liver glycogen.

The data in Table III show that the observed content of glycogen (~0.6% of wet-muscle weight) compares reasonably with previous reports. The high content of protein (15%) is a characteristic of muscle-glycogen preparations. Removal of this protein requires fairly drastic procedures[14,15,27]. However, the bulk of the protein does not affect the size distributions on sucrose density-gradients, in that much of the protein is "floated off" from the glycogen during centrifugation because of its lower density[14]. However, some protein is intimately involved in the structure. The 2-mercaptoethanol experiments (Table II) indicate that muscle glycogen is partially built on a protein backbone held together by disulphide bonds. Certainly, some of the other protein associated with muscle glycogen seems to be more firmly bound than that associated with liver glycogen. However, the large amount of associated protein suggests that there may be more than a casual relationship between muscle glycogen and protein (*cf.*, for example, hyaluronic acid and chondroitin sulphate and their relationship in connective tissue[36]).

These results indicate that glycogen in muscle is heterogeneous with respect to molecular size in a manner similar to that observed for liver[1–5]. This finding is contrary to a long-held belief[13–15] that muscle glycogen consists solely of β-particles. It had been reported[37] that glycogen extracted from autopsy or biopsy specimens of human muscle includes a component of high molecular weight. However, that study did not indicate polydispersity of glycogen from either rat liver or rat skeletal muscle. Muscle glycogen appears to be at least partially built on a protein backbone held together by disulphide bonding similar to the situation in liver glycogen[4,5].

TABLE II

EFFECT OF DISRUPTION OF DISULPHIDE BONDS IN GLYCOGENS WITH RESPECT TO SEDIMENTATION COEFFICIENT

Glycogen source	*Treatment (before dialysis)*	*Glycogen content (%)*	
		<2000S	*>2000S*
Liver[a]	None	80	20
	2-mercaptoethanol	85	15
	2-mercaptoethanol + iodoacetamide	98	2
Liver[b]	None	81	19
	2-mercaptoethanol	86	14
	2-mercaptoethanol + iodoacetamide	97	3
Muscle 1	None	72	28
	2-mercaptoethanol	66	34
	2-mercaptoethanol + iodoacetamide	80	20
2	None	66	34
	2-mercaptoethanol	61	39
	2-mercaptoethanol + iodoacetamide	85	15
3	None	71	29
	2-mercaptoethanol	80	20
	2-mercaptoethanol + iodoacetamide	84	16
4	None	69	31
	2-mercaptoethanol	63	37
	2-mercaptoethanol + iodoacetamide	84	16

[a]From Chee and Geddes[5]. [b]Performed with the same time and conditions as the muscle-glycogen preparations, to act as a control.

TABLE III

PROTEIN CONTENT AND GLYCOGEN YIELD

Glycogen source	*Glycogen content of total homogenate (% of wet-tissue wt.)*	*Protein content of purified product (as % of glycogen content)*
Liver[a]	—	1 6
Muscle 1	0 58	13.8
2	0.57	12.2
3	0.66	15.3
4	0.54	18.1
Average	0.59	14.9
Rabbit muscle[b]	0.5–0.8	5–30[c]
Insect flight-muscle[d]	1–1.5	29[e]

[a]Control; experiments performed with the same time and conditions. [b]Calculated from Table I, ref. 14. [c]Prior to treatment with chloroform and urea. [d]From Childress *et al.*[27]. [e]Calculated from Table I, ref. 27. Prior to modified Sevag procedure.

Differences between the liver and muscle molecules appear to be confined to the extent of disruption by disulphide bond-breaking reagents and the large amount of protein associated with muscle glycogen. Since muscle is the largest storage area for glycogen in mammals (liver has the highest concentration), if glycogen of high molecular weight is found to be associated within the lysosomal compartment, as in liver[1,38], there are profound metabolic implications particularly for the Cori cycle[39].

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REFERENCES

1 R. GEDDES AND G. C. STRATTON, *Biochem. J.*, 163 (1977) 193–200.
2 R. GEDDES AND G. C. STRATTON, *Carbohydr. Res.*, 57 (1977) 291–299.
3 R. GEDDES, J. D. HARVEY, AND P. R. WILLS, *Biochem. J.*, 163 (1977) 201–209.
4 R. GEDDES, J. D. HARVEY, AND P. R. WILLS, *Eur. J. Biochem.*, 81 (1977) 465–472.
5 N. P. CHEE AND R. GEDDES, *FEBS Lett.*, 73 (1977) 164–166.
6 H. M. BULLIVANT, R. GEDDES, AND P. R. WILLS, *Biochem. Int.*, 6 (1983) 497–506.
7 P. C. CALDER AND R. GEDDES, *Carbohydr. Res.*, 116 (1983) 233–238.
8 P. DROCHMANS, *J. Ultrastruct. Res.*, 6 (1962) 141–163.
9 P. DROCHMANS, *La Morphologie Du Glycogène*, Editions Arscia A.S., Brussels, 1966
10 J. MORDOH, C. R. KRISMAN, AND L. F. LELOIR, *Arch. Biochem. Biophys.*, 113 (1966) 265–272.
11 S. A. ORRELL AND E. BUEDING, *J. Biol. Chem.*, 239 (1964) 4021–4026.
12 A. A. BARBER, S. A. ORRELL, AND E. BUEDING, *J. Biol. Chem.*, 247 (1967) 4040–4044.
13 J. P. REVEL, *J. Histochem. Cytochem.*, 13 (1965) 104–114.
14 J.-C. WANSON AND P. DROCHMANS, *J. Cell Biol.*, 38 (1968) 130–150.
15 J.-C. WANSON AND P. DROCHMANS, *J. Cell Biol.*, 54 (1972) 206–224.
16 O. KJOLBERG, D. J. MANNERS, AND A. WRIGHT, *Comp. Biochem. Physiol.*, 8 (1963) 353–365.
17 N. P. CHEE, R. GEDDES, AND P. R. WILLS, *Biochim. Biophys. Acta*, 756 (1983) 9–12.
18 R. B. COXON, E. C. GORDON-SMITH, AND J. R. HENDERSON, *Biochem. J.*, 97 (1965) 776–781.
19 B. I. KHAIKINA AND V. E. YAKUSHKO, *Fed. Proc.*, 24 (1965) 991–994.
20 R. GEDDES, *Proc. Aust. Biochem. Soc.*, 62 (1969) 124.
21 C. R. KRISMAN AND R. BARENGO, *Eur. J. Biochem.*, 52 (1975) 117–123.
22 R. CHING AND R. GEDDES, unpublished results.
23 M. MANTLE AND A. ALLEN, *Biochem. Soc. Trans.*, 6 (1981) 607–609.
24 T. BRITTAIN AND R. GEDDES, *Biochim. Biophys. Acta*, 543 (1978) 258–263.
25 M. R. STETTEN AND D. STETTEN, *J. Biol. Chem.*, 207 (1954) 331–340.
26 H.-G. HERS, *Biochem. J.*, 86 (1963) 1–8.
27 C. C. CHILDRESS, B. SACKTOR, I. W. GROSSMAN, AND E. BUEDING, *J. Cell Biol.*, 45 (1970) 83–90.
28 G.ROSATI *J. Ultrastruct. Res.*, 18 (1967) 444–455.
29 R. GEDDES AND K. B. RAPSON, *FEBS Lett.*, 31 (1973) 324–326.
30 K. A. MOORTHY AND M. K. GOULD, *Arch. Biochem. Biophys.*, 130 (1969) 399–407.
31 O. H. LOWRY, J. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265–275.
32 P. C. CALDER, R. GEDDES, AND P. R. WILLS, unpublished data.
33 B. G. JOHNSON AND W. A. ROWLEY, *J. Insect Physiol.*, 18 (1972) 2375–2389.
34 M. L. SMITHERMAN, A. LAZAROW, AND R. L. SORENSON, *J. Histochem. Cytochem.*, 20 (1972) 463–471.

35 C. A. KRISMAN, *Anal. Biochem.*, 4 (1962) 17–23.
36 Y. MIKUNI-TAKAGAKI AND B. P. TOOLE, *J. Biol. Chem.*, 256 (1981) 8463–8469.
37 W. J. POLGLASE, D. M. BROWN, AND E. L. SMITH, *J. Biol. Chem.*, 199 (1952) 105–112.
38 R. GEDDES, D. E. OTTER, G. K. SCOTT, AND J. A. TAYLOR, *Biochem. J.*, 212 (1983) 99–103.
39 A. DUNN, M. CHENOWETH, AND L. D. SCHAEFFER, *Biochemistry*, 6 (1967) 6–11.