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The Heterogeneity of the Protein Content of Liver and Muscle Glycogens

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The portions of both liver and muscle glycogen that have a high protein content have been investigated. In liver the high molecular weight portions of glycogen may be rendered insoluble by treatment with trichloroacetic acid. This shows that reported desmo- (or insoluble) glycogen is an artefact of the extraction process and therefore of no physiological significance. In contrast, muscle glycogen insolubility is not associated with any specific molecular size range. Insolubility of muscle glycogen is shown to be related to partial degradation of the polysaccharide and to the high protein content remaining after the gentle extraction procedure. Since the molecular weight profile is unaltered by the removal of the insoluble glycogen it does not interfere with the interpretation of metabolic studies.

It is well known that glycogen, carefully extracted from liver or muscle tissue, has significant amounts of protein associated with it [1-8]. Further, we have established, following on from the original suggestion by Krisman and Barengo [4], that both liver and muscle glycogen have, at least partially, a proteoglucan nature [9-11]. These studies have shown that the high molecular weight glycogen is built up on a protein backbone (possibly through a tyrosine residue [12, 13], and is formed covalently into very high molecular weight molecules by the joining of these backbones through disulphide bonds.

The existence of protein-bound glycogen led to the revival of the concept of insoluble (or desmo-) glycogen in addition to soluble (or lyo-) glycogen [8, 14-17]. This concept was popular 20 or 30 years ago but has been ignored in most recent studies [1-4, 18-21]. Shuld desmo-glycogen exist in the cell, i.e. if it is not an artefact of the extraction processes, then there could be profound implications for the interpretation of metabolic experiments on glycogen (for example see [14]). Our recent work showed that high molecular weight glycogen has a relatively high protein content [3, 11] and, in addition, it is well-established as being located in the lysosomal compartment of the cell [18-23]. We decided to extend our investigations of protein-bound glycogen. In addition, muscle glycogen was utilised, particularly because of its high relative protein content [3, 5-7].

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Table 1. Effect of trichloroacetic acid upon liver glycogen.

Preparation mode	Total soluble glycogen (mg/g liver)	Relative yield (%) (phenol=100)	Protein/ glycogen (%)
1. Phenol extraction	53.6	100	2.40
2. TCA extraction	31.1	58	0.36
3. TCA treatment of purified phenol extraction	29.0	54	0.32
	Glycogen content (%) in molecular weight ranges ($\times 10^{-6}$)		
	<250	250-500	>500
1.	46.4	21.8	31.7
2.	88.7	3.8	7.5
3.	90.0	2.7	7.3

Materials and Methods

Sprague-Dawley rats were used in all experiments. Liver [22-24] and muscle [3, 11] glycogens were extracted and purified as already described. Purified glycogens were analysed on sucrose density gradients as previously described [2, 3, 9, 10, 11, 18-23, 25, 26]. Protein content was measured by the coomassie blue micro-assay of Bradford [27] and glycogen concentrations were measured by a calcium chloride enhanced iodine-iodide assay [28]. Total glycogen in tissue was determined by the method of Kemp and van Heijningen [29]. Total carbohydrate was assessed by a phenol-sulphuric acid method [30]. Chain lengths and β-amylolysis limits were determined by standard methods [31, 32] using β-amylase (Boehringer, Mannheim, W. Germany) and Pullulanase (Boehringer) as previously described [2, 11, 33].

Extraction or treatment utilising trichloroacetic acid (TCA) (BDH Chemicals, Poole, U.K.) essentially followed standard methodology [34] with the exception (see Table 1) that 30 min was adopted as the extraction time, there being no additional loss of polysaccharide between 4 and 30 min.

Results and Discussion

Liver glycogen extracted by the normal phenol/water process has a protein content averaging about 2% [2, 5, 21] but this varies by a factor of approximately four [5, 21] over the vast molecular size range of glycogen [9, 22, 23, 25] with the high molecular weight species having consistently the higher content of protein. Exposure of whole liver

Table 2. The loss of soluble purified muscle glycogen on dialysis against water. The dialysate was centrifuged 1000 × *g* × 15 min prior to analysis.

	Glycogen[a]		Total carbohydrate[b]		Protein[c]	
	Total (g/kg muscle)	Relative (%)	Total (g/kg muscle)	% Glycogen	Total (g/kg muscle)	as % Glycogen
Pre-dialysis	4300 ± 700	100	4400 ± 100	98	600 ± 50	14
Post-dialysis	3450 ± 500	80	3500 ± 200	101	300 ± 100	9

[a, b, c] Analyses by the methods of Krisman [28] (a), Dubois [30] (b), and Bradford [27] (c).

glycogen to 10% TCA at 4°C for 4 min causes a loss of 42% of soluble glycogen. Prolonged exposure to the acid (up to 30 min) does not result in any significant increase of this loss. Table 1 shows that the effect of TCA upon liver glycogen, whether purified or in the extraction process from tissue, is to cause insolubilisation of the high molecular weight, high protein content glycogen [3, 11, 18, 33]. This results in a loss of approximately 85% of glycogen-bound protein with corresponding loss of only medium and high molecular weight polysaccharide ($>250 \times 10^6$). Thus it is clear that desmo-glycogen from liver is an artefactual product of the TCA-extraction process, and that this glycogen is normally found in the soluble product extracted by the mild, modern methods [9, 22-25, 35]. As a check that no degradation of the polysaccharide had resulted from the treatment, samples were spectrophotometrically tested by the calcium chloride enhanced iodine-iodide assay [28]. Since this assay depends on the outer chain lengths of the α(1-4)-linked glucose units of the glycogen [36] any degradation of this type would be clearly indicated. No evidence of any degradation was found.

Thus we can conclude that desmo-glycogen of liver tissue is clearly an artefact of the extraction processes and cannot, as has been suggested [8], have a separate physiological existence. By corollary, lyo-glycogen is therefore also an artefact since it is selected negatively by the TCA extraction.

Studies of insoluble glycogen were then extended to muscle tissue. Recently, high molecular weight glycogens have been identified in muscle [3, 11, 33] and shown to be constructed in apparently the same manner as in liver. However, muscle glycogen has a very high protein content [3, 5-7, 11] which may be related, at least partially, to its extraction process. Muscle glycogen is extracted in aqueous 3% mercuric chloride [3] in contrast to liver, where aqueous 45% phenol is used [9, 22, 23, 25].

When freshly prepared muscle glycogen is dialysed, a water insoluble product is formed which contains approximately 20% of the purified glycogen and approximately 50% of the protein (Table 2). As with liver glycogen, removal of the insoluble material did not affect the shape of the Iodine-Iodide-$CaCl_2$ spectra. However, in contrast to the situation with the liver material, removal of the precipitate does not significantly alter the molecular weight profile of the glycogen. In fact, if the insoluble material is resuspended with soluble glycogen and a sucrose density centrifugal analysis is performed, there is no difference observed in the molecular weight spectrum, as the insoluble material is solidly pelleted at the bottom of the centrifuge tube and therefore is not analysed.

Table 3. Solubilisation of water-insoluble muscle glycogen. Solubilisation was determined as the total soluble carbohydrate [30] after centrifugation (1000 × *g* × 15 min).

Treatment (37°C × 48h with shaking)	Glycogen solubilised (%)
H_2O	16
Buffer alone (T.1 M sodium phosphate, pH 7.0)	22
Buffer containing:	
0.1% sodium dodecyl sulphate	44
1.0% sodium dodecyl sulphate	68
10.0% sodium dodecyl sulphate	82
8 M urea	75
10% triton X-100	62
10% tween 80	63
10% sodium deoxycholate	51

Table 4. Effect of decreasing the amount of associated protein on the solubility of purified, water insoluble, glycogen.

	% recovery as soluble material after treatment with:	
	45% phenol [22]	Sevag [37] (chloroform/octanol, 3/1 by vol)
glycogen	42	65
protein	3.8	3.5
% protein/glycogen	5.1	5.4

N.B. The original protein content of the insoluble glycogen is always high but very variable (see Table 2). The original % protein content for phenol- and Sevag-treated insoluble glycogens was 56.4% and 100.3%, respectively.

Table 5. Chain lengths and β-limits of solubilised, water-insoluble, muscle glycogen.

	β	Overall chain length	External chain length	Internal chain length
Soluble muscle glycogen (control)	55	12.7	9.5	2.2
Various Sevag preparations of insoluble glycogen				
1	39	9.9	6.4	2.5
2	36	9.4	5.9	2.5
3	38	9.3	6.0	2.3
Average	38	9.5	6.1	2.4

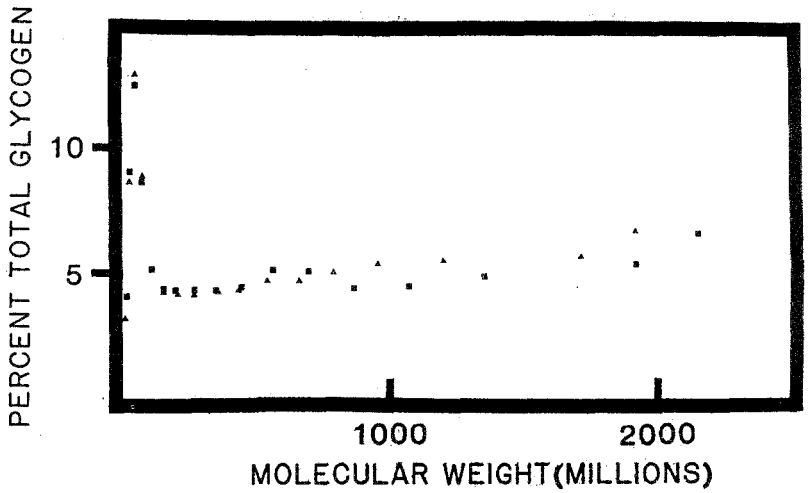

Figure 1. Molecular weight profiles of soluble muscle glycogen before (■) and after (▲) removal of excess protein by the Sevag procedure. Each experimental point represents the percentage content of glycogen with the average molecular weight indicated.

Attempts were made to solubilise the glycogen pellet as indicated in Table 3. Incubation with hydrogen bond breaking reagents could solubilise up to 82% of the water insoluble material. However, a high percentage of protein remained associated with the solublised material and, regardless of the technique used, all of the glycogen reprecipitated upon removal, by dialysis, of the solubilising agent. Therefore it can be concluded that, as with TCA-insoluble liver glycogen, protein is a factor in the insolubility.

In order to solubilise the glycogen in a form where further analyses could be performed it was decided to remove protein impurities by treatmen with aqueous phenol [22] or by the Sevag procedure [7, 37] (Table 4). Both procedures removed much of the protein and gave a soluble glycogen product with a relatively low protein content (approximately 5%). On performing sucrose density gradient centrifugation, the deproteinised material exhibited a molecular weight profile (Fig. 1) very similar to that for "normally" soluble glycogen. Thus the insolubility is not associated with any specific size range, in contrast to the desmo-glycogen of liver tissue.

To investigate the possibility that the insolubility of this glycogen arose from variations in the structure of the polysaccharide portion, further analyses were performed. Glycogen solubilised by the Sevag procedure was precipitated with ethanol, redissolved in buffer and treated with β-amylase and pullulanase. From Table 5 it is clear that the glycogen has short outer chains as evidenced by its low β-amylolysis limit (38%) and low average external chain length (ECL), which was 64% of the control. This is also confirmed by the Iodine-Iodide-$CaCl_2$ absorbance spectrum (Fig. 2), where the solubilised glycogen exhibits an absorption maximum of 400 nm, a characteristic of a partially

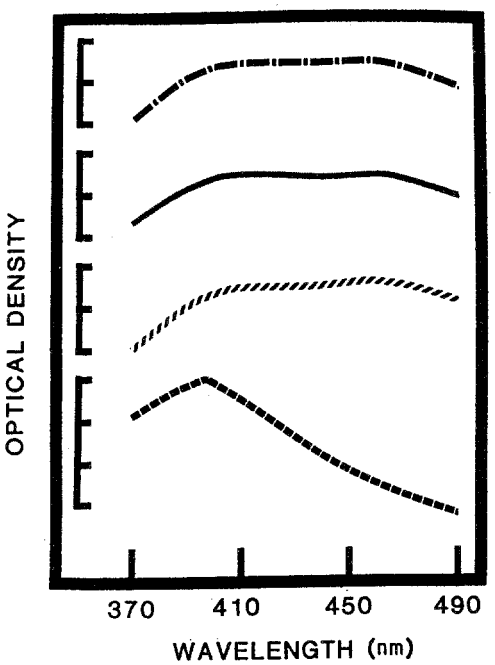

Figure 2. Relative optical density versus wavelength profiles for various glycogens treated with Iodine-Iodide-Calcium Chloride solution. The O.D. at 410 nm was been assigned the same value in all cases. (▮▮▬▮▮▬) liver glycogen, (▬▬▬▬▬▬▬▬) soluble muscle glycogen, (▰▰▰▰▰▰) whole muscle glycogen prior to removal of insoluble material by centrifugation, (▪▪▪▪▪▪▪▪▪▪▪▪▪▪) insoluble material solubilised by the Sevag procedure.

degraded glycogen molecule [36]. Thus the insoluble, or desmo-glycogen of muscle tissue arises from the extraction of material in which the polysaccharide portion is partially degraded, and has a very high protein level associated with it upon extraction. The reason for the extensive polysaccharide degradation most probably lies in the very rapid post-mortem hydrolysis of the glycogen. After death, muscle glycogen is degraded at four to five times the rate of its liver counterpart (Calder PC, Geddes R, experiments in progress, [26]). Thus, like its counterpart in the liver, the insoluble muscle glycogen is an artefact of the extraction process. Since in liver tissue TCA extraction is an outmoded procedure which has been largely replaced by aqueous procedures [21] and in muscle tissue loss of the "desmo-glycogen" does not cause any preferential size selection effect on the soluble extract, only a reduced yield, the existence of this artefact may be ignored in future metabolic and structural studies.

In conclusion, the desmo-glycogen of liver tissue has been clearly demonstrated to be an artefact of the extraction process and to have no separate physiological existence [8]. The insoluble glycogen from muscle is associated with both a high protein content, and partial degradation of the polysaccharide. The latter may well be associated with the very rapid post-mortem changes in the tissue [3, 26, 38, 39] which we are continuing to investigate.

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References

1 Bullivant HM, Geddes R, Wills PR (1983) Biochem Int 6:497-506.
2 Calder PC, Geddes R (1983) Carbohydr Res 118:233-38.
3 Calder PC, Geddes R (1985) Carbohydr Res 135:249-56.
4 Krisman CR, Barengo R (1975) Eur J Biochem 52:117-23.
5 Wanson JC, Drochmans P (1968) J Cell Biol 36:130-50.
6 Wanson JC, Drochmans P (1972) J Cell Biol 54:206-24.
7 Chidress CC, Sacktor B, Grossman IW, Bueding E (1970) J Cell Biol 45:83-90.
8 Butler NA, Lee EYC, Whelan WJ (1977) Carbohydr Res 55:73-82.
9 Geddes R, Harvey JD, Wills PR (1977) Eur J Biochem 81:467-72.
10 Chee NP, Geddes R (1977) FEBS Lett 73:164-66.
11 Calder PC, Geddes R (1985) Glycoconjugate J 2:365-73.
12 Aon MA, Curtino JA (1985) Biochem J 229:269-72.
13 Rodriguez IR, Whelan WJ (1985) Biochem Biophys Res Commun 132:829-36.
14 Lourau M, Meyer F (1960) J Physiologie 52:835-45.
15 Louray M, Meyer F (1958) J Physiologie 50:5-17.
16 Russell JA, Bloom WL (1955) Amer J Physiol 183:345-55.
17 Stetten D, Stetten MR (1960) Physiol Rev 60.503-37.
18 Geddes R, Taylor JA (1985) Biochem J 228:319-24.
19 Ching R, Geddes R, Simpson SA (1985) Carbohydr Res 139:285-91.
20 Geddes R, Taylor JA (1985) Biosci Rep 4:315-20.
21 Geddes R (1985) in The Polysaccharides, Vol 3, ed. Aspinall GO, Academic Press, New York, p 284-336.
22 Geddes R, Stratton GC (1977) Biochem J 163:193-200.
23 Geddes R, Stratton GC (1977) Carbohydr Res 57:291-99.
24 Laskov R, Margoliash E (1963) Bull Res Council Isr All 351-62.
25 Geddes R, Harvey JD, Wills PR (1977) Biochem J 163:201-9.
26 Geddes R, Rapson KB (1973) FEBS Lett 31:324-6.
27 Bradford M (1976) Anal Biochem 72:248-54.
28 Krisman CA (1962) Anal Biochem 4:17-23.
29 Kemp A, van Heijningen AJMK (1954) Biochem J 56:646-52.
30 Dubois M, Gillies KA, Hamilton JK, Rebers PA, Smith F (1956) Anal Chem 28:350-56.
31 Lee EYC, Whelan WJ (1966) Arch Biochem Biophys 116:162-67.
32 Kjolberg O, Manners DJ, Wright A (1963) Comp Biochem Physiol 8:353-65.
33 Calder P, Geddes R (1986) Carbohydr Res 148:173-77.
34 Stetten MR, Katzen HM, Stetten D (1956) J Biol Chem 222:587-99.
35 Bueding E, Orrell SA (1964) J Biol Chem 239:4018-20.
36 Drochmans P (1966) in La Morphologie du Glycogene, Editions Arscia SA, Brussels, p 131-41.

37 Sevag MG (1934) Biochem Z 273:419-29.
38 Newbold RP, Lee CA (1965) Biochem J 97:1-6.
39 Tarr HLA (1968) J Fish Res Board Canada 25:1539-54.