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Note

Digestion of the protein associated with muscle and liver glycogens

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In continuing our investigation of the proteoglucan nature of liver[1,2] and muscle[3,4] glycogen, the effect on the polysaccharide structure of removal of the core protein[2,5,6] has been investigated by using (*a*) hot anaerobic alkali, which is also known to attack the C-chain of the polysaccharide moiety[7,8], and (*b*) Proteinase K which, since it was found to lack saccharase activity, will affect only the protein backbone[8]. Method (*a*) has been used to distinguish between the Meyer[9] and Whelan[10] models for the β-particle subunits of glycogen[8]. Anaerobic alkali will digest the protein and then degrade the polysaccharide C-chain stepwise from the reducing end by "peeling off" successive glucose residues as D-glucoisosaccharinic acid[7,8]. Random scission of glycosidic bonds only occurs in the presence of oxygen. As has been reported[2,8], there was a total loss of high-molecular-weight material, resulting in a product of narrow size distribution with a sedimentation coefficient peak of ~200 S. Table I shows clearly that this massive structural change in the high-molecular-weight α-particles is not accompanied by any surface modification of the constituent β-particles[11]. This conclusion is also confirmed by the lack of alteration in the iodine–iodide–calcium chloride spectra before and after treatment with alkali[12]. These spectra are sensitive to alterations in the outer chains of glycogens[13]. Thus, the removal of protein and the splitting of the Whelan β-particles show no effect on the fundamental structure of the polysaccharide and may be taken, at least circumstantially, as support for the proposed model for high-molecular-weight glycogen[9]. The results further emphasise the similarity of liver and muscle glycogen. The variable recovery of carbohydrate is directly related to the difficulties of handling glycogen in anaerobic strong alkali. Exposure to even low concentrations of dilute alkali can cause rapid degradation. Thus, precipitation and neutralisation procedures must be performed rapidly and, hence, with <100% efficiency.

Table II shows the dramatic effect of Proteinase K on the protein associated with glycogen. There is a 90% loss of protein from both liver and muscle glycogen. Fig. 1 illustrates the great loss of the high-molecular-weight species in liver and muscle glycogen. In liver, 70% of glycogen of mol. wt. $>250 \times 10^6$ was converted into low-molecular-weight species; the corresponding value for muscle was 50%. In

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TABLE I

EFFECT OF ANAEROBIC KOH DIGESTION ON GLYCOGEN STRUCTURE

Tissue		*Glycogen recovery (%)*	*Protein recovery (%)*	*Beta-limits (%)*	*Average chain-lengths*		
					Overall	*External*	*Internal*
Liver	1	94	5.1	47	11.9	8.1	2.7
(individual	2	95	6.3	52	14.7	10.2	3.5
preparations)	3	94	5.3	47	12.5	8.4	3.1
	4	98	5.5	54	11.8	8.9	1.9
Average		95	5.6	50 ±4	12.7 ±1.3	8.9 ±0.9	2.8 ±0.7
Control liver				56 ±3	13.2 ±0.6	9.9 ±0.7	2.2 ±0.3
Muscle	1	92	7.3	55	11.1	8.6	1.5
(individual	2	98	5.9	52	12.1	8.8	2.3
preparations)	3	92	5.8	46	11.9	8.0	2.9
	4	96	4.9	50	15.8	10.5	4.4
Average		94	5.9	50 ±4	12.7 ±2.1	9.0 ±1.1	2.8 ±1.2
Control muscle[a]				54 ±3	12.7 ±1.8	9.5 ±0.6	2.2 ±0.4

[a]Values from ref. 26.

TABLE II

EFFECT OF PROTEINASE K DIGESTION ON GLYCOGENS

	Tissue	
	Liver	*Muscle*
Glycogen (% recovery)	99	91
Protein (% recovery)	6.9	12.8
% Protein/glycogen	0.14	1.24

each experiment, the disappearance of glycogen of mol. wt. $>500 \times 10^6$ was 75%. These massive changes in the molecular weight distributions were not accompanied by any changes in the β-particle construction as monitored by the calcium chloride-enhanced iodine–iodide spectra (Fig. 2).

The above results are further evidence, firstly, of the proteoglucan nature of glycogen[2,14,15] and, secondly, of the strong structural parallel between muscle and liver glycogen. Since the high-molecular-weight glycogen of liver is closely associated with the lysosomal compartment[16–19] and since the metabolic response to the genetic lack of (1→4)-α-D-glucosidase is paralleled by liver and muscle[20], it seems possible that the muscle lysosomes may participate significantly in normal carbohydrate metabolism. We are currently investigating the implications of that with respect to the Cori cycle[26].

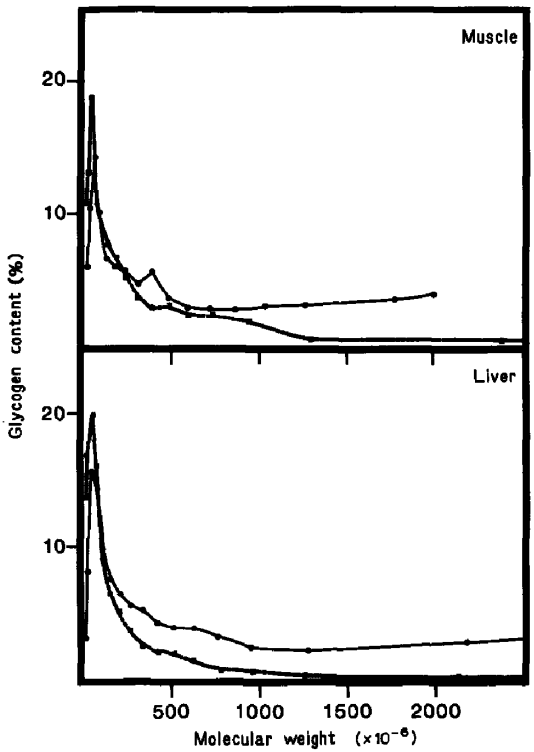

Fig. 1. The effect of treatment with Proteinase K on the molecular-weight-distributions of muscle and liver glycogens: ——, control; ▬▬▬, treated. Strictly speaking, these results should be presented in histogram form[15] but, for ease of presentation, a continuous curve has been drawn through the experimental points.

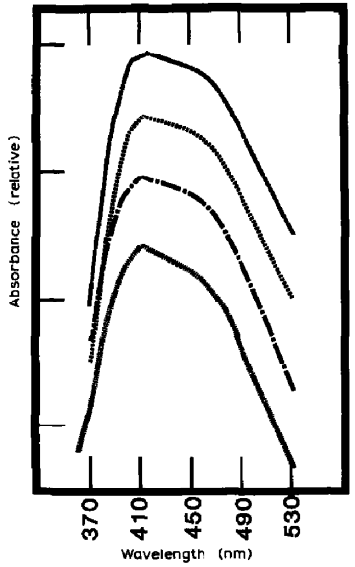

Fig. 2. The effect of treatment with Proteinase K on the iodine–iodide–$CaCl_2$ spectra of liver and muscle glycogen: ▬▬▬, muscle control; ▬▬▬▬, muscle + Proteinase K: ▬▬▬, liver control; , liver + Proteinase K. Individual spectra are presented at essentially the same concentration, but vertically displaced for clarity of presentation.

EXPERIMENTAL

Sprague–Dawley rats were used in all experiments. Liver glycogen was extracted and purified[16] by using aqueous 45% phenol. Cubes of adductor magnus tissue were rapidly frozen in liquid nitrogen and muscle glycogen was extracted with aqueous mercuric chloride[4]. Size distributions of glycogens were measured on sucrose density gradients[22]. Glycogen concentrations were measured by a calcium chloride-enhanced iodine–iodide assay[12] or, in the absence of sucrose, by a phenol–sulphuric acid method[23]. Chain lengths and beta-amylolysis limits were determined by standard methods[3,24] using pullulanase and beta-amylase (Boehringer). Proteinase K (EC 3.4.21.14, Boehringer) was shown[8] to have no significant saccharase activity. Glycogen solutions (1 mg/mL) in borate buffer (0.1M, pH 7.5) were incubated with ~1 ng of the purified enzyme (37°, 6 h). The reaction was terminated by brief exposure to boiling water.

Protein content was measured by the method of Bradford[25].

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