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ORDERED SYNTHESIS AND DEGRADATION OF LIVER GLYCOGEN INVOLVING 2-AMINO-2-DEOXY-D-GLUCOSE

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ABSTRACT

The incorporation of 2-amino-2-deoxy-D-glucose from precursor 2-amino-2-deoxy-D-galactose into liver glycogen has been shown to be a metabolically inhomogenous process after starvation. The protein-to-polysaccharide ratio is also heterogeneous with respect to molecular size, and enhanced overall as compared to normal glycogen. The results are discussed from the viewpoint of a molecular order in the synthesis and degradation of liver glycogen.

INTRODUCTION

It is well established that 2-amino-2-deoxy-D-glucose (D-glucosamine) can be incorporated into liver glycogen by injection of 2-amino-2-deoxy-D-galactose (D-galactosamine)[1–5], which, after conversion into UDP-D-galactosamine, followed by epimerisation to UDP-D-glucosamine, can then act as a substrate for glycogen synthase[3]. This incorporation does not result in a metabolic block in glycogenolysis[5] nor does it appear to affect the course of glycogen synthase, glucoamylase, isoamylase, or beta-amylase actions, although it affects the rate of the enzymic attack[3,5]. Further, D-galactosamine is metabolised in liver by the same pathway as D-galactose[1–3]. In the situation where D-galactosamine is provided to a previously starved liver, resynthesis appears to be associated with the ribosomes or rough endoplasmic reticulum[3], an observation already reported for resynthesis from D-glucose under similar circumstances[6]. Indeed, when large amounts of D-galactosamine are injected, there is a significant enhancement of the protein–polysaccharide ratio[4].

Unfortunately, even though the metabolic inhomogeneity of glycogen, with respect to size, is well established in liver[7–12], as well as other tissue[13], all of the above studies used methods known to cause extensive degradation of the glycogen molecule[14–16]. In addition, degradation of the glycogen molecule will modify or remove protein from the glycogen and, since backbone protein [17] is preferentially associated with the fraction of high molecular weight[10,18], it seemed important to investigate the incorporation of D-glucosamine into glycogen without extensively de-

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grading the latter. Further, glycogen of high molecular weight is known to be associated, in liver, with the lysosome[19,20], and is the material preferentially degraded post-mortem[21] or, indeed, chemically.

EXPERIMENTAL

New Zealand white-rats were starved for 24 h and injected intraperitoneally with 2-amino-2-deoxy-D-[^{14}C]galactose (6 μCi, >45 mCi/mmol, Amersham) and D-glucose (750 mg). The rats were allowed to feed *ad libidum* for 4 or 13 h after injection and then sacrificed[5]. Control experiments with non-starved (14-μCi injection) rats were also performed. Livers were removed rapidly, and plunged into liquid nitrogen in order to eliminate the effects of rapid, and metabolically inhomogeneous, post-mortem degradation[21]. Glycogen was isolated by a cold-water extraction method and fractionated on sucrose density-gradients as previously described[19]. Diffusion coefficients for the glycogen fractions had been measured previously by laser intensity-fluctuation spectroscopy[22,23]. Molecular weights were determined by application of the Svedberg equation[22].

Glycogen concentrations were determined by an iodine–iodide reaction[19], and protein by a modification of the method of Lowry[24].

For the beta-amylase digestion of fractions from sucrose gradients, equivalent fractions were combined, and the sucrose was removed by dialysis against running water. The glycogen was precipitated with ethanol, collected by centrifugation, and resuspended in 10mM sodium acetate buffer (pH 4.8). Half of each sample was used as a control; to the other half was added 50 μL of beta-amylase solution [beta-amylase (Boehringer Mannheim; 5 mg/mL) diluted 1/200 with BSA (0.5 mg/mL)]. Each sample was rotated for 20 h at 37° in equilibrium-dialysis cells against the buffer. All compartments were measured for radioactivity, buffer compartments for maltose (3,5-dinitrosalicylate), and glycogen compartments for glycogen (phenol–H_2SO_4).

RESULTS AND DISCUSSION

As observed previously, starvation causes a dramatic decrease in the glycogen content of the liver, and this depletion (and the subsequent resynthesis on refeeding) is a grossly heterogeneous process with respect to molecular size[9,11]. As can be seen from Fig. 1, in confirmation of our earlier results[11], early resynthesis (4 h) favours the production of material of high molecular weight, whereas continued resynthesis (13 h) results in an overproduction of material of low molecular weight. Regardless of the overall size-distribution, the glycogens showed no structural variations, as measured by beta-amylolysis, with respect to size as compared with glycogen isolated from a normal liver (Table I), nor did they show any significant variation in metabolism with respect to size, as assessed by the incorporation of radioactivity from D-[^{14}C]galactosamine (Table I).

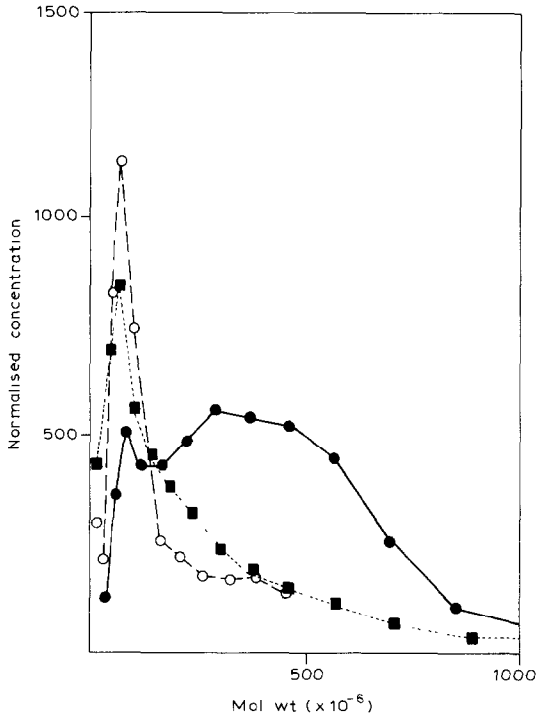

Fig. 1. Molecular weight distributions of the various glycogens normalised to the same area: ···■···, normal; —●—, SR 4; —○—, SR 13.

TABLE I

DIGESTION OF FRACTIONATED GLYCOGENS BY BETA-AMYLASE[a]

Normal glycogen (N3)			*Starved/re-fed glycogen (SR4)*		
Mol. wt. range ($\times 10^{-6}$)	*Beta-limits (%)*		*Mol. wt. range* ($\times 10^{-6}$)	*Beta-limits (%)*	
	Chemical	*Radioactive*		*Chemical*	*Radioactive*
5–70	50	n.d.[b]	5–70	54	43
70–140	46	56	45–140	51	49
140–240	51	50	140–330	48	45
240–370	47	33	300–550	n.d.	45
370–550	43	55	550–1000	48	50
550–800	54	52	1000–3000	48	39
800–1500	42	40			
1500–3000	46	50			

Method	*Beta-limits for whole (unfractionated) glycogens*		
	N3	*SR4*	*SR13*
Chemical	44	46	47
Radioactive	45	54	52

[a]Results expressed as apparent beta-amylolysis limits, as measured by maltose production (chemical) or by loss of radioactivity (radioactive). [b]Not determined.

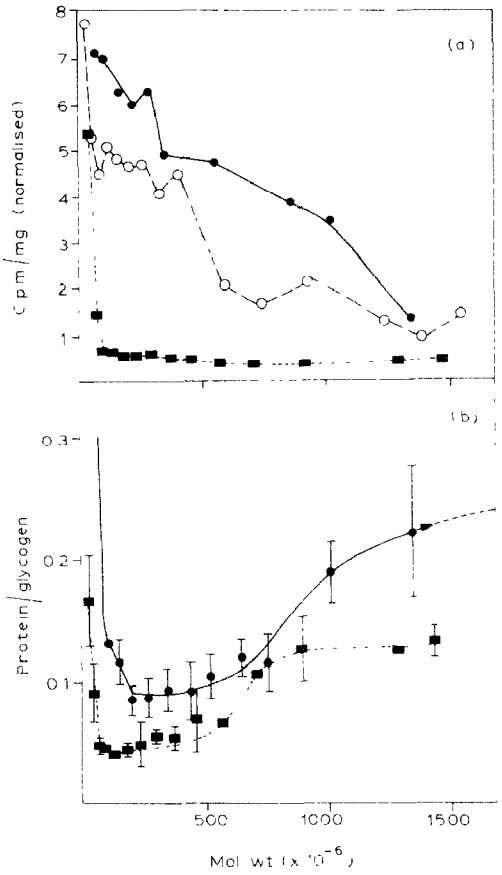

Fig. 2. (*a*) Distribution of radioactive incorporation within the various molecular sizes of the glycogens; (*b*) the heterogeneity of the protein/polysaccharide ratio of glycogen with respect to molecular size for normal and starved/re-fed (4 h) animals; symbols as in Fig. 1

Fig. 2*b* shows a heterogeneity of the protein–polysaccharide ratio similar to that in the well-fed animal[10]. However, the values reported here for the starved–re-fed animal are, on average, double the ratio found for the well-fed animal. The intense, post-starvation resynthesis of polysaccharide at the rough endoplasmic reticulum[3,6] is concerned, at least in the early stages reported here, with the production of material of high molecular weight, which is reflected in doubling of the relative protein content. However, as shown in Fig. 2*a*, the incorporation of D-[^{14}C]glucosamine is far from uniform over the size range, and parallels the short-term incorporation of D-[^{14}C]glucose under similar circumstances[11]. It is obvious that synthesis of glycogen of low molecular weight proceeds initially, at least, very rapidly, since the bulk of the radioactivity is located in these fractions. However, it is known from previously reported[11] experiments with D-[^{14}C]glucose that this incorporation into the glycogen of low molecular weight is passed on to the glycogens of high molecular weight. Fig. 2*b* indicates a relatively low amount of protein in the

region of low molecular weight, which clearly shows that glycogen of that size is being synthesised by two similar, but not distinct, mechanisms: (*a*) synthesis of β-particles on a protein backbone, each β-particle being completed sequentially because the radioactivity is uniformly distributed throughout the particle[25]; these particles later associate to form the large α-particles[18]; and (*b*) complete, sequential synthesis as in (*a*) but without the protein backbone and therefore the subsequent ability to form large α-particles.

A mechanism of this type accords with the observations of Devos and Hers on the sequential synthesis of liver glycogen in starved/re-fed livers[12,25,26] and are further confirmed by the results of beta-amylolytic digestion shown in Table I. However, all of the β-particles, whether free or associated on a protein backbone in the form of the large α-particles[17,18,27], are synthesised and degraded evenly (Table I). (Variations in the calculated beta-limits show no trend either with respect to molecular size or by method of measurement, *i.e.*, chemically or by loss of radioactivity, and may be regarded as random.) Therefore, the tentative suggestions[25] that the sequential synthesis and degradation could be related to (*a*) a defined order of β-particle incorporation into the α-particles that are degraded in a reverse order, or (*b*) a "link" of unknown nature in the α-particle, may be rejected.

Thus, the molecular order in the synthesis and degradation of liver glycogen is related to its well-known metabolic inhomogeneity in this tissue[7,9,11,19,21,28–31]. Glycogen having a relatively high content of protein (Fig. 2*b*)[10] is incorporated into the lysosomal compartment[11,19,20,29], where it can undergo non-phosphorolytic degradation independently of the glycogen particles in the cytosol.

These results also show that the previously reported effects of inhibition of hepatic protein synthesis by D-galactosamine[4,32] do not affect the incorporation experiments reported above and are related to the quantity of D-galactosamine presented to the cell. The unusual glycogens isolated after intraperitoneal injections of huge amounts of D-galactosamine[4] are clearly artefactual, and certainly connected with the rapid conversion of the amino sugar into its phosphate ester (with consequent loss of intracellular inorganic phosphate), its subsequent slow metabolism[33], and its incorporation into glycogen as D-glucosamine.

It is clear that some sugars, related to D-glucose, may be incorporated in small quantities, without harm, into liver glycogen. The incorporation of D-galactose, substituting for ~1 in every 500 D-glucose residues, was reported many years ago[34]. The failure of D-glucosamine, whether administered by intraperitoneal injection or by perfusion, to be directly incorporated into liver glycogen[35,36] is probably a surface-receptor phenomenon and would bear further investigation[37].

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