THE STRUCTURE OF LIVER GLYCOGEN

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1. Introduction

It has recently been shown [1] that protein-bound glycogen may be synthesized in some in vitro experiments and the implications of these experiments have been very clearly stated by Whelan [2]. Since isolated glycogen can be shown to be of very large molecular size – up to 10^9 daltons [3] – and its constituent β-particles are relatively small (approx. 10^7 daltons [3]) it seemed unlikely that the very high molecular weight glycogen were synthesized on a single protein backbone. The protein involved would have to be of considerably greater size than any so far reported. It was therefore decided to study the effect of disulphide bond-breaking reagents upon the size-distribution of glycogen molecules.

2. Experimental

Livers were quickly removed from rabbits (New Zealand White) which had been given an overdose of Nembutal (Abbott Laboratories Ltd., Naenae, New Zealand) and plunged into liquid nitrogen. Glycogen was then isolated from the tissue by cold-water extraction [4,5] and fractionated on a sucrose density gradient [3,5]. Sedimentation coefficients of the fractions were determined by the method of Martin and Ames [6]. A partial specific volume of 0.63 ml/g for glycogen was used [3].

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Some samples of purified glycogens were treated with disulphide-bond breaking reagents. Solutions of glycogen (approx. 10 mg/ml) in 8 M urea (pH 8.5, 0.1 M Tris buffer) were treated with excess (0.1 ml/ml) β-mercaptoethanol (B.D.H. Chemicals, Poole, England) for 30 min. These solutions were then dialysed against running water overnight or treated with excess (0.3 g/ml) iodoacetamide (B.D.H. Chemicals, Poole, England) and dialysed as before.

Protein was determined by the method of Lowry et al. [7] and glycogen by an iodine–iodide reaction [4].

3. Results

In confirmation of earlier experiments [8] no significant change in the sedimentation coefficient distributions was caused by the addition of 8 M urea.

Figure 1 and table 1 indicate that treatment with β-mercaptoethanol has caused a small but appreciable loss of high molecular weight material and a corresponding increase in low molecular weight material. (As in a previous paper [4], the division between 'high' and 'low' molecular weight glycogen has been arbitarily set at 2000 S. However since the previous paper [4] used a partial specific volume of 0.68 ml/g no exact comparison of data can be made.) Additionally, when the reduced sulphydryl groups are blocked with iodoacetamide and cannot therefore randomly reform, there is a practically total elimination of material of large molecular weight. (It should be noted that in a control experiment omitting the β-mercaptoethanol no significant change in the distribution was noted.) It is therefore clear that high

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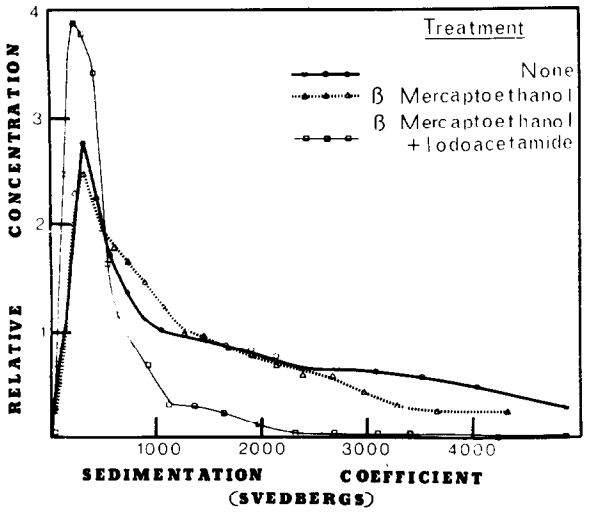

Fig.1. The effect of breaking disulphide bonds on the sedimentation coefficient distribution of liver glycogen. The relative concentration scale has been normalised (against fraction volume not sedimentation coefficients) to allow direct comparisons to be made between the samples. These results from one liver glycogen have been essentially duplicated in three subsequent experiments. Sedimentation coefficients are calculated as $s^0_{20,w}$ values [3].

molecular weight glycogen is constructed on a protein backbone which is itself held together by disulphide bridges. The fact that there is no massive increase of material in the very lowest molecular weight fractions after treatment with β-mercaptoethanol and iodoacetamide indicates that, at least, the bulk of the low molecular weight glycogen is unaffected by the breaking of disulphide bridges. The difference in the nature of the two glycogen populations, 'large' and 'small', is further highlighted by fig.2 where it can be seen that the two populations of polysaccharide have different amounts of protein associated with them. The large increase in the amount of protein associated with the very low molecular weight glycogen indicates that this material is either what remains after degradation by the polysaccharidases or is the skeleton upon which larger glycogen will be built. Some of this protein is enzymic [9] and is associated with the synthesis and degradation of the polysaccharide but the remainder must be structural.

Table 1
Effect of the breaking of sulphydryl bonds upon glycogen distributions

Sample treatment	Glycogen Contents (%)	
	0–2000 S	> 2000
None or 8 M urea	80	20
β-Mercaptoethanol	85	15
β-Mercaptoethanol + iodoacetamide	98	2

Values calculated from the data in fig.1

Since the glycogen distributions are unaffected by hydrogen-bond breaking reagents such as 8 M urea these results confirm that in vivo, at least some of the liver glycogen population is synthesized on a protein backbone. Moreover, in high molecular weight material the polysaccharide is built up on protein chains which are themselves held together by disulphide bridges. In addition this large glycogen has a greater amount of protein associated with it and this is probably related to the large amounts of disulphide bridges which must be involved. The protein content of the polysaccharide is possibly also a controlling factor in the well-described metabolic inhomogeneity of glycogen [10,11].

In conclusion it is clear that the large particles of glycogen (α-particles), as shown by electron-microscopy [12], composed of the small spherical β-particles, are held together by protein in the manner suggested

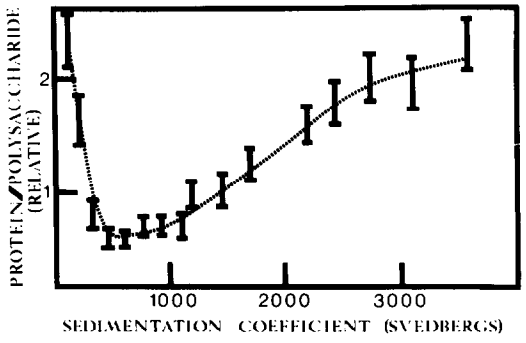

Fig.2. The protein–polysaccharide ratio of various glycogen fractions of the original glycogen. The large errors are associated with the small amount of protein and, in some fractions, with the relatively small amount of polysaccharide.

by Krisman and Barengo [1] with the addition of disulphide bridges holding various fundamental glycogen–protein associations together. The fundamental glycogen–protein particles which associate to form the large molecular weight species have sedimentation coefficients of approx. 200–400 S and this would correspond to molecular weights of approx. $15–40 \times 10^6$ [13]. The fundamental unit upon which the large molecular weight material is based is therefore composed of 2–3 β-particles per protein unit [3]. This method of producing large polysaccharide molecules should be investigated in other systems where polysaccharides are known to be associated with proteins (e.g., chondroitin sulphate, starch).

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