Journal of Chromatography, 387 (1987) 485–492
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 044

Note

Gel filtration of glycogens and their hydrolysis products

M. L. GARCIA*, M. R. FERRER, O. VALLS and S. GARCIA
Department of Biological Physical Chemistry, Faculty of Pharmacy, Barcelona University, Barcelona (Spain)
(First received March 20th, 1986; revised manuscript received August 28th, 1986)

Glycogen, and homopolymer of α-D-glucopyranose, constitutes the energetic reserve of mammalian tissues. Inside living cells it is strongly bonded to proteins and other substances, which makes it difficult to obtain with a high degree of purity.

Column fractionation of glycogen is a frequently employed chromatographic technique separations on diethylamino-Sephadex[1], DEAE-cellulose 2, concavalin A-Sepharose 4B[3] and glutaraldehyde-Bio-Gel P-10[4] having been carried out. Gel filtration of different kinds of glycogen has been described by Marshall[5] using 1% sodium chloride as eluent. This technique permits the purification of this macromolecule and the determination of its average molecular weight. Norman *et al.*[6] reported the gel filtration of the products of isoamylolysis of branched (1→4) α-D-glucans. The fractionation of rat liver glycogen on a Sephacryl S-1000 gel column was studied by Konishi *et al.*[7] in order to establish some structural properties of the different fractions.

In this work, gel filtration of several kinds of glycogen on agarose, at different pH values and ionic strengths of the eluent, has been carried out in order to determine the behaviour of glycogen under different experimental conditions. Gel filtration of glycogen hydrolysis products (GHPs), on dextran at different temperatures, is also described, and the thermodynamic parameters, ΔG°, ΔH°, ΔS°, for these fractions have been calculated.

EXPERIMENTAL

Apparatus

The chromatographic column, K 16/70 (70 cm × 16 mm), was equipped with temperature control and adapter Type A-16 to maintain constant the gel volume in the column (Pharmacia, Up-psala, Sweden). A Gilson peristaltic pump, Minipuls HP-4, operating at flow-rates in the range 0.5–1300 ml/h, and a Gilson fraction collector, Microcol TDC-80, were also employed.

Reagents

Different kinds of glycogen, obtained from Sigma, were examined: Types III and X from rabbit liver (RLG) and Type VII from mussel (*Mytilus edulis*) muscle (MMG), prepared by a modification of the Bell procedure[8,9].

0021-9673/87/$03.50

The products (GHPs) resulting from the hydrolysis of glycogen with the enzyme amyloglucosidase from *Aspergillus niger* (Boehringer Mannheim), for 30 min at 37°C[10] were also analysed. This enzyme breaks α (1-6) glycosidic links comprising the points of branching of the glycogen macromolecule.

As eluents, solutions of 1% sodium chloride and buffered solutions of borates and acetates were used.

Standards

Dextrans of known molecular weights (MW), T10 (MW = 10 000), T40 (MW = 42 100), T70 (MW = 70 400), T500 (MW = 472 000) and Blue Dextran T2000 (MW = $2 \cdot 10^6$), obtained from Pharmacia were employed. Glucose was used to determine the permeated volume, V_p, and Blue Dextran T2000 was used to measure the exclusion volume, V_o.

Analyses

The content of Blue Dextran T2000 was determined by UV spectrophotometry at 254 nm. Glucose, glycogens and GHPs were evaluated by polarimetry at 365 nm by means of a method for the determination of the rotatory power developed in our laboratory[11].

RESULTS

The Blue Dextran 2000 and the glucose elution profiles, on Sepharose 2B (a) and Sephadex G-100 (b), are shown in Fig. 1, the eluent in both cases being 1%

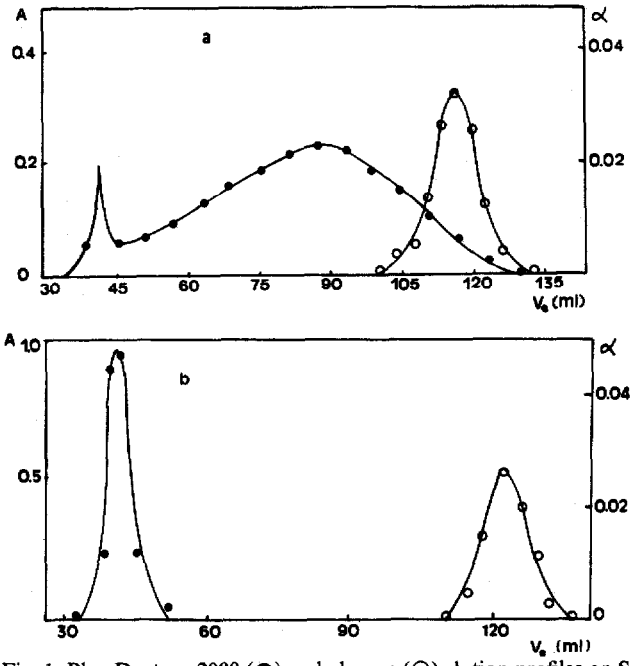

Fig. 1. Blue Dextran 2000 (●) and glucose (○) elution profiles on Sepharose 2B (a) and Sephadex G-100 (b). Eluent: 1% sodium chloride. α = Rotary power; V_e = elution volume.

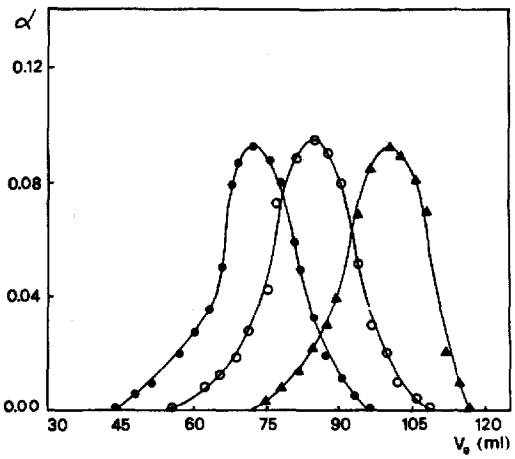

Fig. 2. Elution profiles of the three types of glycogen analysed in Sepharose 2B. Eluent as in Fig. 1. Glycogens: ●, MMG, Type VII; ○, RLG Type III; ▲, RLG Type X.

sodium chloride (I = 0.17 and pH 5.6) at flow-rates of 7.5 and 24 ml/h, respectively; factions of 3 ml were collected.

The elution profiles of the three types of glycogen analysed on Sepharose 2B, with 1% sodium chloride (I = 0.17 and pH 5.6) as eluent, are in Fig. 2 the elution volume, V_e, being maximum for the RLG Type X and minimum for the MMG, Type VII. In Table I, the statistical deviations of V_e can be appreciated, which were calculated in ten consecutive trials. The mean distribution constants, K_{av}, are also reported, being maximum for the RLG, Type X and minimum for the MMG, Type VII.

The influence of the pH on the distribution constant, K_{av}, is shown in Fig. 3; for all glycogens, this parameter increases at extreme values of pH (acidic and alkaline) within the studied interval (4.0–8.6). Borate or acetate buffered or non-buffered sodium chloride solutions were used as eluents.

TABLE I

STATISTICAL PARAMETERS AND DISTRIBUTION CONSTANTS FOR THE GLYCOGENS ANALYSED ON SEPHAROSE 2B

δ = deviation, μ = median average.

Type of glycogen	$\bar{V}$ *(ml)*	σ	μ^{95}, μ^{99}	$K_{av} = (V_e - V_o)/(V_p - V_o)$
MMG VII	72.0	0.471	72.0 ± 0.337, 72.0 ± 0.484	0.40
RLG III	84.5	0.408	84.5 ± 0.292, 84.5 ± 0.419	0.56
RLG X	103.0	0.577	103.0 ± 0.412, 103.0 ± 0.593	0.59

TABLE II

V_e, K_{av} AND ΔG° VALUES CALCULATED FOR THE GHPs ANALYSED ON SEPHADEX G-100, AT DIFFERENT TEMPERATURES

T (K)	GHP_{VIII}			GHP_{III}			GHP_X		
	$V_e(ml)$	K_{av}	$\Delta G^\circ(cal/mol)$	$V_e(ml)$	K_{av}	$\Delta G^\circ(cal/mol)$	$V_e(ml)$	K_{av}	$\Delta G^\circ(cal/mol)$
298	107.0	0.800	132.69	108.0	0.814	122.37	111.0	0.850	96.64
308	105.0	0.780	152.70	106.0	0.802	135.60	109.5	0.835	110.82
318	102.0	0.759	174.98	103.5	0.777	160.10	107.0	0.819	126.70
328	99.5	0.751	187.41	100.5	0.764	176.18	104.5	0.812	136.30
338	97.0	0.730	212.25	98.5	0.749	194.93	102.5	0.796	153.30

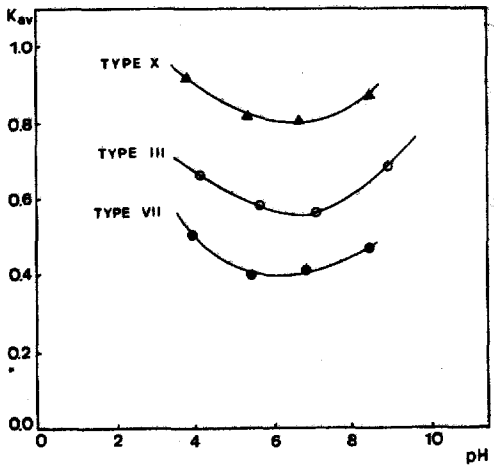

Fig. 3. Influence of pH on the distribution constant, K_{av}, for all glycogens studied here (see Fig. 2). Buffers: pH 4.0, acetic acid–sodium acetate; pH 5.6 and 6.9, hydrochloric acid–sodium chloride; pH 8.6, boric acid–sodium tetraborate.

The effect of the eluent ionic strength, I, on the elution volume was found not to be significant for the analysed glycogens, in the range $I = 0.17$–0.68 with sodium chloride as eluent.

The K_{av} values of the glycogen hydrolysis products GHP_{VII}, GHP_{III}, and GHP_X on dextran gel (Sephadex G-100) were determined at several temperatures (25–65°C); this parameter was found to decrease with increasing temperature (Table II).

DISCUSSION

The distribution constants, K_{av}, obtained from the agarose gel (Sepharose 2B) data, which were 0.40 for the MMG, Type VII, 0.56 for the RLG, Type III and 0.81 for the RLG, Type X, were within the order expected for polysaccharides with similar molecular weights[12,13].

The statistical study of the accuracy of the method indicates an excellent reproducibility of the results in ten consecutive trails carried out under the same experimental conditions, for the polymers studied.

A characteristic curve (Fig. 4) was obtained on plotting the K_{av} values obtained for dextrans against their molecular weights, which allows the calculation of the mean molecular weights of the analysed polymers[14,15]. The values obtained, $2 \cdot 10^6$, $1 \cdot 10^6$ and $5 \cdot 10^4$ for the MMG, type VII, RLG, Types III and X, respectively, were in inverse relationship to their distribution constants[16] and they involved an error of 3–7% with respect to the values calculated by other analytical techniques[17]; this error is of little significance in comparison to the magnitude of these molecular weight. The lowest molecular weight obtained for the rabbit liver glycogen Type X may be attributed to the extraction procedure for this polymer which involves hot water, whereas the other glycogens were extracted with cold water and methanol. The M_w value obtained for the mussel muscle was higher that for the rabbit liver, which is

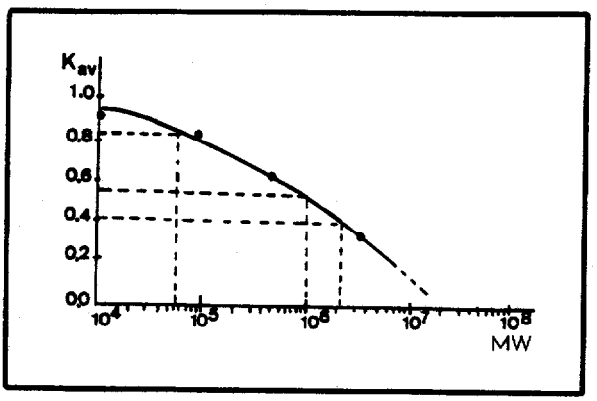

Fig. 4. Variation of the distribution constant, K_{av}, *versus* the molecular weight for different types of dextran.

not in accord with the results of Marshall[5], but the RLG analysed by this author was extracted using the mercuric chloride procedure.

The increase of K_{av} for glycogen at extreme values of pH may be attributed to a charging process according to which this polysacharide, which is neutral in non-electrolytie solutions, acquires a definite electric charge in salt solutions[18,19]. On the other hand, in solutions of some oxacid salts, cyclic complexes are formed with vicinal OH groups. Thus borates react with vicinal OH groups of glycogen to form bis-diol-glycogen borate complexes[20] (Fig. 5).

The K_{av} of the GHPs, calculated from the V_e values obtained, on Sephadex G-100, was higher for the RLG, Type X and lower for the MMG, Type VII. The molecular weight average, calculated by means of different dextrans[21–23], was $2 \cdot 10^3$, which is in agreement with the expected value when the size of this glycogen fraction (formed by chains of 8–14 glucoses) is taken into account.

The K_{av} values also decrease with increasing temperature, within the interval (25–65°C) studied. Taking into account the establishment of an equilibrium between the solute in the mobile phase and the solute in the interior of the gel, the change in the Gibbs standard free energy, $\Delta G°$, between the two states will be given by

$$\Delta G° = - RT \ln K_{av} \quad (1)$$

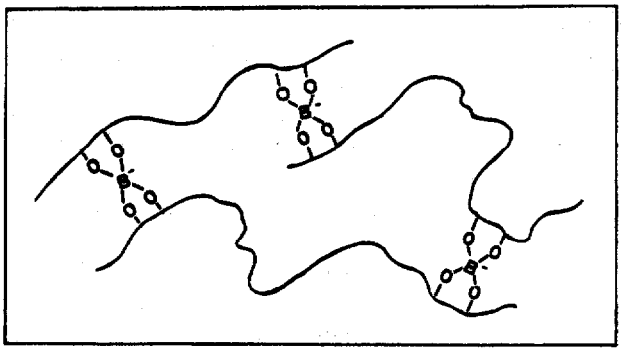

Fig. 5. Bis-diol-glycogen borate complex.

Where R is the gas constant and, T is the absolute temperature. The ΔG° values obtained (Table II) increase with increasing temperature found previously[25,25].

The entropy and enthalpy changes associated with the reversible binding of the eluent by the stationary phase are defined by applying the van 't Hoff relationship to the chromatographic process:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{2}$$

Coming eqns. 1 and 2 yields:

$$-\ln K_{av} = \frac{\Delta H^\circ}{RT} - \frac{\Delta S^\circ}{R} \tag{3}$$

A plot of $-\ln K_{av}$ *versus* $1/T$ was, a line (Fig. 6), and indicates a negative enthalphy for the interaction between the solute and the gel matrix, similar to that obtained by Dellweg *et al.*[24].

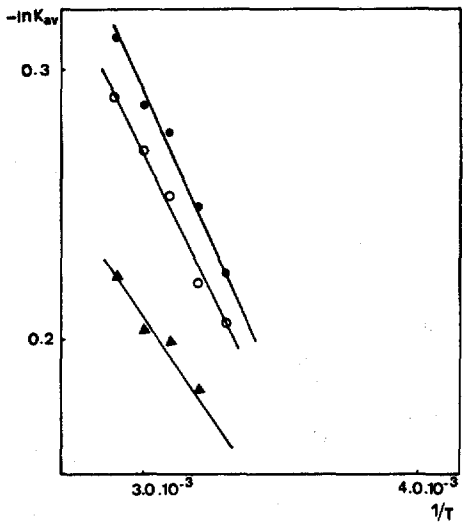

Fig. 6. Graphical determination of ΔH° and ΔS°. Glycogens as in Fig. 2.

The enthalpy, according to Dellweg *et al.*[24], which must be evaluated by use of the differential form to the eqn. 3, is obtained at each temperature from the slope of a tangent, $d(\ln K_{av})/d(1/T)$, to the curve which describes the experimental data in the van 't Hoff plot:

$$\frac{d\ (\ln K_{av})}{dT} = \frac{\Delta H^\circ}{RT^2} \tag{4}$$

The corresponding entropy change at each temperature may be estimated by use of eqn. 2.

The ΔG°, ΔH° and ΔS° values calculated here at 35°C, are listed in Table III together with the values obtained by Dellweg *et al.*[24] for maltodextrins on Bio-Gel

TABLE III

THERMODYNAMIC PARAMETERS

	ΔG° (cal/mol)	ΔH° (cal/mol)	ΔS° (cal/mol · K)
Maltotetrose*	+268.00	−370.00	−2.14
Maltopentose*	+341.00	−469.00	−2.72
GHP_{VII}	+152.70	−497.93	−2.11
GHP_{III}	+135.60	−440.34	−1.87
GHP_X	+110.82	−351.62	−1.50

* Values calculated by Dellweg *et al.*

P-2 at 25°C. Compared with the results reported by Dellweg *et al.*[24] and considering the number of glucose units that constitute the GHPs, the ΔH° and ΔS° values obtained are similar but the ΔG° values are sligtly lower than expected. This fact can be attributed to the different chromatographic column bed utilized in the two sets of experiments.

ACKNOWLEDGEMENT

We are indebted to Professor M. Espina for carrying out the calculations.

REFERENCES

1 P. A. Ockerman, *Biochem. Biophys. Acta*, 74 (1963) 58.
2 L. N. Bobrova and B. N. Stepanenko, *Dokl. Akad. Nauk. SSSR*, 191 (1970) 468.
3 E. H. Donnelly and I. J. Goldstein, *Biochem. J.*, 118 (1970) 679.
4 J. F. Kennedy and A. Rosevear, *J. Chem. Soc., Perkin Trans. 1* (1973) 2041.
5 J. J. Marshall, *J. Chromatogr.*, 77 (1973) 201.
6 P. T. Norman, L. E.Macaskie and K. K. Grewell, *Carbohydr. Res.*, 144 (1983) 338.
7 Y. Konishi, Y. Ninomiya and H. Fuwa, *Agric. Biol. Chem.*, 48 (1984) 215.
8 D. J. Bell, H. Gutfreund, R. Cecil and A. G. Ogston, *Biochem. J.*, 28 (1934) 882.
9 B. W. Brigman, *J. Am. Chem. Soc.*, 64 (1942) 1349.
10 T. Iwamasa, *J. Electronmicr.*, 19 (1972) 371.
11 T. Montero, M. L. García, O. Valls and S. García, *Cir. Farm.*, 289 (1985) 255.
12 M. L. Fishman and R. A. Barford, *J. Chromatogr.*, 52 (1970) 494.
13 D. Malchow and O. Luderitz, *Eur. J. Biochem.*, 2 (1967) 469.
14 F. G. Lehmann, *Clin. Chim. Acta*, 28 (1970) 335.
15 J. Marrink and M. Gruber, *Febs Lett.*, 2 (1969) 242.
16 S. Hjertén, *Arch. Biochem. Biophys.*, 99 (1962) 466.
17 M. L. García, O. Valls, A. Alsina, S. García, P. Sauve and R. Verger, *XXI Reunión Bienal de Química, Santiago de Compostela, 1986.*
18 J. L. Frahn and J. A. Mills, *Aust. J. Chem.*, 12 (1959) 65.
19 H. Weigel, *Adv. Carbohydr. Chem.*, 18 (1959) 61.
20 B. N. Stepanenko and L. B. Uzdenikova, *Carbohydr. Res.*, 25 (1972) 526.
21 D. M. W. Anderson and J. F. Stoodart, *Anal. Chim. Acta*, 34 (1966) 401.
22 P. E. Squire, *Arch. Biochem. Biophys.*, 107 (1964) 471.
23 K. A. Granath and B. E. Kvist, *J. Chromatogr.*, 28 (1967) 69.
24 H. Dellweg, M. John and G. Trenel, *J. Chromatogr.*, 57 (1971) 89.
25 S. Hjertén, *J. Chromatogr.*, 50 (1970) 189.