DISTRIBUTION OF α-AMYLASE-RESISTANT REGIONS IN THE GLYCOGEN MOLECULE*†

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ABSTRACT

Exhaustive degradation of glycogen by α-amylase yields sugars, branched oligosaccharides, and a highly branched, relatively high-molecular-weight "macrodextrin" The macrodextrin formed by action of porcine pancreatic α-amylase on shellfish glycogen β-amylase limit-dextrin had a molecular weight of about 6,000 and was obtained in 25% yield This molecular size and yield indicate that there are about 42 macrodextrin units or clusters of dense branching per 10^6 daltons of the glycogen β-amylase limit dextrin, or 23 macrodextrin regions per 10^6 daltons of the original glycogen Graded hydrolysis of the β-dextrin by α-amylase released low-molecular-weight products and macrodextrins, with gradual decrease in molecular size of the residual β-dextrin It is concluded that the macrodextrin regions are randomly dispersed throughout the glycogen molecule rather than being concentrated in the "core" Molecular weights of glycogens and limit-dextrins were shown to be related to the sedimentation coefficient, s, by the equation mol wt $= 5 \times 10^3 s^{3/2}$

INTRODUCTION

Glycogen has long been known to possess an average chain length of 10–12 D-glucose units and to have average external and internal chain lengths of 7–8 and 3–4 glucose units, respectively[1] Early evidence for internal chain lengths of less than 3–4 glucose units was obtained by Roberts and Whelan[2] who treated both amylopectin and glycogen with human salivary α-amylase to obtain α-amylase limit-dextrins (α-dextrins) that, based upon further analysis using R-enzyme, were concluded to have internal chain lengths of 1–2 glucose units In an attempt to find a maximal α-dextrin,

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Heller and Schramm[3] used porcine pancreatic α-amylase to produce a macrodextrin from glycogen The macrodextrin was prepared from a deproteinized and dialyzed digest by gel filtration and was characterized by size, yield, and susceptibility to further α-amylolytic degradation Heller and Schramm concluded that macrodextrins represent regions of the glycogen that are so densely branched that few, if any, linkages are susceptible to further cleavage by α-amylase A typical macrodextrin, prepared essentially according to the method of Heller and Schramm by Dr Walter Verhue in our laboratories, had an average size of 34 glucose units and an average chain length of 4 glucose units Such macrodextrin regions might constitute the resistant "cores" of glycogen molecules, with the less-branched, more metabolically active part of the molecules located in the periphery. Alternatively, the macrodextrin regions might be arranged either regularly or randomly throughout the structure, the random arrangement being appropriate for a "statistical" model of glycogen[4] The work reported here was undertaken to determine how many densely branched, macrodextrin-yielding regions exist in a glycogen molecule and how such regions are arranged within the glycogen molecule

MATERIALS AND METHODS

Glycogen was obtained from Sigma Chem Co (St Louis, Missouri, glycogen from shellfish, practical grade, type II, lot No 75B-1600) Further information from Sigma indicates that the glycogen had been obtained through an unidentified supplier from Pacific coast oysters by water extraction, solvent precipitation, and drying Crystalline porcine pancreatic (1→4)-α-D-glucan 4-glucanohydrolase (E C 3 2 1 1, α-amylase) and crystalline sweet potato (1→4)-α-D-glucan maltohydrolase (E C 3 2 1 2 ; β-amylase), were obtained from Worthington Biochem Corp (Freehold, New Jersey) Other chemicals were of reagent grade Glass-distilled water was used throughout

Glycogen was equilibrated with laboratory air, and its moisture content was determined by drying it in a vacuum oven Glycogen solutions of known concentration were obtained by dissolving weighed amounts of the air-dry glycogen with gentle warming (not over 60°)

Glycogen β-amylase limit-dextrin was prepared approximately as suggested by Whelan[5] Shellfish glycogen (10 g) was dissolved with gentle warming in water (150 ml), and the solution was filtered through glass wool into a dialysis bag The solution was made 0 2M in sodium acetate and 10mM in 2-mercaptoethanoic acid (mercaptoacetic acid), and acidified to pH 4 65 β-Amylase (0 1 ml of crystalline suspension) was added, and the digest was continuously dialyzed against a slow stream of distilled water for 5 days Finally, trichloroacetic acid was added to the digest to a concentration of 2%, and the suspension was centrifuged to remove proteins, dialyzed overnight, and lyophilized (yield, 5 35 g) Four portions of the glycogen β-dextrin were degraded with α-amylase under conditions varying only in the time of exposure to α-amylase and amount of enzyme The substrate concentration in all digests was

10 mg/ml Sodium glycerophosphate, 20mM, pH 6 8, was used as a buffer The incubation temperature was 40° The enzyme concentrations used were 75, 7 5, 0 75, and 0 075 milliunits (mU) per ml. The durations of exposure to the enzyme were 10, 20, 40, 70, and 100 min for the three digests with the lower enzyme concentrations and 100 and 1000 min for the digest with the highest enzyme concentration Enzyme activity in international units (1 U of enzyme will hydrolyze one micromole of bonds per min) was determined from the linear increase in reducing value with time of the digest containing the lowest concentration of enzyme The degrees of exposure of the β-dextrin to α-amylase were expressed in terms of the log (Et/S), where E is enzyme activity in U/ml, t is time in min , and S is the substrate concentration in mg/ml

Three sets of samples were taken (a) For reducing value measurement, enzyme activity was stopped by adding 4 vol of 2% sodium carbonate (b) For gel-filtration analysis, enzyme activity was stopped by adding a few drops of conc ammonium hydroxide, and the sample was preserved by freezing (*c*) For ultracentrifugal analysis, enzyme activity was stopped and the relatively high-molecular-weight carbohydrate was precipitated by addition of 2 vol of hot ethanol

Reducing values were measured by the alkaline ferricyanide procedure with a Technicon AutoAnalyzer[6] Reducing values were expressed as % apparent maltose, *i e* , the maltose concentration having a reducing value equivalent to that of the sample, times 100, divided by the substrate concentration For gel-filtration analysis, a 10-mg sample was separated on a Sephadex G-50 column The carbohydrate profile of the fractionated column effluent was determined by the phenol–sulfuric acid method[7]

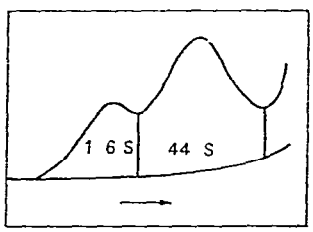

Fig 1 Tracing of an ultracentrifuge plate showing bimodal distribution of macromolecules, characteristic of degradation of glycogen β-amylase limit-dextrin by porcine pancreatic α-amylase Direction of sedimentation, left to right, with a double sector synthetic boundary cell The major peak (right, 44S) is the high-molecular-weight, partly degraded β-amylase limit-dextrin, the smaller peak (left, 1 6S) is macrodextrin The sample was taken at log $(Et/S) = -3$ 13 The vertical lines indicate the sectioning of the diagram for quantitative estimation of the areas under the macrodextrin and residual β-amylase limit-dextrin peaks The base line was obtained from the second sector of the cell, containing water only

A similar profile had been determined for a column-fractionated, 10-mg sample from a large-scale α-amylase hydrolyzate of glycogen The amount of carbohydrate in the large-scale hydrolyzate that was soluble in 66% ethanol was determined by the addition of 2 vol of ethanol to a known amount of the hydrolyzed glycogen, and by collecting, drying, and weighing the precipitate A cut-off point in the column-elution profile

was chosen such that the column, high-molecular-weight fraction approximated the fraction of carbohydrate precipitated from the large-scale hydrolyzate by 66% ethanol Sedimentation behavior was observed at 20° on aqueous solutions containing approximately 1% carbohydrate, in a Spinco Model E analytical ultracentifuge employing a Schlieren optical system Sedimentation coefficients were determined by the maximum-ordinate method[8] No corrections were made for concentration dependence, and sedimentation-coefficient distributions were not determined The relative amounts of the differently sedimenting populations were calculated from planimeter measurements of a projected and traced Schlieren image (Fig 1) The effect of radial dilution was negligible (2% or less)

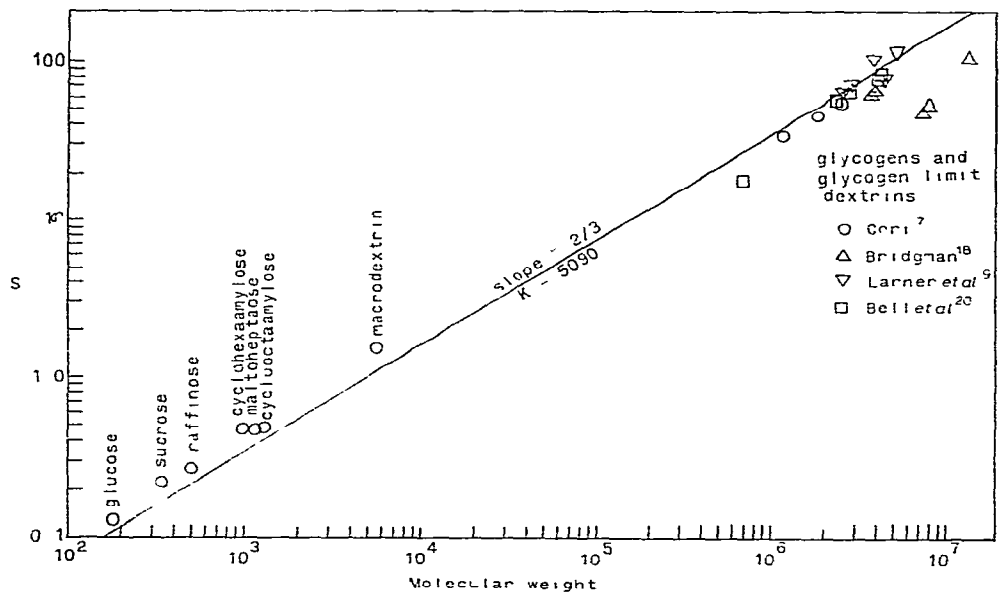

Fig 2 Relationship between sedimentation coefficient, s, and molecular weight for sugars dextrins, and glycogens The straight line is calculated for the relationship mol wt $= Ks^{3/2}$ for K = 5090, with s expressed in Svedberg units This corresponds to a roughly spherical molecule with about 1 8 g of water per g of carbohydrate

An estimate of the mol wt associated with a given sedimentation coefficient was made graphically (Fig 2) The rationale behind Fig 2 is presented in the Appendix Densities of a dilution series were determined pycnometrically, and the partial specific volume was derived graphically ($\bar{v} = 0\ 62 \pm 0\ 01$)

The hydration of glycogen in solution was determined approximately by assuming that the sedimentation-pellet volume, per g of glycogen, is equivalent to the hydration volume Solutions containing known amounts of glycogen (generally about 10 mg of glycogen in 0 7 cm^3 of water) were layered over Fluorocarbon FC-43 in a single-sector ultracentrifuge cell After being centrifuged at various speeds in the range 30,000–60,000 r p m (60,000–260,000 $\times g$), the pellet appeared to reach an

approximately constant volume which was measured by use of the ultracentrifuge Schlieren system together with the ultracentrifuge cell geometry Numerical values were obtained in the range 2 2–3 6 cm^3 per g of glycogen, with the most reproducible value of about 2 44 cm^3/g Errors in determination of the pellet volume stemmed primarily from the difficulty in measuring the exact positions of the top and bottom menisci, owing to the very big refractive-index change between supporting fluid, pellet, and solvent If it is assumed that, in the pellet, the glycogen has an effective specific volume of 0 62 and water of 1, there would be 1 82 g of water of hydration per g of glycogen (range 1 6–3)

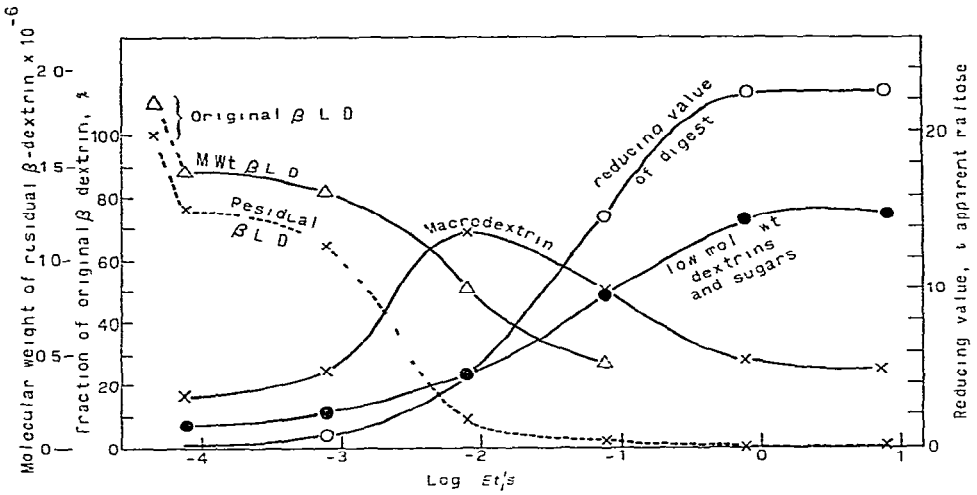

Fig 3 Time course of the conversion of shellfish-glycogen β-dextrin by porcine pancreatic α-amylase to macrodextrin and low-molecular-weight products For experimental details see text The abscissa is given as the logarithm of the product of enzyme concentration (U/ml) times time (min) divided by substrate concentration (mg/ml) △, mol wt of residual β-dextrin (βL D), X----X, amount of residual β-dextrin, X——X, amount of macrodextrin, ●, amount of low-molecular-weight dextrins and sugars, O, reducing value of digest expressed as percent apparent maltose

RESULTS

Table I is a compilation of some of the parameters of the initial shellfish-glycogen β-dextrin and the products resulting from action of porcine pancreatic α-amylase The course of the reaction is presented graphically in Fig 3 The samples were selected to cover, in equal log (Et/S) steps, a very wide range of exposure of the β-dextrin to the α-amylase On α-amylase action, the β-dextrin rapidly declined in amount and molecular size As the β-dextrin was degraded, a macrodextrin was formed as the major initial product, together with small amounts of low-molecular-weight dextrins and sugars The amount of macrodextrin increased to 68% of the total carbohydrate at log (Et/S) = −2 13, then declined as some of the less resistant macrodextrins were further converted into lower-molecular-weight products How-

ever, even after exhaustive treatment with α-amylase [at log $(Et/S) = +0\ 87$, representing 1000 times the extent of exposure at log $(Et/S) = -2\ 13$], the macrodextrin accounted for about 25% of the initial β-dextrin

TABLE I

ACTION OF PORCINE PANCREATIC α-AMYLASE ON SHELLFISH-GLYCOGEN β-DEXTRIN

Extent of reaction log (Et/S)	*Reducing value of digest (as % maltose)*	*Residual β-dextrin (% of initial β-dextrin)*	$s_{20,w}$ *of residual β-dextrin*	*Mol wt of residual β-dextrin* $(10^{-6})^a$	*Macrodextrin (% of initial β-dextrin)*	*Low mol wt dextrins and sugars (%)*
Original β-dextrin		100	51	1 85	0	0
−4 13	0 17	76	44	1 49	17	7
−3 13	0 71	64	42	1 39	25[b]	11
−2 13	5 4	9	31	0 88	68	23
−1 13	14 6	2	20	0 46	50	48
−0 13	22 5				28[c]	72
0 87	22 5				26	74

[a] $M = 5090\ s^{3/2}$ [b] $s_{20,w}$ 1 6 S~mol wt 6090 [c] $s_{20,w}$ 1 4 S~mol wt 5000

The molecular weight of the final, highly resistant macrodextrin, was obtained by reducing value (mol wt 5,500) and by sedimentation and diffusion (mol wt 6,140)

Using 6×10^3 as the provisional mol wt, the yield of macrodextrin from β-dextrin (25%), the molecular weight of the β-dextrin ($1\ 85 \times 10^6$), and the yield of β-dextrin from glycogen (54%), we calculate that there are approximately $0\ 25 \times 1\ 85 \times 10^6/(6 \times 10^3) = 77$ macrodextrin regions per original β-dextrin molecule [and hence per original glycogen molecule of mol wt. $1\ 85 \times 10^6/(0.54) = 3\ 4 \times 10^6$] The same analytical values indicate that there are $0\ 25 \times 10^6/(6 \times 10^3) = 42$ macrodextrin regions per 10^6 daltons of β-dextrin, or $0\ 25 \times 0\ 54 \times 10^6/(6 \times 10^3) = 22\ 5$ macrodextrin regions per 10^6 daltons of the parent glycogen

DISCUSSION

Our results clearly corroborate and extend the reports of Heller and Schramm[3] and Schramm[9] that glycogen contains regions of dense branching ("macrodextrin") resistant to α-amylase These macrodextrins may be expected to vary substantially in molecular size and degree of resistance to α-amylase, depending on the type of glycogen used, the type of α-amylase, and the extent or severity of the treatment by α-amylase

Shellfish glycogen was used for this study because of its availability and because it gives a high yield of macrodextrin, in comparison with rabbit-liver glycogen[9] We do not know if the original state of this glycogen was the α or β form[10], nor do we know how much, if any, degradation occurred during its commercial preparation For the purposes of this work, the difference between original α or β form is of no consequence Any degradation that may have occurred during the extraction procedure would not alter the substance of our argument

We have employed crystalline porcine pancreatic α-amylase as the degradative agent because of its availability and because its specificity with regard to branching is well understood The linear parts of glycogen—the peripheral chains and long interbranch regions — are converted into D-glucose, maltose, and maltotriose, the maltotriose eventually being converted into D-glucose and maltose For the enzyme to cleave between branch points, it is only necessary that there be a chain of two α-D-(1→4)-linked glucose units between the D-glucose units holding the α-D-(1→6)-linkages[11] Isolated branches are converted into low-molecular-weight branched oligosaccharides, in the range of 4–7 glucose units Where two or more branch points are separated by less than two D-glucose units, such regions are converted into multiple-branched oligosaccharides[12] Our initial study of salivary-amylase macrodextrin indicates that it simply consists of larger-molecular-size clusters of dense branching built on the same structural principles as the multiple-branched oligosaccharides[12] Thus, there is a spectrum of multiple-branched dextrins, resistant to α-amylase, of increasing molecular size and branching complexity We have arbitrarily designated the macrodextrins as those compounds precipitated by 66% ethanol from an α-amylase-digest of glycogen or β-limit-dextrin Correlation between the fractionation into high- and low-molecular-weight populations by gel filtration and alcohol precipitation was good Gel-filtration fractionation of the 66% ethanol precipitate from a large-scale glycogen hydrolyzate resulted in 97% of the material being classified as high-molecular-weight

Until now, it has not been known whether the macrodextrins constitute an amylase resistant "core" of the glycogen molecule, or whether they are arranged more or less randomly in the structure, as would be expected if glycogen has a "statistical" structure[4] From the "core" hypothesis, one would expect the glycogen molecule to be rapidly attacked at the periphery with formation of low-molecular-weight sugars and simple dextrins, leaving a single, complex high-molecular-weight core, destined to become the macrodextrin From the statistical model, one would expect macrodextrins to be produced as initial products, together with other lower-molecular-weight sugars and dextrins

From the ultracentrifuge patterns (Fig 1), it is obvious that there is a distribution of the macromolecular degradation products into two major peaks, the residual high-molecular-weight, partly degraded β-dextrin, and a new slow-moving peak, the macrodextrin peak Formation of a macrodextrin as an initial product is inconsistent with the single core hypothesis As enzymic degradation proceeds, the macrodextrin peak increases in size, while the residual β-dextrin peak decreases both in size and mobility Although we have not attempted to determine the molecular-size distribution in the residual β-dextrin, it appears that amylase "carves" small fragments (macrodextrins) from the parent β-dextrin molecule. If the amylase had cleaved the β-dextrin into two approximately equal, large fragments, one would expect to see a single broad peak, and no macrodextrin formation Alternatively, one might suppose that as a single α-dextrin molecule is attacked, it is entirely converted into macrodextrins and lower-molecular-weight products Such an interpretation would suggest the prompt formation of macrodextrin, as observed But, the molecular weight of the

residual β-dextrin should remain more or less constant Our measurements indicate that the molecular weight of the β-dextrin decreases progressively during the degradation

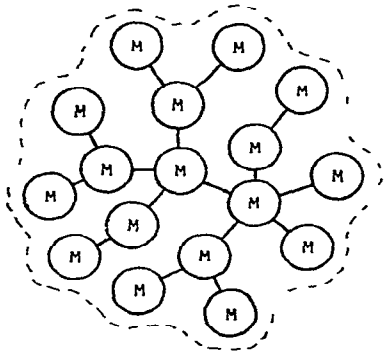

Fig 4 Schematic 2-dimensional representation of shellfish-glycogen β-dextrin, mol wt 410,000 (equivalent to mol wt 760,000 of the original glycogen) The diagram may be regarded as a section of an actual polysaccharide molecule, the entire particle being many times larger than the section indicated The regions labelled M are densely branched, and on α-amylase action are converted into macrodextrin Organization is such that α-amylase initially cleaves the molecule into large fragments or clusters, subsequently breaking these down to macrodextrin and oligosaccharides The reducing group of the original molecule might be attached to any one of the M groups, not necessarily at the center The dotted outline represents the periphery of the original glycogen molecule

From these results we suggest that the β-dextrin (and its parent glycogen) can be depicted as a "bunch" of macrodextrin regions (M) of dense branching, held together into a single molecule by regions of low-branching density (Fig 4) The peripheral chains of the glycogen (dotted lines) are readily removed by β-amylase, leaving the β-dextrin α-Amylase attacks the β-dextrin initially at the outer, less branched regions The peripheral M regions, being most accessible, are liberated first, thereby exposing the more interior M regions to further attack As amylase action continues, eventually all the accessible glycosidic bonds are cleaved, leaving only the most resistant macrodextrins, branched oligosaccharides, and sugars Some of the less resistant macrodextrin fragments initially formed are themselves degraded in the later stages of the reaction, *i e* after $\log (Et/S) \sim -2$

The Meyer model of glycogen is based on chain-length measurements, averaged over the entirety of all the glycogen molecules examined[13] Similarly, the results reported here are averages for all types of glycogen molecules in the preparation Presumably, glycogen is a population of similarly constituted molecules, so the number of densely branched, macrodextrin-yielding regions per 10^6 daltons of glycogen would be more or less independent of molecular size If this is true, degradation of the native glycogen during its preparation and conversion into the β-dextrin would have little or no effect on macrodextrin formation The average shellfish-glycogen molecule of molecular weight 3.4×10^6 corresponds to an average of 77 densely branched

regions Any of several factors could account for further heterogeneity of the number of densely branched regions per molecule within a particular glycogen population One such factor could be the "metabolic age" of a glycogen molecule in terms of the number of successive synthesis–degradation cycles of the animal's glycogen stores Increased branching density could result from a random placement of new branches upon existing structures during the synthesis cycle and a relative stability of the more complexly branched regions during the degradation cycle The number of densely branched regions in a particular native glycogen molecule also could be affected by fluctuations in the availability of substrates, the relative activities of elongating and branching enzymes[14], or the relative activities of competing, degradative enzymes during net glycogen synthesis In addition, such variables as species differences, feeding schedules, individual age, time of day, or method of glycogen isolation could contribute to differences in the complex branching patterns of glycogen Moreover, even with a given glycogen preparation, different specificities of different α-amylases (*e g* bacterial α-amylase in comparison with pancreatic α-amylase) could well give different modes of degradation and significant differences in the structure and yield of macrodextrin Further work would be necessary to examine the role played by any of these factors

This work has confirmed the observations of Roberts and Whelan[2] and of Heller and Schramm[3] that there are α-amylase-resistant regions of glycogen much more densely branched than the Meyer model with its uniform inner-chain lengths would indicate Further, we have been able to estimate the average number of macrodextrin-yielding regions per glycogen molecule under conditions where only the largest of such densely branched regions were examined Whatever the distribution about the average may be, it does seem clear that there are at least several tens of densely branched regions per molecule It also is clear that the glycogen β-limit dextrin begins to fragment rapidly after exposure to α-amylase with the early appearance of low-molecular-weight macrodextrins together with high-molecular-weight residual β-dextrin Therefore, it seems that glycogen is constructed of several large, densely branched regions connected by less densely branched, easily hydrolyzed segments This more open structure contains, in addition to linear portions that give D-glucose, maltose, and maltotriose, segments containing as many as 4 or 5 branches, resistant to further α-amylolysis but not included in the macrodextrin fraction Since the macrodextrins appear early during α-amylolysis, the densely branched regions examined here must have been arranged randomly, in contrast to any kind of a concentration of densely branched regions or a dense core which would have resulted in macrodextrins appearing only after extensive α-amylolysis

APPENDIX

Relation between sedimentation coefficient and molecular weight for glycogen and glycogen dextrins — Determination of molecular weight by velocity ultracentrifugation[8] through use of the Svedberg equation $M = Nfs/(1 - \bar{v}\rho_0)$ requires experi-

mental values for f, s, $\bar{v}$ and ρ_0 For glycogen and glycogen dextrins of various molecular sizes, one expects that the frictional coefficient f and the sedimentation coefficient s would be highly variable, whereas $\bar{v}$, the partial specific volume of the solute, and ρ_0, the solvent density, would be essentially constant The frictional coefficient f can be calculated for spheres by using Stokes' law $f = 6\pi\eta r$, where η is the viscosity of the solvent and r is the radius of the particle For a particle of (hydrated) specific volume v, the radius is given by $r = (3\ Mv/4\pi\ N)^{1/3}$ and therefore f, the frictional coefficient is $f = 6\pi\eta\ (3\ Mv/4\pi\ N)^{1/3}$ We can, therefore, rewrite the Svedberg equation

$$M = \left\{\frac{N\,s}{(1-\bar{v}\rho_0)}\right\}(6\,\pi\eta)\left(\frac{3\ Mv}{4\ \pi N}\right)^{1/3} \text{ or } M^{2/3} = \left\{\frac{s\eta}{(1-\bar{v}\rho_0)}\right\}v^{1/3}\ (N\pi)^{2/3}\ \{6(3/4)\}^{1/3}$$

To the extent that v and $\bar{v}$ are independent of the molecular size of the various polysaccharides, we have $M = Ks^{3/2}$, where K is a collection of the constants and experimentally invariant terms To evaluate K we have measured $\bar{v}$ (0 62–0 63) and have taken the sediment volume of glycogen [total volume occupied by one gram of glycogen fully hydrated and centrifugally packed, at speeds in the range 30,000–60,000 rpm (60,000–260,000 $\times g$), av 2 44 with a range of 2 2–3 6 cc/g] to be roughly equivalent to the specific volume v The molecular weight is proportional to the square root of the specific volume, therefore even an approximate value of the specific volume gives a reasonably accurate value for the molecular weight Generally, the increase in f due to asymmetry is relatively small in comparison with hydration effects, except for highly asymmetric molecules Electron micrographs[10 15] indicate that native glycogen is not highly asymmetric, although the "surfaces" of the particles usually show a rough texture which would increase the frictional coefficient We have arbitrarily included "roughness" in the hydration effect, and for the purpose of calculation we have ignored asymmetry Evaluating all factors, we obtain $M = 5\ 09 \times 10^3\ s^{3/2}$, where s is given in Svedberg units

This approach was checked empirically by calculating K from literature values of M and s for several glycogen and dextrin samples (Table II) The numerical value of K remains surprisingly constant over a range of molecular sizes including limit dextrins and very high-molecular-weight glycogens Thus, the relationship has both an empirical and a theoretical validity The average K value for D-glucose, oligosaccharides, and macrodextrins is about 3×10^3, indicating that these materials are much less hydrated and carry less trapped water than the high-molecular-weight glycogens and limit dextrins For an unhydrated sphere, $K = 2\ 57 \times 10^3$ A graph showing the relationship between s and M is given in Fig 2

The agreement between observed and calculated K values cannot be expected to be perfect Glycogen samples which have been examined are polydisperse Sedimentation and diffusion coefficients measured for such mixtures are different kinds of average values With very high-molecular-weight samples, it is especially difficult to obtain accurate values of the diffusion coefficients, which in some cases were time-

TABLE II

RELATIONSHIP BETWEEN SEDIMENTATION COEFFICIENT AND MOLECULAR WEIGHT FOR GLYCOGEN, DEXTRINS, AND LOW-MOLECULAR-WEIGHT SUGARS

Carbohydrate	s (*Svedbergs*)	M	$K = Ms^{-3/2}$	*Reference*
D-Glucose	0 13	180	3840	16
Sucrose	0 22	342	3314	
Raffinose	0 27	504	3592	
Cyclohexaamylose	0 48	972	2923	
Maltoheptaose	0 48	1152	3464	
Cyclooctaamylose	0 49	1296	3778	
Macrodextrin	1 55	5500[a]	2850	This work
Macrodextrin	1 55	6140[b]	3181	
Glycogen	54	$2\,6\times10^6$	6552	17
Glycogen fraction	44 3	$1\,9\times10^6$	6443	
Glycogen fraction	33 8	$1\,2\times10^6$	6107	
Glycogen fraction	25 2	$0\,8\times10^{6c}$	6324	
Glycogen fraction	18 4	$0\,5\times10^{6c}$	6335	
Glycogen	65	$4\,1\times10^6$	7824	18
Glycogen	61	$3\,9\times10^6$	8186	
Glycogen	100	$13\,9\times10^6$	13,900	
Glycogen	82	$5\,2\times10^6$	7003	
Glycogen	73	$4\,6\times10^6$	7375	
Liver glycogen	99	$4\,0\times10^6$	4061	19
Liver glycogen fraction 1	109	$5\,4\times10^6$	4745	
Liver glycogen fraction 2	70	$3\,0\times10^6$	5122	
Muscle glycogen fraction 1	76	$4\,6\times10^6$	6943	
Muscle glycogen fraction 2	61	$2\,7\times10^6$	5669	

[a]By reducing-value method [b]By *s* and *D* measurements [c]Obtained by assuming that these fractions have the same f/f_0 as the higher-molecular-weight preparations

dependent Moreover, different investigators have used values of $\bar{v}$ ranging from 0 60 to 0 65, with 0 65 being used most frequently[18,20] Although this introduces only a minor error, it contributes to the overall uncertainty in the molecular weight, and hence in the K value

The authors believe that changes in the sedimentation coefficient alone accurately reflect changes in the relative molecular weights of the various glycogen and dextrin samples, and that, at least for comparative purposes, it is unnecessary to obtain the less meaningful and unresolved diffusion coefficients

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