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SPECIFIC ADSORPTION OF STARCH OLIGOSACCHARIDES IN THE GEL PHASE OF STARCH GRANULES*

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ABSTRACT

The gel phase of native starch-granules is penetrable by such low-molecular-weight solutes as oligosaccharides, amino acids, and salts [Lathe and Ruthven, *Biochem.* J., 62 (1956) 665]. Molecules larger than about 1000 daltons are effectively excluded. Starch oligosaccharides (maltotriose through maltoheptaose and perhaps higher) exhibit anomalous behavior in that they are taken up by the gel phase far in excess of the amount expected on the basis of their molecular size. Adsorption was measured by using radioactive starch oligosaccharides and counting weighed amounts of solution before and after equilibration with starch granules. The measurements were corrected for water sorption by the starch granules and for exclusion effects as ascertained by controls with nonstarch types of oligosaccharides.Maximum adsorption was observed with maltotetraose. The results indicate a specific binding between the starch oligosaccharides and molecular chains in the starch, presumably those chains in the gel phase. We suggest that these chains constitute interbranch regions of branched molecules, or segments of linear molecules in the gel or amorphous phase, the segments being of sufficient length to form a double helix or other association with the linear oligosaccharides.

INTRODUCTION

In 1956, Lathe and Ruthven[1] showed that native starch granules can act as a molecular sieve for uncharged molecules in the size range up to 1000 daltons. Although the performance of starch-granule columns was poor by today's standards, such columns could be used for analysis or separation of molecules in this size range. Lathe and Ruthven examined the behavior of several mono- and oligosaccharides on

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potato-starch columns. They noted that most sugars behaved "normally"; that is, they gave reasonably sharp elution diagrams and were retarded on the column by an amount that decreased with incresing molecular size. However, the starch oligosaccharides, maltotriose and maltotetraose, were reported to behave anomalously in that they showed extensive peak broadening or tailing, and they were retarded on the column more than would be expected on the basis of their molecular weights. If one views the Lathe and Ruthven results from the standpoint of gel-permeation chromatography[2], one would conclude that the starch granule (or a portion of it) is behaving as a permeable gel and that the starch oligosaccharides react physically or chemically with starch. The present paper is a report on our attempts to study these phenomena more closely, with the object of learning more about the nature of the gel phase of starch granules and the association of starch with starch oligosaccharides. Initial experiments with starch columns gave poor results with starch oligosaccharides, owing probably to the slow equilibration between the solute in the mobile phase and that adsorbed in the interior of the starch granule. Therefore, we resorted to a batch method. Also, it was necessary to determine carefully the water uptake and the accessible volume of water within the starch granule for solutes of various molecular size under the conditions used for the starch oligosaccharide investigations.

EXPERIMENTAL AND RESULTS

Potato starch was a commercial Idaho potato starch, Hallmark Powdered Potato Starch, kindly donated by Stein–Hall and Company. In one batch, the smaller granules were removed by repeatedly suspending the starch in a dishpan of distilled water, allowing the larger granules to settle for 20–25 min and then discarding the supernatant suspension of mainly small granules. The remaining starch was filtered off, air-dried for several days, sieved (60 mesh), and stored in airtight containers. To ascertain possible effects of nonstarch components, one batch of potato starch was extracted with 85% methanol ("defatted" potato starch)[3], washed with water, and air dried. The moisture content of each batch was determined by drying overnight in a vacuum oven at 105°.

Unlabeled sugars and cycloamyloses (Schardinger dextrins) were pure commercial or laboratory preparations. Labeled starch oligosaccharides were prepared by the *Bacillus macerans* transglycosylase coupling-reaction[4] followed by paper chromatography, radioautography, excision of the individual radioactive zones, rechromatography and elution. Scintillation chemicals were obtained from Packard. Blue dextran was from Sigma. A solution having optical density ($o.d._{620}$) of about 1 was centrifuged for 15 min at 27000 *g* to remove aggregated or particulate matter that might separate on subsequent centrifugation.

For the starch-granule columns, a weighed amount of starch was stirred overnight with 0.002% sodium azide solution. The columns were prepared essentially by the method of Lathe and Ruthven[1], by using a Pharmacia K 16/70 column with A 16 flow adapters. A Buchler polystaltic pump was used to pump the solvent (0.002%

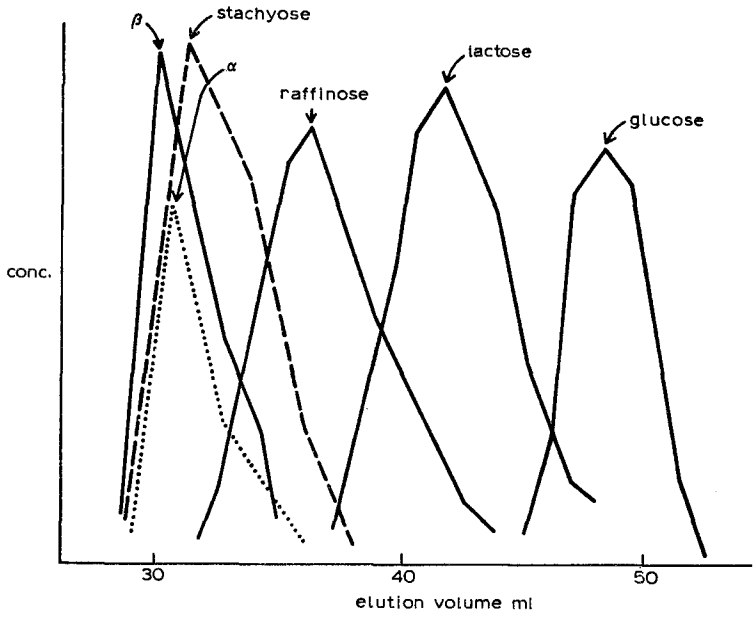

Fig. 1. Typical elution diagram of D-glucose and oligosaccharides on a potato-starch (fines removed), upward-flowing column; α: cyclohexaamylose; β: cycloheptaamylose. The void volume for this column, as determined by blue dextran, was 30 ml.

sodium azide) upward through the column. Between the pump and the column were located a Pharmacia LV-4 four-way valve, containing a sample loop, and an LV-3 three-way valve, to flush the connecting tubing after filling the sample loop. The void volume was determined by using blue dextran. Fractions (1 ml) were collected and assayed for total carbohydrate by the automated phenol–sulfuric acid method[5] or the orcinol–sulfuric acid method[6], or for blue dextran by absorbance at 620 nm. Distribution coefficients were calculated by the method of Lathe and Ruthven[1] and are presented in Table I and Fig. 2.

TABLE I

K_d VALUES FROM COLUMN DATA

Sugar	*Potato starch*	*Defatted potato starch*	*Large-granule potato starch*
Monosaccharide	0.73 (D-Mannose)	0.68 (D-Mannose)	0.56 (D-Glucose)
Lactose	0.40	0.39	0.35
Raffinose	0.18	0.24	0.17
Stachyose	0.08	0.09	0.05
Cyclohexaamylose	0.05	0.06	0.01
Cycloheptaamylose	0.02	0.02	0.00

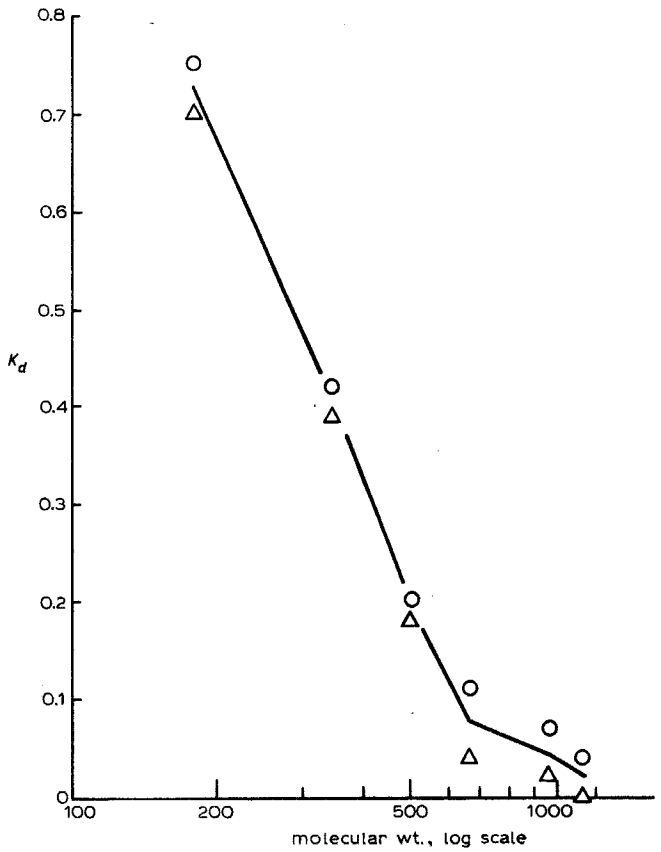

Fig. 2. Distribution coefficients (K_d) for D-glucose and oligosaccharides calculated from potato-starch, upward-flowing column. The solid curve is the average of two runs.

Water absorption of starch granules was determined by weighing 10 g of air-dry starch and 25 g of blue dextran solution into a flask or beaker. All weighings were made on an analytical balance. After 30 or 60 min of gentle stirring, the suspension was allowed to settle, and the supernatant was centrifuged for 15 min at 1085 *g*. The absorbance of this supernatant and the original blue dextran were measured at 620 nm on a Beckman double-beam spectrophotometer equipped with a Gilson digital readout. Water absorption was calculated by using the formula:

Total absorbed H_2O per g of dry starch = [(g H_2O absorbed) + (g initial moisture in starch)]/(g dry starch),

where g H_2O absorbed = g blue dextran solution × [1 − o.d. initial/o.d. final]

The results were: potato starch, 0.483 ±0.010 g water/g dry starch; defatted potato, 0.533 ±0.020; potato starch (fines removed), 0.512 ±0.033.

Adsorption of starch oligosaccharides could not be determined by the column method owing to broadening and skewing of the elution peaks. Therefore, the batch

method was used as described in the preceding paragraph. Individual, radioactive, starch oligosaccharides were diluted to give about 1000 c.p.m. per ml, and then 10 g of starch and 25 g of oligosaccharide solution were weighed out and stirred. After settling and centrifuging the suspension, portions of the supernatant and original oligosaccharide solution were weighed into scintillation vials and counted (Packard Tri Carb 3003).

The adsorption experiments were executed three times, Experiment I using large-granule potato starch with 30 min of stirring at room temperature, Experiment II using potato starch with 60 min of stirring at 30°, and Experiment III using defatted potato starch with 60 min of stirring at 30°. Owing to the necessity of determining the amount of material adsorbed from the differences between the initial and final concentrations, each experiment was repeated two or three times, the countings were carried out to an error of 1% or less, and the data were handled by standard statistical methods.

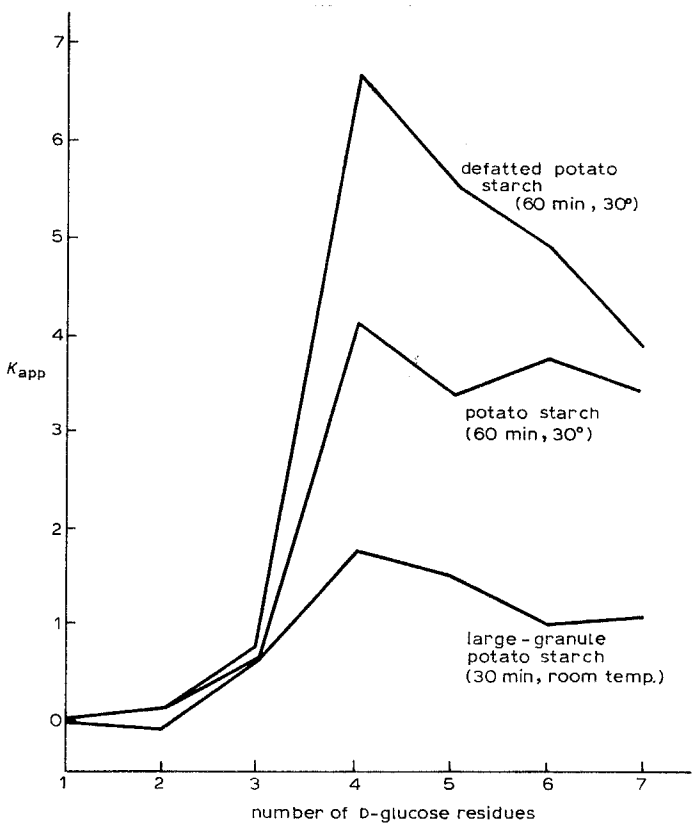

Fig. 3. Apparent adsorption coefficients (K_{app}), batch method, for radioactive D-glucose and starch oligosaccharides, corrected for nonspecific, gel-permeation inclusion.

The adsorption of radioactive starch oligosaccharides* (G_n) by starch was calculated by the formula:

$$K_{eq} = [\text{starch-}G_n \text{ complex}]/[\text{uncomplexed starch}]\,[G_n]$$

$$= [\text{c.p.m. adsorbed}]/[\text{g starch}]\,[\text{c.p.m. not adsorbed/ml effective volume}].$$

The effective volume for a given oligosaccharide is the free volume plus the restricted volume within the starch granule that is accessible to an unadsorbed oligosaccharide of the same molecular size. The free volume is the total amount of solution added, less the amount of water taken up by the air-dry starch, as obtained from the blue dextran, moisture-uptake measurement. The restricted volume per gram of starch was obtained from the smooth curve in Fig. 2 and represents a small increase in the total volume accessible to a given G_n. It was assumed that the concentration of uncomplexed G_n within the restricted volume was the same as that in the free volume. The unadsorbed G_n is the c.p.m./ml (really c.p.m./g) of supernatant times the total effective volume. The c.p.m. adsorbed is then the difference between c.p.m. originally added and the c.p.m. in the effective volume.

The results from the three sets of experiments are presented in Fig. 3.

DISCUSSION

Water absorption. — Our values for water uptake varied with the different starch batches, but are in the range of those obtained by other workers[7–11]. Our variations may have resulted from differences in time or temperature of equilibration, or from differences in granule-size distribution. With the defatted potato starch, higher water uptake may have arisen from granule damage resulting from the defatting procedure. Optical microscopy revealed many cracks in the defatted starch-granule, possibly as a consequence of water removed from the granule interior with concomitant shrinking and stress within the gel phase. Similar cracking can be induced by drying native starch-granules. Alternatively, although potato starch is generally regarded as being essentially lipid-free, removal of a small amount of lipid might uncover a few water-binding sites.

Access of small solutes to the bound water. — Our column results (Figs. 1 and 2) are in agreement with those of Lathe and Ruthven[1] and indicate that saccharides up to about 1000 daltons can penetrate starch granules. However, even with D-glucose or D-mannose, a significant fraction of the bound water is inaccessible as a solvent. We are reluctant to interpret this bound water as "water of crystallization", inasmuch as other studies have cast substantial doubt on the necessity of water in the crystalline part of potato-starch granules[12]. We are more inclined to think that the inaccessible, bound water is a type of "surface water" bound to the external surfaces of starch chains, which is partly inaccessible as a solvent simply because it cannot surround a relatively large, solute molecule, even such a small molecule as a monosaccharide.

*The abbreviation G_n is used in this paper to refer to a starch-derived oligosaccharide.

Specific binding of starch oligosaccharides. — The results presented in Fig. 3 indicate clearly that starch oligosaccharides G_3—G_7 are bound to starch, rather than being merely dissolved in the restricted volume. The exterior surface of a starch granule is totally inadequate to account for this binding as a surface phenomenon[8]. Instead, we interpret the specific adsorption as an interaction with the free, molecular starch-chains within the amorphous or gel phase of the starch granule. Although the results themselves give little clue as regards the nature of the interaction, we are inclined to evoke the double-helix hypothesis[12]. Conformational analysis of starch chains has uniformly indicated that such chains are helices[13], and examination of models indicates that double helices are readily formed. Although with G_3 or G_4, it is clearly impossible to make a complete turn of a helix, there can be enough contact between such oligosaccharide chains and longer starch-chains that a stable interaction may occur. This particularly true if the chains are wound about each other, giving a type of topological interlocking that is more stable than a simple side-by-side association of essentially linear chains (Fig. 4).

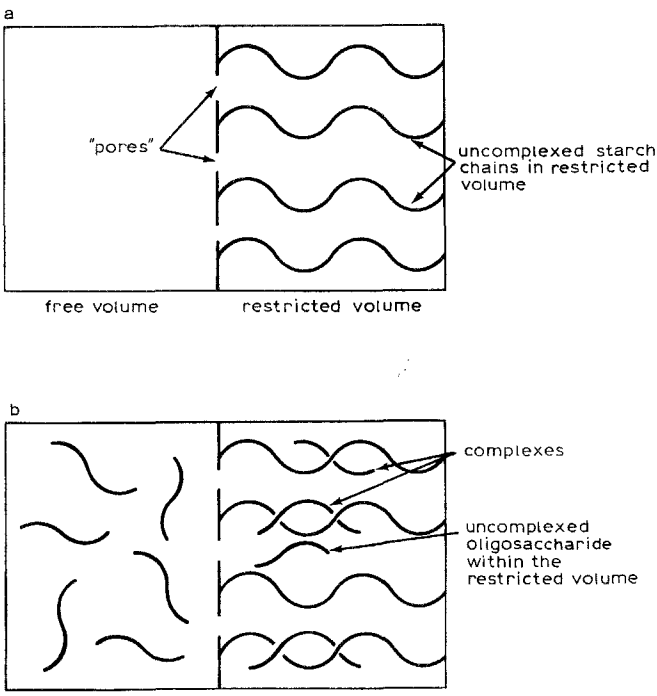

Fig. 4. Scheme (*a*) showing "pores", free volume, restricted volume, and free (uncomplexed) starch chains in a starch granule; (*b*) oligosaccharides (~) are able to penetrate pores into restricted volume and may form "complexes" with starch chains.

It is tempting to relate the decrease in binding of chains larger than G_4 to the drop-off in length of interbranch segments in amylopectin. However, it is also possible

that the longer, interbranch starch-chains are themselves twisted together and thus inaccessible for complex formation with oligosaccharides.

Also, from Fig. 2, it is very clear that sugars larger than stachyose (a tetrasaccharide) are mainly excluded from the starch granule. Indeed, a linear extrapolation of the data of Fig. 2 for D-glucose through stachyose would give an exclusion limit of about 800 daltons (about a pentasaccharide). That the cycloamyloses (Schardinger dextrins) are admitted to a small extent into starch granules may reflect their cyclic and compact structures, having relatively small Stokes radii[14].

Within the limits of our experiments, it is clear that true binding-equilibrium may not have been attained, so that it is impossible to draw valid, quantitative, thermodynamic conclusions. Nevertheless, it seems very likely that the binding *trend* of oligosaccharides for uncomplexed starch-chains increases with oligosaccharide chain-length. The change in binding energy calculated from the 60-min data of Fig. 3, in passing from G_3 to G_4, represents an increment in $-\Delta G^\circ$ of about 1160 cal per additional D-glucose residue. This is probably a minimum value, inasmuch as one might reasonably expect that equilibration is more rapid for G_3 than for G_4 and higher oligosaccharides. Moreover, binding sites accessible to G_4 are certainly accessible to G_3, whereas the reverse is not necessarily true.

Validity of the equilibrium-constant concept for a heterogeneous system. — Suppose that the gel contains a distribution of i classes of binding sites (S_i) such that, per gram (dry weight) of gel, there are a moles of type S_a, b moles of type S_b, c moles of type S_c, and so on. Each site is characterized by an interaction with each G_n; for example

$$S_a + G_n \rightleftharpoons \underset{\text{(complex)}}{G_n \cdot S_a},$$

for which it is possible to write an equilibrium constant:

$$K_{a,n} = [\text{moles of complex of type } a,n \text{ per gram of gel}]/[\text{moles of uncomplexed site of type } a \text{ per gram of gel}] \cdot [\text{concentration of } G_n \text{ in moles/l}]. \quad (1)$$

The numerical value of $K_{a,n}$ is the reciprocal of that concentration of G_n that gives half saturation of the a-type sites. For starch granules, one might reasonably expect a large number of small sites and a decrease in the number of sites as they get larger and larger. Moreover, presumably the smaller oligosaccharides could complex with sites of any size above a critical minimum. Binding of two or more oligosaccharides to a single, large site would not be excluded, but the likelihood of such multiple binding would be very small for low concentrations of oligosaccharide.

The total observed binding at a given G_n concentration would be the sum of binding at all types of sites. Thus, the total binding would be:

$$\sum_i [\text{moles of complex}]_{i,n} = [G_n] \sum_i K_{i,n} \times [\text{moles of uncomplexed sites of type } i \text{ per gram of starch}].$$

In the absence of any knowledge about the numerical value of the quantity in the right-hand brackets, it does not seem possible to evaluate the right-hand sum to convert the equation into the usual form of an equilibrium constant.

However, if the number of uncomplexed sites is large in comparison with the number of complexed sites, so that in effect it remains constant, Eq. *1* may be written:

$$K'_{a,n} = [\text{moles of complex of type } a,n \text{ per gram of gel}]/[\text{concentration of } G_n \text{ in moles/l}], \quad (2)$$

where the units of $K'_{a,n}$ are $l.g^{-1}$.

For example, if the adsorption experiments are carried out by using tracer amounts of radioactive oligosaccharides, presumably only a trivial proportion of the binding sites will be occupied, so that the term in Eq. *1* [moles of uncomplexed site of type *a* per gram of gel], although unknown, remains essentially constant and can be incorporated into an apparent equilibrium constant $K'_{a,n}$.

As before, the total observed binding at a given G_n concentration would be:

$$\sum_i [\text{moles of complex}]_{i,n} = [G_n] \times \sum_i K'_{i,n}$$

$$\text{If } K''_n = \sum_i K'_{i,n},$$

$$\text{and } [\text{total moles of complex}]_n = \sum_i [\text{moles of complex}]_{i,n},$$

one may write an apparent equilibrium constant:

$$K''_n = \frac{[\text{total moles of complex}]_n}{[G_n]}.$$

This equation is identical in form to Eq. *2*, and is equally as valid because it incorporates no additional assumptions. Therefore, it is evident that, by using radioactive-tracer methods, one may obtain an experimental value for K''_n representing the weighted total K'_n value ($K'_{a,n}$ contains a weighting factor for the number of sites of type S_a) for all types of binding sites.

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