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# PARTITIONING OF OLIGOGLUCANS IN SEPHADEX G-15 IN RELATION TO THEIR CONFORMATIONAL STRUCTURE

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## SUMMARY

There is good evidence that, in Sephadex gels, oligosaccharide series show (positive) linear free energy relations with respect to the degree of polymerisation. The distribution coefficients of disaccharides are thus a measure of the differences between the polyglucan series to which they belong. The partitioning in Sephadex G-15 of eight diglucosides was studied. Their distribution coefficients could be arranged in four pairs. The eight corresponding polyglucan series can also be classified into four pairs, both members of each pair having a similar conformational structure. Further, the order of the pairs of distribution coefficients seemed to be well matched to the conformational pairings. This partitioning pattern is probably not confined to the homogeneous disaccharides, because differences between the diglucosides were similar in two cases to differences between corresponding heterogeneous disaccharides. However, it is concluded that it would be incautious to attribute the order of the disaccharide distribution coefficients purely to different degrees of steric exclusion.

## INTRODUCTION

In both Sephadex[1,2] and polyacrylamide[3–5] gels, polar homologous series such as the oligosaccharides exhibit a positive linear free energy relationship (LFER) with respect to the degree of polymerisation (DP). The fact that the logarithm of the distribution coefficient ($K_d$) is related linearly to DP suggests that a steric factor may be the major determinant of the differences between the $K_d$ values of different members of a series; it also suggests that any gel–solute interactions that occur are also additive and, at least approximately, the same for each monomeric residue in the oligomer.

The fact that although different oligoglucans exhibit LFER, they do not necessarily have the same slopes, is not unexpected because there are also marked conformational variations[6] associated with the different glycosidic linkages. In this

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paper, data on the $K_d$ values of disaccharides and the higher members of some series are presented and discussed in terms of the conformational structure.

## EXPERIMENTAL

### *Materials*

Sephadex G-15 (Batch No. 2014) was kindly donated by the manufacturer, Pharmacia, Uppsala, Sweden, as also was dextran 500. Deuterated water ($^2H_2O$) was from Norsk Hydro, Norway.

Glucose, maltose and cellobiose were from BDH, Poole, U.K. Isomaltose, malto- and isomaltotriose, nigerose and laminaribiose were from Sigma, St. Louis, MO, U.S.A., and turanose, lactose and melibiose were from Pfanstiehl, Waukegan, IL, U.S.A. Cellotriose was generously given by Dr. D. A. I. Goring, and isomaltotetraose and -pentaose were generously given by Dr. A. de Belder. All the other sugars were from Koch-Light, Colnbrook, Bucks, U.K.

### *Column chromatography*

Distribution coefficients were determined by elution through a gel column, 35 × 1 cm I.D. The loading volume was 0.5 ml and the linear flow-rate never exceeded 2.5 cm $h^{-1}$. The column was prewashed and eluted with water purified in a Milli-Q 4 H system (Millipore, Bedford, MA, U.S.A). The effluent concentrations were measured with a differential refractometer (Optilab 901, B. Philip, Stockholm, Sweden).

### *Calculations*

The dynamic distribution coefficient $K_d$ is defined[7] as:

$$K_d = (V_e - V_0)/(V_w - V_0) \quad (1)$$

where $V_e$, $V_0$ and $V_w$ are the peak elution volumes of a test solute, a void volume indicator (dextran 500) and water measured as $H_2{}^{18}O$ (ref. 8), respectively. It is, however, more convenient to use deuterium ($^2H_2O$) as the internal water volume reference, but owing to hydrogen isotope exchange between the gel hydroxy groups and the eluted hydrogen isotope, the distribution coeffient of deuterium ($K_d^D$) is a little higher than unity. Thus:

$$K_d = K_d^D (V_e - V_0)/(V_D - V_0) \quad (2)$$

where $V_D$ is the peak elution volume of deuterium, and $K_d^D$ in this gel is 1.075[9].

## RESULTS AND DISCUSSION

Fig. 1 gives data on some lower members of the isomalto (6-O-$\alpha$), malto (4-O-$\alpha$) and cello (4-O-$\beta$) dextrins. Although the range of DP values is much more limited than in other studies[3–5], they indicate that the $K_d$ value of a disaccharide (Table I) may be regarded as representative for the relative partitioning behaviour of that particular series.

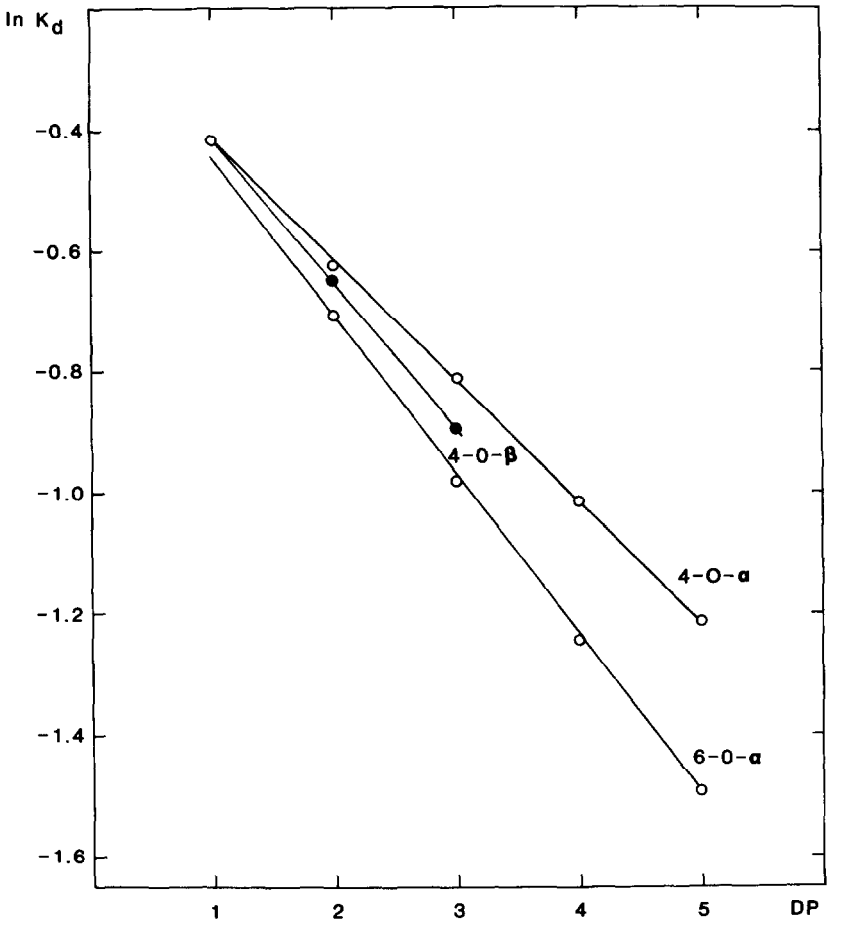


Fig. 1. The relation between ln $K_d$ and the degree of polymerisation for three oligosaccharide series. Temperature, 25°C.

The LFER were postulated by Martin[10], who assumed that the standard free energy of transfer of an *n*-mer into chromatographic support material would be the sum of the contributions from its constituent units. Thus, for a homogenous polymer such as a polyglucan[11]:

$$-\ln K_d = 1/RT\,(\Delta G_1^0 + \Delta G_n^0 + \sum_{2}^{n-2} \Delta G_i^0) \qquad (3)$$

where $\Delta G_1^0$ and $\Delta G_n^0$ refer to the two terminal residues, and $\Delta G_i^0$ to the intermediate ones. Thus, for the oligoglucans, the LFER will include the disaccharide and higher oligomers, and in some cases may even extend to include glucose.

If steric exclusion were the only factor determining the partitioning behaviour,

TABLE I

$K_d$ VALUES OF SOME DIGLUCOSIDES IN RELATION TO THE CONFORMATIONAL TYPE OF THE CORRESPONDING OLIGOGLUCAN CHAIN

| *Type* | *Link* | *Diglucoside* | $K_d$* | *S.E.M.* |
|---|---|---|---|---|
| Extended and ribbon-like | 3-O-α | Nigerose | 0.5190 (6) | 0.0010 |
| | 4-O-β | Cellobiose | 0.5234 (6) | 0.0015 |
| Flexible and helical | 3-O-β | Laminaribiose | 0.5354 (6) | 0.0005 |
| | 4-O-α | Maltose | 0.5362 (9) | 0.0021 |
| Rigid and crumpled | 2-O-α | Kojibiose | 0.5107 (7) | 0.0023 |
| | 2-O-β | Sophorose | 0.5056 (6) | 0.0020 |
| Flexible and rather extended | 6-O-α | Isomaltose | 0.4933 (6) | 0.0010 |
| | 6-O-β | Gentiobiose | 0.4664 (6) | 0.0015 |

* The mean $K_d$ value is followed by the number of measurements in parenthesis and then by the standard error of the mean (S.E.M.).

and if energetic interactions between the gel and anhydroglucose residues were absent, *i.e.* if the standard transfer enthalpy ($\Delta H^0$) were zero, then:

$$\Delta G^0 = -T\Delta S^0 \tag{4}$$

The entropy ($\Delta S^0$) can then be equated with the relative number of the conformations of the solute within the gel[12,13]; thus:

$$\Delta S^0 = R \ln \Omega/\Omega_0 \tag{5}$$

where $\Omega/\Omega_0$ is the ratio of the numbers of attainable conformations within the imbibed water and those in the bulk water outside the gel bead. There should thus be a simple relation between the conformational ratio and the distribution coefficient, *viz*:

$$K_d = \Omega/\Omega_0 \tag{6}$$

The poly (or oligo) glucans are very interesting compounds as regards partitioning in gels such as Sephadex or polyacrylamide; they are series built up of the same monomer, and the only primary structural difference is the position of the glycosidic link.

Conformationally the polyglucans can be arranged, depending on the glycosidic linkage, in four pairs[6] (Table I); further, as also shown in the Table, the $K_d$ values appear to fall also into four pairs.

Both the α and β forms of the 6-O-linked glucosides are specially flexible because, in contrast to the other linkages, rotation is possible at two angles instead of one[6]. This means that there is a much greater number of possible conformations and hence, unconstrained in free solution, a flexible but extended structure is most probable. A consequence of this is a relatively greater restriction on the number of conformations attainable in the confines of the interstitial spaces, and so these disaccharides would be expected to have the lowest $K_d$ values, as is in fact the case.

TABLE II

COMPARISON OF DIFFERENCES BETWEEN DIGLUCOSIDES AND BETWEEN HETEROGENOUS DISACCHARIDES

Galp = galactopyranose, glcp = glucopyranose, glc = glucose and fru = fructose.

| *Disaccharide* | | $K_d$ | *ln* $K_d$ | $\Delta \ln K_d$ |
|---|---|---|---|---|
| | (4-O-β)-(6-O-α) | | | |
| Cellobiose | 4-O-β-Glcp-glc | 0.5234 | −0.6474 | 0.0592 |
| Isomaltose | 6-O-α-Glcp-glc | 0.4933 | −0.7066 | |
| Lactose | 4-O-β-Galp-glc | 0.5088 | −0.6757 | 0.0614 |
| Melibiose | 6-O-α-Galp-glc | 0.4785 | −0.7371 | |
| | (3-O-α)-(6-O-α) | | | |
| Nigerose | 3-O-α-Glcp-glc | 0.5190 | −0.6559 | 0.0507 |
| Isomaltose | 6-O-α-Glcp-glc | 0.4933 | −0.7066 | |
| Turanose | 3-O-α-Glcp-fru | 0.5372 | −0.6214 | 0.0523 |
| Palatinose | 6-O-α-Glcp-fru | 0.5098 | −0.6737 | |

At the other end of the scale, those disaccharides that are flexible and compactly regular (helical), *i.e.* 3-O-β-laminaribiose (nomenclature as described in ref. 14) and 4-O-α-maltose[15], have the highest $K_d$ values, as would be expected. The less flexible pairs, 3-O-α-nigerose and 4-O-β-cellobiose and the α and β 2-O-members, kojibiose and sophorose, have intermediate values. A similar partitioning pattern may well apply to the heterogenous series. Thus, as shown in Table II, the difference between the 4-O-β (cellobiose) and 6-O-α (isomaltose) diglucosides was quantitatively similar to that between the corresponding galacto-glucosides, lactose (4-O-β-Galp-glc) and melibiose (6-O-α-Galp-glc). Similarly there was nearly the same difference between nigerose (3-O-α-Glcp-glc) and isomaltose as between the corresponding glucofructosides, turanose (3-O-α-Glcp-fru) and palatinose (6-O-α-Glcp-fru).

Although the correlation between the $K_d$ values and conformational type appears reasonable, it would be incautious to attribute this exclusively to steric exclusion. Thus the behaviour of the 4-O-α-maltodextrins is considerably changed if they are cyclic instead of linear. The small chemical change resulting from cyclisation can itself hardly explain the changed order, with the larger β-cycloheptadextrin eluting later, and the smaller α (hexa) member earlier, both being eluted after glucose[16]. It should be noted, however, that in contrast to the case with the non-cyclic forms, cyclic amyloses can present only one aspect of their ring surface to the gel. This apparent reversal does not affect all three cyclodextrins, however, because an order of ascending elution volumes γ, α, β has been reported[17], which is also the order of descending aqueous solubilities[18].

This phenomenon, where a more bulky solute has a higher distribution coefficient, suggests a sorptive (interactive) process[11]. It is also evident in a gel such as Sephadex G-15 that, even if steric exclusion is the dominant factor in determining the slope of a postive LFER, this may be modified by other (interactive) factors[1].

Interactions are probable in all the series discussed here because most, if not all, exhibit non-zero $\Delta H^0$ values[2,17,19]. Such non-zero $\Delta H^0$ values do not necessarily

mean that a gel–saccharide interaction occurs. The $\Delta H^0$ change may be due to a changed hydration of the solute within the interstices of the matrix.

There are considerable grounds for supposing that solute–water interactions play a not unimportant role in partitioning. For example, the isomaltodextrins are much more water-soluble than the cellodextrins[20], and the greater exclusion of the former might thus be consequence of their greater preference for water.

This solubility difference indicates that, in addition to the effect on the conformation, there may be other consequences of the glycosidic linkage. Thus intramolecular (inter-residue) effects, such as hydrogen bonding in cellulose[21] or hydrophobic interactions in maltose[22–25], may occur and may, in turn, be expected to modify the surface of the oligosaccharide and hence its interactions with the gel–water system.

It may thus be relevant that Miyajima *et al.*[26] concluded, from a study of the viscosity $B$ coefficients, that the 4-O-α-glycosidic linkage reduced the water-structure-promoting effect of this saccharide series.

The hydration of saccharides composed of D-glucose residues may be expected to be complex, because in the preferred $^4C_1$ conformation, the pyranose residue has both polar and non-polar domains[22]. In fact, glucose and disaccharides have been classified both as water-structure makers[27–29] and as structure breakers[30,31].

It must also be borne in mind that, owing to its nearness to the matrix, the water inside the gel may be ordered differently from that in the bulk state outside[32], and that the effect of this on saccharide hydration energies may profoundly influence partitioning. It should also be noted that, although the Sephadex gel G-15 is a cross-linked dextran (mainly 6-O-α-linked), its surface is by no means purely saccharide-like, but contains an appreciable fraction of 1,4-dioxane rings owing to intramolecular cross-linking[33].

The potentially important role of water is underlined by the close correlation between the partitioning of oligosaccharides in Sephadex G-15 and in paper[34] and thin-layer chromatography on Kieselguhr[35]. Partitioning in paper chromatography has also been reported to be correlated with the mole fraction of water in the solvent mixture[36,37]. Thus a plot of xylo- and cellodextrin log $K_d$ values on Sephadex G-15 against $R_M$ values on Kieselguhr fitted a single straight line[35].

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