Biochimica et Biophysica Acta, 1065 (1991) 35–41

ADONIS 000527369100218T

BBAMEM 75199

Hydrophobic surfaces in oligosaccharides: linear dextrins are amphiphilic chains

C. Sivakama Sundari, B. Raman and D. Balasubramanian

Centre for Cellular and Molecular Biology, Hyderabad (India)

(Received 24 July 1990)
(Revised manuscript received 3 January 1991)

Key words: Oligosaccharide; Chain conformation; Amphiphilic surface; Hydrophobic bonding; Lectin-sugar binding; Antigen–antibody interaction; Cell surface oligosaccharide

Polysaccharide chains are usually considered to be highly hydrophilic, since they have no obvious nonpolar moieties in them. Yet, it is possible to realise conformations in these chains wherein all the hydroxy groups are disposed in one side or face of the chain and the hydrogens disposed in the other. We experimentally demonstrate that such an amphiphilic surface is present in linear oligomeric dextrins, i.e., α-1,4-linked D-glucosides, but not in α-1,6-D-glucosides (dextrans) or in β-1,4-D-glucosides (cellulose). This amphiphilicity is generated as a consequence of the stereochemical constraints, which vary with the structure of the sugar and with the type of linkage. Oligosaccharide chains that can adopt incipient helical structures might display amphiphilicity. This property might be relevant to intermolecular recognition on cell surfaces, lectin-sugar binding, antigen–antibody interactions and the like, and might be manifested more in hetero-molecular recognition processes than as homomolecular self-aggregation.

1. Introduction

At first glance a sugar molecule would be thought to be only hydrophilic in nature, because of the presence of several hydroxyl groups and no obviously apolar groups in it. Yet, amphiphilicity can be generated in sugar chains based on the conformation of the monomeric sugars, the epimeric structure, the stereochemistry of the inter-sugar glycosidic bond and the chain conformation. An excellent example are the cyclodextrins, namely cyclo(oligoamyloses), which are bracelet-like molecules that possess a hydrophobic interior and a polar outer surface. This amphiphilic feature of cyclodextrin molecules arises from the $^4C_1$ conformation of the monomeric α-D-glucose units and the 1a,4e-glycosidic linkage, which disposes all the hydroxyl groups on the outer rim of the bracelet, leaving the inner cavity less polar. Consequently these molecules are able to solubilize or include one or more molecules of nonpolar substances such as aromatic hydrocarbons within this inner cavity [1,2].

Correspondence: D. Balasubramanian, Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India.

We now ask the question whether linear oligomeric saccharides could also display amphiphilicity. Would one side or face of the molecule be hydrophobic and the other surface of the ribbon hydrophilic? We have attempted to answer these questions by studying aqueous solutions of glucose, sucrose, maltose, and of linear dextrin, i.e., 1a,4e-linked oligomers of α-D-glucopyranoside, and comparing them with those of dextran (1,6-linked α-D-glucose units) and of cellulose (β-1e,4e-linked D-glucose units), as well as of xylan (β-1,4-linked D-xylopyranose). Our results suggest that dextrin chains are amphiphilic ribbons whose hydrophobic surfaces are recognized by other hydrophobic or amphiphilic molecules under appropriate conditions. It also appears that, in general, oligosaccharide chains which can adopt incipient helical type curved surfaces might be amphiphilic.

Neal and Goring [3] had suggested earlier that the smallest oligomeric dextrin, maltose, can adopt a conformation in which one face of the molecule is hydrophilic due to the disposition of the hydroxyl groups therein, while the other face is relatively apolar. In contrast, the β-1e,4e-linked dimer of glucose, namely cellobiose, should be less capable of such intramolecular hydrophobic folding since the β-1e,4e-glycosidic link is less flexible than the α-1a,4e-link [3]. One may expect

such segregation of the polar and apolar faces to be accentuated with higher oligomers of maltose. Some support for this possibility arises from the observations of Janado and Yano [4] that the solubility of naphthalene in water is progressively enhanced in the presence of dextrins of increasing chain length. These authors have suggested that these dextrins form curved nonpolar surfaces similar to the internal surface of the cyclodextrins which interacts favorably with apolar compounds. It is well known that α-amylose, which is the high molecular weight polymer of these oligodextrins, folds into several helical forms [5–8], whose inner cavity is relatively apolar; this enables amylose to display hydrophobic binding towards stilbene-substituted fatty acids as inclusion complexes [9,10]. The inclusion complex of molecular I_2 with α-amylose is a classical example much studied in the past.

Earlier, Nakatani et al. [11] have reported that fluorescence probes experience a partial hydrophobic environment when they interact with oligoamyloses beyond a chain length of six or so. We explore these points further in this paper and present evidence which suggests that even short-chain dextrins are amphiphilic ribbons that enter into hydrophobic interaction with appropriate partners. This finding is of relevance to cell surface polysaccharides, glycoproteins and lectin–sugar interactions, since it suggests a possible nonpolar or hydrophobic component in such interactions.

2. Materials and Methods

Linear Dextrin-20, which is hexameric α-1,4-linked D-glucose and Dextrin-10, the corresponding decamer, as well as Dextran 4, which is α-1,6-D-glucopyranoside of molecular weight 4000–6000, were obtained from Serva Chemical Co. All the other sugars and chemicals were obtained from Sigma Chemical Co. Estimation of the changes in the environmental polarity, brought about by the sugars, was done using the fluorescent probes 8-anilino-1-naphthalenesulfonate (ANSA), pyrene, and 5-(dimethylamino)-1-naphthalenesulfonamide (dansamide) which was synthesized in our laboratory. The relative intensity ratio of the first and the third vibronic bands (Ham effect) of the fluorescence of pyrene is sensitive to the medium polarity [12], while with ANSA and dansamide, it is the band position and the quantum yield that are polarity sensitive [14]. The critical micelle concentrations (cmc) of cetyltrimethylammonium bromide (CTAB) and Triton X-100 were determined in water and in the presence of the additives using surface tensiometry or the pyrene fluorescence probe method [13]. The solubilities of L-tyrosine in pH 7.1 Tris-HCl buffer, in the absence and presence of the various sugars were determined at 298 K using the molar absorption coefficient value of 1370 at 275 nm for the amino acid, and the procedure adopted was the same as

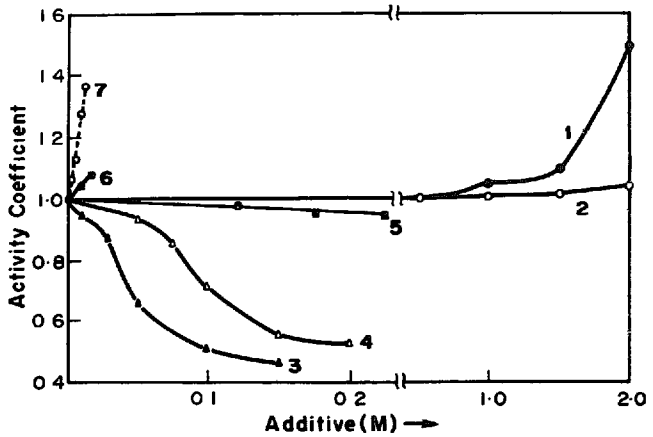

Fig. 1. Solubilization of L-tyrosine by aqueous sugar solutions at 298 K. Curve (1) is for sucrose, (2) for glucose, (3) for Dextrin 10, (4) for Dextrin 20, (5) for cellobiose, (6) for Dextran 4, and (7) for xylan (however, here the data points are for xylan concentrations of 0.05%, 0.025% and 0.0125% w/v, rather than in molarities, since the molecular weight of the xylan was not specified). Activity coefficients were calculated as the ratio of the solubility in water to that in the additive test solution.

that of Lakshmi and Nandi [15]. 11-Bromoundecanoyltryptophan (BUT), an amphiphile that is known to self-coil in water, was synthesized in our laboratory and its fluorescence intensities were measured in water and in the presence of various sugars. The Hitachi Model F4000 spectrofluorimeter and Model 330 spectrophotometer were used, and all measurements were done at ambient temperature, unless otherwise specified.

3. Results and Discussion

Sugars are conventionally thought to reduce the solubility of lipophilic compounds in water; the term 'sugaring out' has been used in this connection [15], akin to the salting out phenomenon. However, should dextrins display amphiphilic tendency, one might expect them to solubilize or enhance the solubility of lipophilic substances in water. Janado and Yano [4] have shown earlier that oligomeric maltoses solubilize neutral arenes better than glucose or water. Fig. 1 shows such 'sugaring in' effects of Dextrin 10 and Dextrin 20 towards L-tyrosine in aqueous solutions. The activity coefficient f ($= c_0/c$, where c_0 is the solubility in water and c is that in the presence of the additive) of tyrosine is decreased significantly by the dextrins, since they enhance its solubility in water. While monomeric glucose does not significantly affect the activity coefficient, oligomeric glucoses are able to do so, but as Fig. 1 shows, this is true only with α-1,4-linked glucose; the limited data that we could get at the accessible solution concentrations of the α-1,6-linked dextran chain suggest that it has little or no solubilizing effect. We found that neither does cellobiose, which is the β-1,4-linked dimer

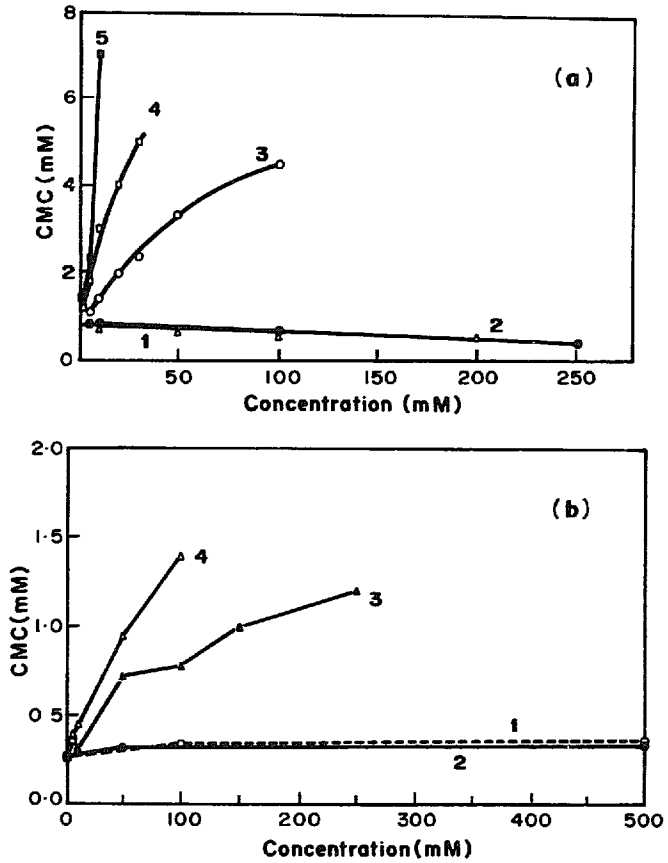

Fig. 2. (Panel a) The effect of various saccharides on the cmc of CTAB. Curve (1) is for glucose, (2) for trehalose, (3) for Dextrin 20, (4) for Dextrin 10, and (5) for α-cyclodextrin. (Panel b) The effect of various saccharides on the cmc of Triton X-100 in water. Curve (1) is for glucose, (2) for maltose, (3) for Dextrin 20, and (4) for Dextrin 10. The limited data points that we collected with solutions of cellobiose, dextran and xylan all fell right on curves (1) and (2) of both panels. The cmc values were measured using the pyrene fluorescence probe method [13].

of D-glucose. The free energies of transfer of tyrosine from water to 200 mM dextrin solutions, calculated from the activity coefficient values and at 298 K, were estimated to be −380 cal/mol for Dextrin 20 and −460 cal/mol for 150 mM Dextrin 10; in contrast, these values are negligible for cellobiose, and slightly positive in the case of 2 M glucose (+25 cal/mol), 2 M sucrose (+240 cal/mol) and 20 mM Dextran-4 (+70 cal/mol). The behaviour of maltose was similar to glucose. The other polymer tried, namely xylan (0.05%) where the value was +160 cal/mol, is also a poor solubilizer of tyrosine, which it 'sugars out' of water.

Another way of monitoring the hydrophobic or amphiphilic character of the sugars is to study their effects on the critical micellar concentration (cmc) values of conventional surfactants in solution. Fig. 2a reveals that while glucose and trehalose (α-1,1-linked dimer of D-glucose) marginally advance the cmc of cetyltrimethylammonium bromide (CTAB) in water (from 0.9 mM to a limiting value of about 0.6 mM), oligomeric dextrins increase the cmc substantially. An additive that increases the cmc of a surfactant is thought to do so by weakening the hydrophobic self-association (micellization) of the surfactant. In the case of the dextrins, this could arise through direct interaction between the dextrin molecules and the surfactant molecules, which would tilt the micellization equilibrium in the direction of free monomers, and thus postpone the cmc. This interaction between dextrins and CTAB would necessarily have to be significantly hydrophobic in character, since glucose itself does not seem to interact with CTAB. In support of this point we note that α-cyclodextrin is very effective in delaying the micellization; 10 mM α-cyclodextrin postpones the cmc of CTAB by almost a factor of eight, namely from 0.9 mM to 7 mM. The cyclodextrin molecule is known to interact with fatty acids and related amphiphiles and include them within its apolar cavity [2].

This effect of dextrins on the cmc of surfactants appears to be general and not confined to CTAB. Fig. 2b shows that glucose and maltose have negligible effect on the cmc of the nonionic detergent Triton X-100 (*t*-octylphenyl decaethyleneglycol), while Dextrin-20 and Dextrin-10 postpone the cmc value substantially. Gratzer and Bevan [16] have shown earlier that sucrose advances the cmc of Triton X-100 slightly; this effect has been considered to be due to increased hydrophobic interaction between nonpolar moieties in the presence of sucrose, an additive that is thought to enhance water structure. The effect of the linear dextrins could therefore be thought of as the opposite, namely a weakening of this hydrophobic self-association.

In this connection, the standard free energy of the transfer of the detergent from water to aqueous solutions of the sugars can be estimated in much the same way as was done for tyrosine above, excepting that the cmc values are used in place of the concentrations here [16], namely using the equation $\Delta G^0_{tr} = -RT \ln[\text{cmc}]_s/[\text{cmc}]_w$, where the subscripts s and w refer to the aqueous sugar solution and water, respectively. Table I lists the values of the transfer free energies of CTAB and Triton X-100 from water to various sugar solutions. As with tyrosine, the values of the transfer free energies indicate in these cases that α-cyclodextrin and linear dextrins solubilize the amphiphilic substances, while mono- and disaccharides behave oppositely. Dextran and xylan behave quite differently from dextrin; they advance the cmc of CTAB and affect that of Triton X-100 little.

The weakening of the hydrophobic self-aggregation and the enhanced solubilization by the dextrins can occur either because they tend to break water structure, or because of a possible heteromolecular association between the dextrins and the nonpolar chains of the surfactant molecules. Some support for the latter possibility of a hydrophobic interacting surface in these

TABLE I

Standard free energy of transfer of some detergents from water to aqueous sugar solutions at 298 K

Solvent system	Transfer free energy from water (cal/mol)	
	CTAB	Triton X-100
10 mM α-Cyclodextrin	−1220	-
50 mM Dextrin 10	−1040	−720
50 mM Dextrin 20	−770	−530
200 mM Maltose	−30	-
250 mM D-Glucose	+240	negligible
1 M Sucrose	+410	+200
200 mM Cellobiose	+240	-
10 mM Dextran 4	+120	negligible
0.025% Xylan	+350	negligible

oligosaccharides comes from the observations [17,18] that these molecules are partitioned from aqueous solutions into polystyrene gels. Hydrophobic index values [19], i.e., ratio of the hydrophobic and the hydrophilic surface areas of these molecules, and the CH surface area [20] of sugar molecules have been estimated as a measure of their hydrophobicities.

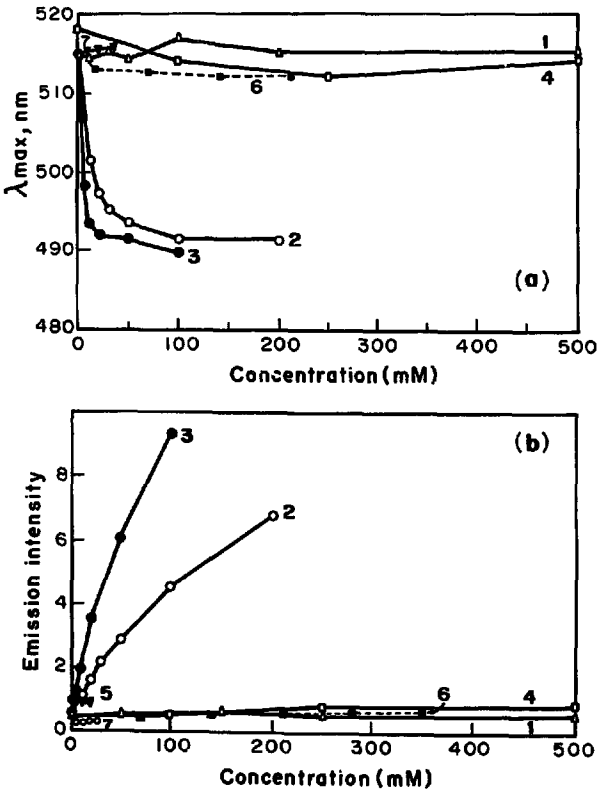

Fig. 3. Variation in the emission band position (a) and the emission intensity (b) of the probe ANSA in water upon the addition of various succharides. Curve (1) is for glucose, (2) for Dextrin 20, (3) for Dextrin 10, (4) for maltose, (5) for Dextran 4, (6) cellobiose and (7) xylan. The excitation maximum was 365 nm.

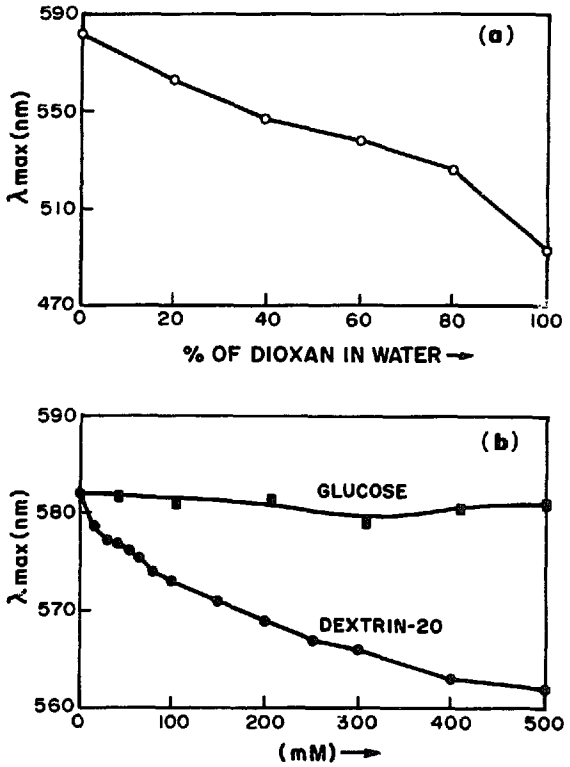

Fig. 4. (Panel a) Variation in the emission band of the neutral dansamide in water: dioxan mixtures. (Panel b) Variation of the emission band of dansamide in solutions of glucose and of Dextrin 20 in water. Excitation was at 323 nm.

Further insight into the hydrophobic character of the dextrins comes from fluorescence probe experiments. Fig. 3 shows the variation in the emission band maximum values and also in the intensity of the fluorescence of the polarity probe ANSA [14] as a function of increasing concentrations of the sugars in water. Here again, it is apparent that even hexameric dextrins offer a medium of decreased polarity to the probe. The emission maximum of ANSA in α- and β-cyclodextrins has been seen to be around 490–495 nm [21]. Other fluorescence probes of polarity such as dansamide and pyrene show similar behavior. Fig. 4 shows that the medium polarity offered by lienar dextrin chains might be roughly comparable to that of 30% dioxane in water. The Ham ratio of the intensities of the third and first vibronic bands of the meission spectrum of pyrene is a polarity index [12], and it was found to increase from 0.58 in water to about 0.67 in 500 mM Dextrin 20; this may be compared with the value of 0.74 seen in micelles of Triton X-100 in water [21]. In contrast, dextran offers a medium polarity that is not any different from that of water; neither do the sugars glucose, maltose, sucrose, cellobiose, or xylan (data not shown).

Based on these results, we suggest that these polarity probes bind to the dextrin chain in a fashion that lets

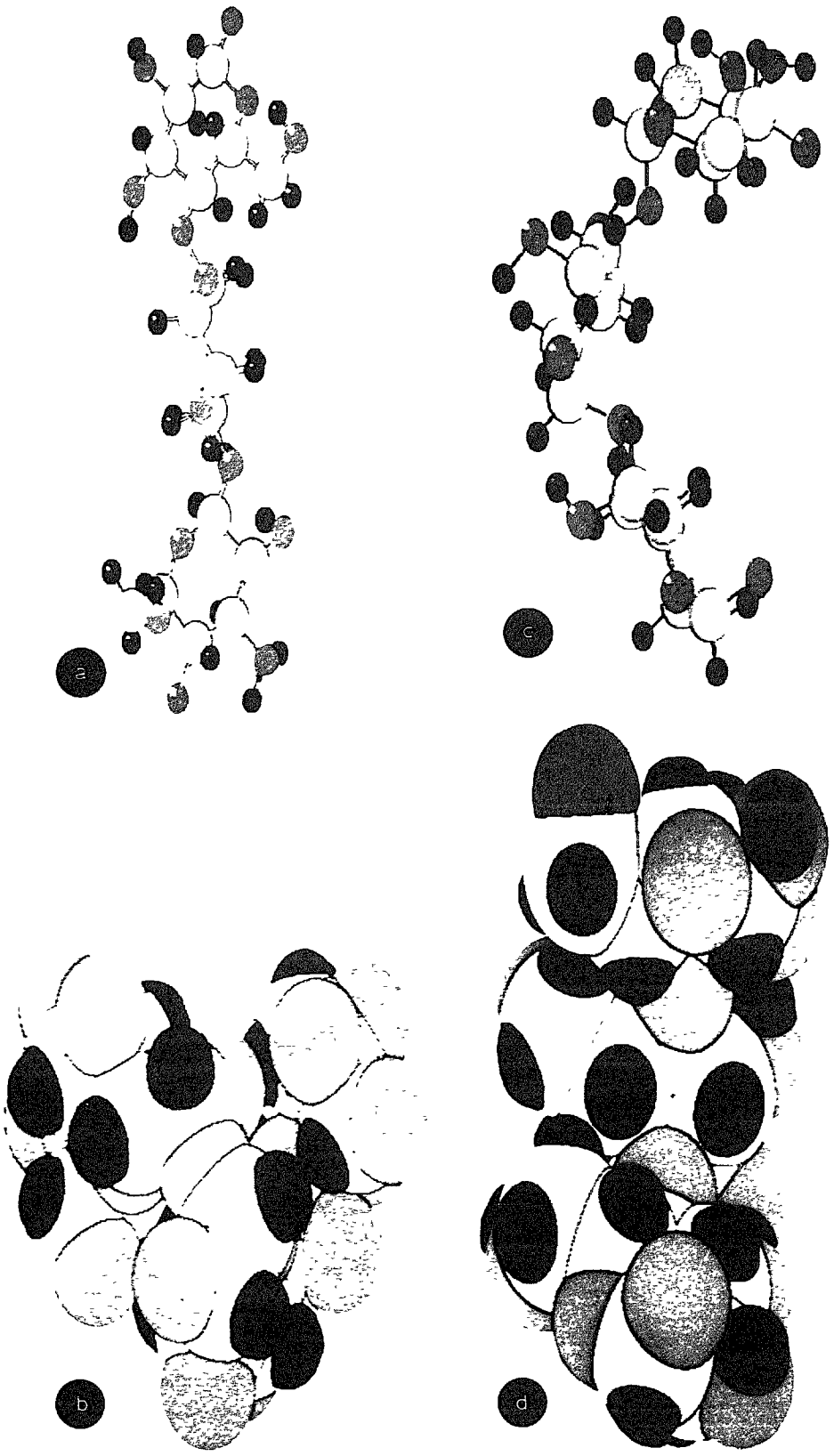

Fig. 5. The energy minimized conformations of: (a), cellulose trimer, shown in the ball and stick format; (b), dextran trimer, shown in the space-filled model format; and (c) and (d), dextrin trimer, shown in both representations. The bond angles and lengths for the $^4C_1$ form of D-glucose were taken from [29] and the energy minimization was done using the procedures given in [27,28]. Dark spheres are hydrogen atoms, grey spheres are oxygen atoms and white balls are carbon atoms.

them experience an immediate environment or *cybotactic* zone that is less polar than the bulk water medium. Such binding can be envisaged if the dextrin chain were to have a nonpolar side comprising the CH groups on one side and all the hydroxyls on the other, i.e., if the chains were to be amphiphilic ribbons or sheets, with one side or face polar and the other surface apolar in character. We already noted how such a possibility can be realized in the lowest oligomeric dextrin maltose [3], and how an apolar face can be generated if the cyclodextrin ring were to be nicked at one place and the resultant chain laid out. Homopolysaccharides are known to take on the extended ribbon (Type A), the flexible helix (Type B), the crumpled ribbon (Type C) and the flexible coil (Type D) conformations, depending on their chain linkage types [8,22]. The α-1,4-glucans such as dextrins take on only the Type B conformation or the helical form, of which three kinds are known. Cyclodextrin is thought to be one turn of one of these, called the V-form [7].

The invocation of a hydrophobic face in linear dextrins and higher polymers might also explain the reported ability of linear amylose to 'uncoil' or straighten out self-coiled amphiphiles such as 16-substituted palmitic acid *p*-nitrophenyl esters [23]. Heteromolecular association between the hydrophobic face of the amylose chain and the alkyl chain of the ester molecule (or inclusion within the nonpolar interior of the helix) might be envisaged as the mechanism. In an effort to test this idea with short dextrin chains, we studied the variation in the fluorescence quantum yields of the molecule 11-bromoundecanoyltryptophan (BUT), which is known to be self-coiled in water [24], upon the addition of various sugars. BUT showed an emission band at 357 nm in 1 M maltose and a relative emission intensity of 1, compared to which its intensity increased to 1.53 and the band blue-shifted to 354 nm in 300 mM Dextrin 20, and an intensity of 1.7 and a band at 351 nm in 150 mM Dextrin 10. We interpret these relative intensifications as arising due to the straightening out of the self-coiled chain of BUT, which would lead to relief from the heavy atom quenching experienced by the tryptophan fluorophore in the molecule. The blue-shifting of the band in the presence of linear dextrin is possibly a polarity effect, since tryptophan emission is polarity sensitive [25].

In polysaccharides, the three dimensional shape is constrained by the geometry of the glycosidic linkage and whether groups are disposed axially or equatorially. For sugars existing in the usual $^4C_1$ chair form, the following possibilities exist [26]. The 1e,4e-linked polymers exist as two-fold (or three-fold) extended chains, the surfaces of which contain polar groups. These will therefore not be amphiphilic (e.g., cellulose or 1,4-linked xylan). Fig. 5a shows the energy minimized conformation of cellulose trimer (based on the procedure of Rao and associates [27,28]), in the ball and stick model form, which exemplifies this point. The α-1,6-dextrans form two-fold crankshaft like structures, and are also not amphiphilic chains; see Fig. 5b for a view of space filled model of dextran trimer, again generated by the energy minimization procedure above, using the bond lengths and angles given for glucose in the $^4C_1$ form [29]. On the other hand, the 1e,3e- and 1a,4e-linked chains form helices with the outer surface different from the inner. The 1e,3e-chains form triple helices with a rather inaccessible interior, while the outer face is solvated. The 1a,4e-chains are the candidates that offer amphiphilic surfaces (e.g. dextrins), as do the 1e,4a-chains (β-1,4-D-galactans). Figs. 5c and d display the energy minimized geometry of dextrin trimer; the amphiphilic feature of the chain is clearly brought out here, in contrast to cellulose or dextran chains. Also notable is the similarity between the inner apolar surface of α-cyclodextrin and that of linear dextrin. The other structures that might display amphiphilicity are β-1,3-D-galactans, α-1,2-, α-1,4-, and β-1,3-D-mannans, and β-1,3- and α-1,4-D-xylans [22]. Unfortunately, these compounds are not readily available in the pure form for us to have studied their amphiphilicity.

We believe that this property might be of value in heteromolecular recognition in glycopeptides, cell surface oligosaccharides, and in lectins and sugar–protein interactions, some of which are thought to have some degree of hydrophobic component [30–33]. The issue of whether saccharide chains enter into hydrophobic interactions has been debated in literature. Kabat et al. [34] had noted that the monoclonal antibody anti-I Ma recognizes a large lipophilic surface presented by the hapten trisaccharide β-D-Galp-(1,4)-β-D-GlcpNAc-(1,6)-D-Gal in its preferred conformation and that, when necessary and stereochemically possible, the carbohydrate will assume intramolecular hydrogen bonds in order to become more compatible for hydrophobic bonding with the antibody. Kronis and Carver [35] had held that hydrogen bonds and van der Waals interactions are more important than hydrophobic forces in such protein–carbohydrate interactions. Later work by Lemieux et al. [36,37] on other antibodies have suggested a pattern of interactions involving the recognition of an amphiphilic surface presented to the protein by the oligosaccharide. In order to provide greater interaction with a complementary lipophilic protein surface, the oligosaccharide might be required to form intramolecular hydrogen bonds.

We believe that in the present instance, the stereochemical constraints imposed on the dextrin chain lead to its adoption of the amphiphilic surfaces, and that the other structures of the glucans such as the α-1,6-, β-1,4-, and the 1,2-type are not restricted conformationally in this fashion, and thus are not amphiphilic. It is also likely that such amphiphilic surfaces are easier recog-

nized in heteromolecular interactions; we could not detect any evidence of homomolecular interactions or self-aggregation in the case of the oligodextrins.

4. Acknowledgements

We are grateful to Professor V.S.R. Rao of Bangalore and Professor E.D.T. Atkins of Bristol for criticism and suggestions. We are deeply grateful to Professor Avadesha Surolia and to Miss Shreeta of the Indian Institute of Science, Bangalore, for their generous advice and help in computer calculations and modelling of the various structures.

References

1 Clarke, R.J., Coates, J.H. and Lincoln, S.F. (1988) Adv. Carbohydr. Biochem. 46, 205–249.
2 Bender, M.L. and Komiyama, M. (1978) Cyclodextrin Chemistry, Springer Verlag, Berlin.
3 Neal, J.L. and Goring, D.A.I. (1970) Can. J. Chem. 48, 3745–3747.
4 Janado, M. and Yano, Y. (1985) Bull, Chem. Soc. Japan 58, 1913–1917.
5 Wu, H.-C. and Sarko, A. (1978) Carbohydr. Res. 61, 27–40.
6 Wu, H.-C. and Sarko, A. (1978) Carbohydr. Res. 61, 7–25.
7 Zugenmaier, P. and Sarko, A. (1976) Biopolymers 15, 2121–2136.
8 Rees, D.A. (1977) Polysaccharide Shapes, Chapman and Hall, London.
9 Hui, Y., Russell, J.C. and Whitten, D.G. (1983) J. Am. Chem. Soc. 105, 1374–1376.
10 Hui, Y., Winkle, J.R. and Whitten, D.G. (1983) J. Phys. Chem. 87, 23–25.
11 Nakatani, H., Shibata, K.I., Kondo, H. and Hiromi, K. (1977) Biopolymers 16, 2363–2370.
12 Nakajima, H. (1976) J. Mol. Spectrosc. 61, 467–469.
13 Kalyanasundaram, K. and Thomas, J.K. (1977) J. Am. Chem. Soc. 99, 2039–2044.
14 Stryer, L (1968) Science 162, 526–533.
15 Lakshmi, T.S. and Nandi, P.K. (1976) J. Phys. Chem. 80, 249–252.
16 Gratzer, W.B. and Bevan, G.H. (1969) J. Phys. Chem. 73, 2270–2273.
17 Yano, Y., Tanaka, K., Doi, Y. and Janado, M. (1988) J. Solut. Chem. 17, 347–358.
18 Janado, M. and Yano, Y. (1985) J. Solut. Chem. 14, 891–902.
19 Miyajima, K., Machida, K. and Nakagaki, M. (1985) Bull. Chem. Soc. Japan 58, 2595–2599.
20 Yano, Y., Tanaka, K., Doi, Y. and Janado, M. (1988) Bull. Chem. Soc. Japan 61, 2963–2964.
21 Shobha, J. and Balasubramanian, D. (1987) Proc. Indian Acad. Sci. Chem. Sci. 98, 469–478.
22 Kennedy, J.F. (1988) Carbohydrate Chemistry, Clarendon, Oxford, U.K.
23 Jiang, X.-K., Fan, W.-Q. and Hui, Y.-Z. (1984) J. Am. Chem. Soc 106, 7202–7205.
24 Shobha, J. and Balasubramanian, D. (1986) J. Phys. Chem. 90, 2800–2802.
25 Lakowicz, J.R. (1983) Principles of Fluorescence Spectroscopy, Plenum Press, New York.
26 Atkins, E. (1986) Int. J. Biol. Macromol. 8, 323–329.
27 Rao, V.S.R., Yathindra, N. and Sundararajan, P.R. (1969) Biopolymers 8, 325–333.
28 Yathindra, N. and Rao, V.S.R. (1970) Biopolymers 9, 783–790.
29 Arnott, S. and Scott, W.E. (1972) J.C.S. Perkin II, 324–335.
30 Lemieux, R.U., Wong, T.C., Liao, J. and Kabat, E.A. (1984) Mol. Immun. 21, 751–759.
31 Lemieux, R.U. (1989) Chem. Soc. Rev. 18, 347–374.
32 Sastry, M.V.K., Banarjee, P., Pantajali, S.R., Swamy, M.J., Swarnalatha, G.V. and Surolia, A. (1986) J. Biol. Chem. 261, 11726–11733.
33 Acharya, S., Patanjali, S.R., Sajjan, S.U., Gopalakrishnan, B. and Surolia, A. (1990) J. Biol. Chem. 265, 11586–11594.
34 Kabat, E.A., Liao, J., Burzynska, M.H., Wong, T.C., Thogersen, H. and Lemieux, R.U. (1981) Mol. Immunol. 18, 873–881.
35 Kronis, K.A. and Carver, J.P. (1985) Biochemistry 24, 834–840.
36 Lemieux, R.U., Hindsgaul, O., Brd, P., Narasimhan, S. and Young, W.W., Jr. (1988) Carbohydr. Res. 178, 293–305.
37 Delbaere, L.T.J., Vandonselaar, M., Prasad, L., Quail, J.W., Pearlstone, J.R., Carpenter, M.R., Smillie, L.B., Nikrad, P.V., Spohr, U. and Lemieux, R.U. (1990) Can. J. Chem. 68, 1116–1121.