Refined 1.8-Å Structure Reveals the Mode of Binding of β-Cyclodextrin to the Maltodextrin Binding Protein[†,‡]

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ABSTRACT: The maltodextrin binding protein from *Escherichia coli* serves as the initial receptor for both the active transport of and chemotaxis toward a range of linear maltose sugars. The X-ray structures of both the maltose-bound and sugar-free forms of the protein have been previously described [Spurlino, J. C., Lu, G.-Y., & Quiocho, F. A. (1991) *J. Biol. Chem. 266*, 5202–5219; Sharff, A. J., Rodseth, L. E., Spurlino, J. C., & Quiocho, F. A. (1992) *Biochemistry 31*, 10657–10663]. The X-ray crystal structure of the maltodextrin binding protein complexed with cyclomaltoheptaose (β-cyclodextrin) has been determined from a single crystal. The structure has been refined to a final *R*-value of 21% at 1.8-Å resolution. Although not a physiological ligand for the maltodextrin binding protein, β-cyclodextrin has been shown to bind with a K_d of the same order as those of the linear maltodextrin substrates. The observed structure shows that the complexed protein remains in the fully open conformation and is almost identical to the structure of the unliganded protein. The sugar sits in the open cleft with three glucosyl units bound to the C-domain at the base of the cleft, in a similar position to maltotriose, the most tightly bound ligand. The top of the ring is loosely bound to the upper edge of the cleft on the N-domain. The sugar makes a total of 94 productive interactions (of less than 4.0-Å length) with the protein and with bound water molecules. Of these there are only four (of less than 3.4-Å length) direct sugar–protein hydrogen bonds, with another four water-mediated hydrogen bonds. Comparison shows that, other than rotation of the C6 hydroxyl groups and some torsional deformations, the structures of β-cyclodextrin and the small molecule crystal structure of β-cyclodextrin are similar.

The maltodextrin binding protein (MBP)[1] acts as the initial component of high-affinity active transport (Furlong, 1987) and bacterial chemotaxis (Macnab, 1987). The three-dimensional structures of both the maltose–bound and unliganded forms of MBP have been reported to high resolution (Spurlino *et al.*, 1991; Sharff *et al.*, 1992). MBP, like all of the periplasmic binding proteins whose structures have been determined to date, is a monomeric protein with two distinct globular domains separated by a two- or three-strand hinge (Quiocho, 1991). In MBP the maltodextrin binding site is located at the base of the cleft between the two domains, identified as the N- and C-domains. In going from the sugar-free structure to the maltose-bound structure, we observe a large rigid-body hinge bending between the two domains of approximately 35°, resulting in cleft closure and sequestering of the sugar (Sharff *et al.*, 1992). By interacting with both domains, the bound sugar stabilizes the closed liganded form. This large conformational change explains the mechanism by which the membrane-bound receptors for both active transport and chemotaxis can differentiate between the unliganded and ligand-bound forms of MBP, a stringent requirement for signal transduction to occur.

MBP will bind and transport linear maltodextrins of up to seven α(1→4)-linked glucosyl units (Ferenci, 1980). In addition to maltose, structures of MBP complexed with maltotriose and maltotetraose are available to better than 1.9-Å resolution (L. E. Rodseth, J. C. Spurlino, and F. A. Quiocho, unpublished data). Extensive analyses of the protein–sugar interactions in all three structures have been carried out (Spurlino *et al.*, 1991; Quiocho, personal communication). In addition to linear maltodextrins, it has been shown that MBP can bind to cyclic maltodextrins such as cyclomaltohexaose (α-cyclodextrin) and cyclomaltoheptaose (β-cyclodextrin) (Miller, 1981; Miller *et al.*, 1983). These are nonphysiological ligands and cannot be metabolized by *Escherichia coli* as they are not transported across the inner membrane nor do they induce a chemotactic response (Ferenci & Boos, 1980). However, there was no direct evidence to suggest that this is due to the nature of their interaction with MBP as they cannot penetrate the outer membrane through either the maltoporin or the λ receptor (Ferenci & Boos, 1980).

Nevertheless, despite the large difference in volume and structure and much restricted conformational flexibility relative to the linear maltodextrins, it is observed that their binding affinities are similar to or even better than many of the linear sugars; see Table I. In particular, the K_d for β-cyclodextrin is half that for maltose and only 10-fold higher than that for maltotriose, the most tightly binding ligand. Detailed kinetic analysis using stopped-flow rapid mixing techniques (Miller, 1981; Miller *et al.*, 1983) has shown that the differences in K_d are determined predominately by the

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[1] Abbreviations: MBP, maltose/maltodextrin binding protein; BCD, β-cyclodextrin (small molecule crystal structure); BCYCLO, β-cyclodextrin (structure complexed with MBP); MTR, maltotriose.

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Table I: Affinities of MBP for Maltose Sugars[a]

sugar	K_d (μM)	sugar	K_d (μM)
maltose	3.50	α-cyclodextrin	4.00
maltotriose	0.16	β-cyclodextrin	1.80
maltotetraose	2.30		

[a] Miller et al., 1983.

Table II: Refinement and Geometry Statistics for MBP Complexed with β-Cyclodextrin[a]

resolution range	10.0–1.8 Å
no. of reflections (with 2σ cutoff)	30840
completeness	91%
R-value	0.210
protein atoms	2862
ligand atoms	77
ordered waters	136
bond distances	0.014 Å (0.020 Å)
angle distances	0.031 Å (0.030 Å)
dihedral distances	0.044 Å (0.050 Å)
planar group distances	0.011 Å (0.020 Å)
peptide ω-angle	2.000° (3.000°)
chiral volume	0.147 Å^3 (0.150 Å^3)

[a] Numbers in parentheses represent the stereochemical restraints applied by PROLSQ.

Table III: (a) χ^5 and (b) ϕ and ψ Angles for BCD, BCYCLO, and MTR

Part a

	BCD	BCYCLO	MTR		BCD	BCYCLO	MTR
g1	−60.80	65.27	80.52	g5	−62.15	76.54	
g2	−70.72	65.76	81.53	g6	70.47	−10.38	
g3	−70.96	45.28	70.97	g7	70.94	106.72	
g4	64.71	53.10					

Part b

	BCD		BCYCLO		MTR	
	ϕ	ψ	ϕ	ψ	ϕ	ψ
g2–g1	107.66	−109.42	106.24	−106.76	99.74	−138.64
g3–g2	110.86	−106.99	102.66	−123.50	103.37	−124.40
g4–g3	120.04	−109.62	140.47	−101.13		
g5–g4	103.02	−125.71	96.41	−114.43		
g6–g5	114.96	−99.10	109.74	−106.10		
g7–g6	110.56	−106.61	112.30	−135.85		
g1–g7	102.52	−121.12	122.44	−111.61		
$\bar{x}$	109.95	−111.22	112.89	−114.20	101.56	−131.52
$\sigma_{x_{n-1}}$	6.28	9.13	14.62	11.93		

rate of ligand dissociation. The association rate constant k' is essentially the same for maltose, maltotriose, and α- and β-cyclodextrins (2.3, 2.5, 3.6, and 2.2 × 10^{-7} M^{-1} s^{-1}, respectively). On the other hand, the differences in the dissociation rate constant k closely shadow the K_d (90.0, 8.4, 110.0, and 46.0 s^{-1}).

Hinge bending suggests the kinetic model

$$P \rightleftharpoons P^* + L \rightleftharpoons P^*L \rightleftharpoons PL$$

where P* represents the open protein conformation, P the closed protein conformation, and L the ligand. However, experiment showed simple one-step kinetic behavior (Miller *et al.*, 1983; Vermersch *et al.*, 1990), indicating that hinge opening/closing is much more rapid than ligand binding/release and its contribution to the observed K_d is negligible.

In this study the seven ring β-cyclodextrin was used. Of the many small molecule crystal structures available, the one described by Lindner and Saenger (1982), β-cyclodextrin dodecahydrate, was used as an initial model and for comparison purposes. In addition to determining the mode of binding of the cyclic sugar and comparing it to linear maltodextrins, it was hoped to be able to identify additional binding sites.

Table IV: Hydrogen Bond and van der Waals Interactions between β-Cyclodextrin and Protein/Solvent

ring	sugar atom	residue	no./type of contact[a]	ring	sugar atom	residue	no./type of contact[a]
g1	O2	Wat504	1 v	g4	C2	Trp340	1 v
	C3	Wat373	1 v		O2	Trp340	1 h, 2 v
	O5	Wat435	1 v		C3	Wat432	1 v
	O6	Tyr210	2 v		O3	Wat426	1 h
		Wat483	1 v			Wat432	1 h
g2	C1	Trp230	3 v		C4	Wat432	1 v
	C2	Trp230	2 v		C6	Wat431	1 v
		Wat427	1 v		O6	Wat374	1 v
	O2	Glu111	1 v			Wat378	1 h
		Trp230	2 v	g5	C1	Wat432	1 v
		Wat375	1 v		O2	Wat426	1 v
		Wat427	1 h			Wat432	1 v
	C3	Wat375	1 v		O4	Wat481	1 v
	O3	Wat375	1 v	g6	C2	Lys42	1 v
		Wat427	1 v			Wat429	1 v
		Wat502	1 h		O2	Lys42	1 h
	C4	Tyr155	4 v		O3	Wat428	1 h
	C5	Tyr155	1 v			Wat434	1 h
	O5	Tyr155	2 v		O6	Wat430	1 v
		Trp230	1 v	g7	C1	Wat428	1 v
	C6	Tyr155	3 v		C2	Wat428	1 v
		Phe156	1 v		O2	Wat428	1 h
	O6	Phe156	1 v		C3	Wat373	1 v
		Wat374	1 v		O3	Wat373	1 v
		Wat483	1 v			Wat504	1 h
g3	C1	Tyr155	2 v		O4	Wat373	1 v
	C2	Trp340	1 v		C6	Wat380	1 v
	O2	Asp65	1 h			Wat435	1 v
		Met330	2 v		O6	Wat435	1 h
		Wat502	1 h				
	O3	Asp65	1 v				
		Wat487	1 v				
	C4	Trp340	1 v				
	O5	Tyr155	3 v				
		Trp340	1 v				
	C6	Glu153	3 v				
		Trp340	1 v				
	O6	Glu153	1 h, 2 v				
		Pro154	2 v				
		Tyr155	3 v				

[a] h = hydrogen bond (≤3.4 Å); v = van der Waals contact (≤4.0 Å). Total contacts = 15 h and 79 v.

EXPERIMENTAL PROCEDURES

MBP was prepared according to a standard protocol, described by Spurlino *et al.* (1991). Large crystals, suitable for high-resolution data collection, were grown using the hanging-drop method, as described by Rodseth and Quiocho (1993). Drops (20–25% PEG 6000, ≈6.7 mg/mL MBP, 20 mM MES, pH 6.2, 0.17 mM β-cyclodextrin) were suspended over 25–30% PEG 6000 in Linbro tissue culture plates at 4 °C. The small crystals which were formed were used to seed drops of 12–13% PEG over 16–18% PEG, giving large usable crystals. Initial studies seeding drops with crystals of MBP complexed with linear sugars were not successful.

Intensity data were collected from a single crystal to 1.73 Å on an ADSC two-area detector system and processed using the Howard *et al.* (1985) software package, implemented by Nielsen for the ADSC. Structure refinement was carried out using XPLOR (Brünger, 1990) and PROLSQ (Hendrickson & Konnert, 1980) according to the procedures described for the structure determination of the unliganded form of MBP (Sharff *et al.*, 1992). The structure was directly phased from the unliganded MBP structure.

RESULTS

Crystallization and Diffraction Data. Despite the fact that the crystallization conditions were similar to those used to

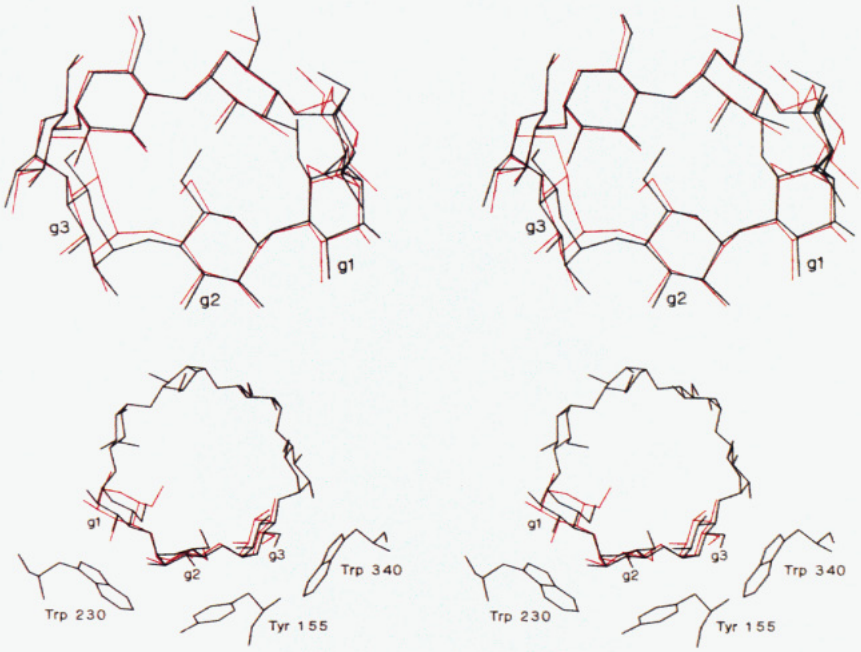

FIGURE 1: Stereoviews of (a, top) superimposed structures of BCD (red) and BCYCLO (black) and (b, bottom) BCYCLO (black) and MTR (red). The three aromatic protein side chains making key stacking interactions with the sugar are shown.

grow crystals of maltose-bound MBP, the crystals of MBP complexed with β-cyclodextrin are triclinic (space group *P*1) and are wholly isomorphous with those of unliganded MBP (Sharff *et al.*, 1992); the cell constants (a = 38.86 Å, b = 44.32 Å, c = 58.31 Å, α = 101.50°, β = 99.30°, γ = 102.20°) differ on average by less than 1%. This immediately suggested that the protein may be binding β-cyclodextrin in the "open cleft" conformation seen for the sugar-free structure. A total of 63 892 observations of 32 945 unique reflections were collected to 1.73 Å (87% complete) with a merging *R*-value of 2.96 and $\langle F/\sigma F\rangle$ = 20. Although data were collected to 1.73 Å as with the crystals of unliganded MBP, the strength and completeness of the shell outside 1.8 Å was such that data outside this resolution were not used in refinement.

Refinement. The initial *R*-value calculated by XPLOR, using the unliganded MBP structure minus solvent, was 32%, confirming the very high degree of isomorphism between the two structures. Inspection of 1.8-Å maps, calculated with $(2|F_o| - |F_c|, \alpha_c)$ and $(|F_o| - |F_c|, \alpha_c)$, revealed cyclic, positive density in the $(|F_o| - |F_c|, \alpha_c)$ map in the ligand binding site, clearly indicating the presence of bound β-cyclodextrin. Although this density was somewhat "fuzzy", the seven sugar rings and overall orientation of the ring were clearly distinguishable. The small molecule crystal structure of β-cyclodextrin (BCD)[1] (Lindner & Saenger, 1982) was manually docked into the density, and several cycles of conventional positional and *B*-factor refinement performed with XPLOR combined with manual manipulation of the sugar reduced the *R*-value to 24.5%. The ligand density was much improved, and it was possible to observe torsions of the rings and determine the exact orientations of the 6′-hydroxyl oxygens. A total of 136 well-defined water molecules were identified and built into the structure. The refinement converged following a final set of cycles with PROLSQ, to a final *R*-value of 21%; see Table II.

The geometry of the refined structure is good and well within accepted limits (Table II). Seven non-glycine residues are in violation of the Ramachandran plot, although two are at the N-terminus and are in poorly resolved density. The Luzatti plot indicates expected coordinate errors around 0.25 Å up to 3.0-Å resolution, rising to around 0.30 Å at 1.8 Å. The overall rms deviation of the protein structure against the unliganded structure, calculated by XPLOR, was 0.38 Å for the backbone and 0.67 Å for the side chains. Comparison of the refined β-cyclodextrin (BCYCLO)[1] structure against the small molecule crystal structure and also the structure of maltotriose bound to MBP (MTR)[1] was performed using MSI's Quanta. Analysis showed an overall rms deviation of 0.77 Å of BCYCLO from BCD and 0.59 Å of the three equivalent sugar residues of BCYCLO from MTR.

DISCUSSION

Comparison of β-Cyclodextrin Structures. Overall both protein-bound and small molecule crystal structures of β-cyclodextrin are very similar. As well as analysis of the overall rms deviation between the two, the χ^5 and ϕ and ψ torsion angles were compared. The exocyclic angle χ^5 is the rotation around the C5–C6 bond and is defined as the torsion angle of the atoms C4–C5–C6–O6 according to IUPAC convention (1980). The endocyclic ϕ and ψ torsion angles are defined by (g2)O5–(g2)C1–(g1)O4–(g1)C4 and (g2)C1–(g1)O4–(g1)C4–(g1)C5, respectively. They are listed in parts a and b of Table III. Of these three key torsion angles χ^5 is subject to the least constraints, allowing relatively free rotation of O6 around the C5–C6 bond; this is clearly apparent by the large variations in χ^5 between the two structures. It is curious that the relative orientations of the 6′-hydroxyls in BCYCLO are the almost exact reverse from BCD; in BCD in χ^5 torsion angles are such that four of the seven 6′-hydroxyls are pointing out of the ring [in the (–)gauche conformation] with two others pointing inward [(+)gauche] (the seventh is disordered). Conversely, in BCYCLO five of the seven 6′-hydroxyls are pointing into the ring with only two facing outward. Notice that the χ^5 angles for sugars g1, g2, and g3 in BCYCLO approximate much more closely to their equivalents in MTR. It should be noted that the numbering of the sugar rings in β-cyclodextrin was changed from the numbering assigned by Lindner and Saenger (1982) such that the sugars which correspond to their equivalents in maltotriose were similarly labeled (g1, g2, and g3). The ϕ and ψ torsions are much more

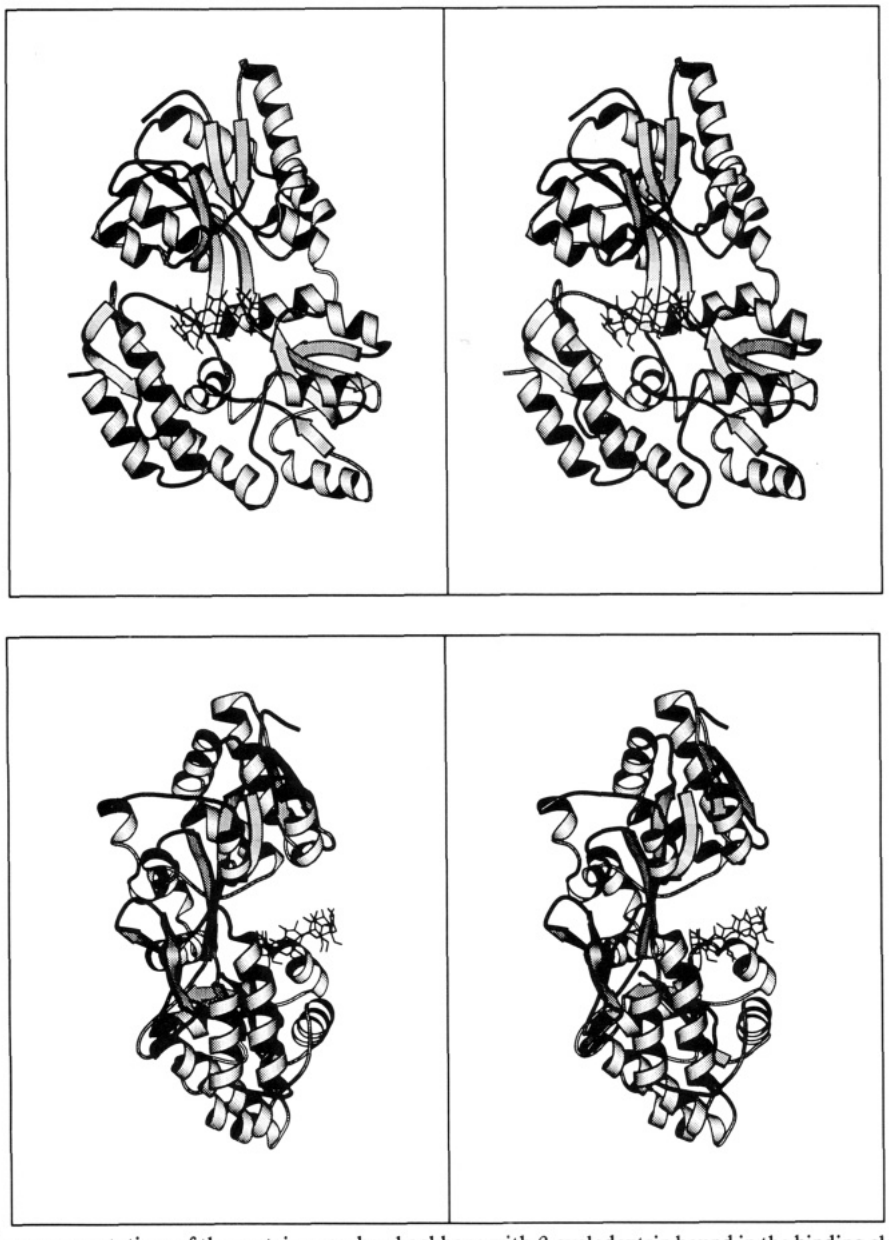

FIGURE 2: Stereo ribbon representations of the protein α-carbon backbone with β-cyclodextrin bound in the binding cleft (stick model), looking (a, top) into the cleft from above and (b, bottom) into the cleft from the side. The N-domain is at the top of each picture. Representations were generated using MOLSCRIPT (Kraulis, 1991).

restricted, especially in a cyclic sugar. This is reflected in the observed values for BCD and BCYCLO. The mean values for both ϕ and ψ are very similar although the standard deviation for ϕ for the protein-bound structure is twice that for the small molecule crystal structure. Table IIIb shows that the ring has undergone a certain degree of torsional stress on binding to MBP. Although all six of the seven sugar linkages show reasonable changes in either ϕ, ψ, or both, only four, namely (g3–g2, g4–g3) and (g7–g6, g1–g7), result in significant changes in sugar conformation; see Figure 1a. Of the two torsion angles in MTR, only that between g1 and g2 differs significantly from BCYCLO, resulting in a twist of g1 of MTR relative to g1 of BCYCLO with the two sugars overlapped; see Figure 1b.

Ligand Binding. The protein structure of the complex of β-cyclodextrin with MBP is essentially unchanged, within the limits of experimental error, from that of the sugar-free form. Even the residues lining the active site cleft are not significantly perturbed, a somewhat unexpected and surprising finding. Furthermore, despite the fact that the protein is in the fully open conformation, the complex would not crystallize under the conditions used to grow crystals of the unliganded protein.

The sugar is sitting in the binding cleft with the plane of the ring along the cleft and the A face against the C-domain (i.e., with the C6 groups facing the C-domain); see Figure 2. Looking into the cleft from the side, the ring is rotated by about $-10°$ from the perpendicular to the hinge, such that the top edge of the ring is tilted over toward the top edge of the N-domain. Quite clearly, the sugar is much more closely associated with the C-domain than with the N-domain, an observation which, although not based on kinetic data, is seemingly in contradiction to previous assumptions that the sugar would bind first to the N-domain (Spurlino *et al.*, 1991), although it must be borne in mind that the cyclic sugar is a nonphysiological ligand for MBP and is quite different in structure from linear maltodextrins. At first glance the association of the ligand with the protein seems rather loose; however, the observed deformations of the sugar, compared to the small molecule crystal structure, clearly suggest that it is bound to the protein. The sugar is heavily hydrated,

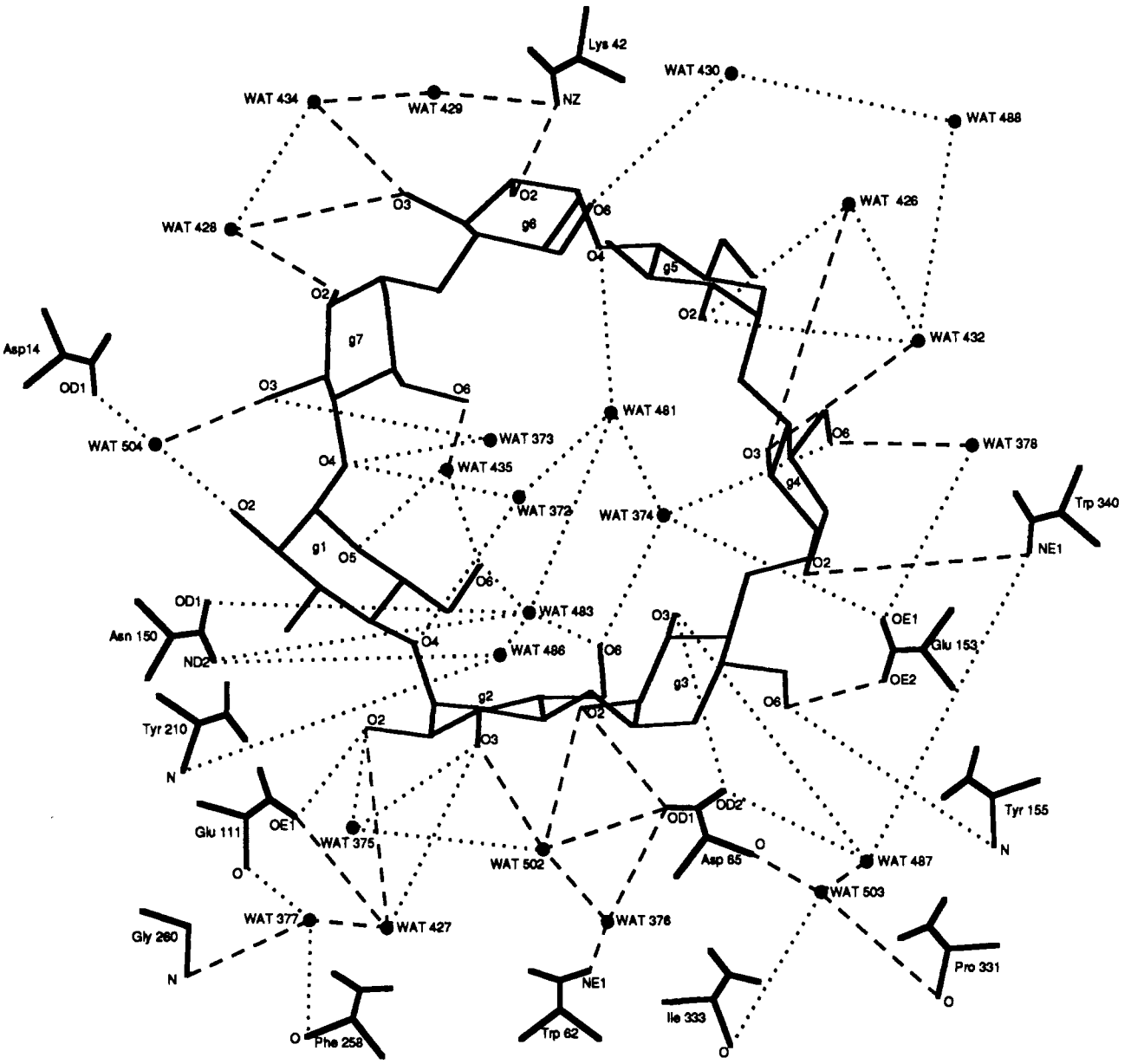

FIGURE 3: Schematic representation of all hydrogen bond (≤3.4 Å) interactions (dashed lines) between β-cyclodextrin and the protein and bound solvent. Potential long-range hydrogen bonds (≤4.0 Å) are indicated by dotted lines.

much more so than the small molecule crystal structure. A total of 20 water molecules are clearly observed, directly associated with the sugar, 9 through hydrogen bond and 12 through van der Waals interactions. There are three water molecules observed binding within the ring although not within normal hydrogen-bonding distance. The observed solvent structure is in contrast with the small molecule crystal structure which showed a high degree of disorder, with 6.5 water molecules distributed over eight sites within the ring cavity and the remaining 5.5 water molecules occupying eight sites between symmetry-related molecules. The positions of the three water molecules included in the ring cavity in the protein structure are similar to the three highest occupancy sites in the small molecule crystal structure; however, there is little additional similarity in the solvent structure. It is noteworthy that although there is less free space within the binding cleft than in the unliganded protein, three times as many ordered water molecules (64) are observed in this structure than the unliganded form (21).

In analyzing the binding of the sugar, comparison was made with the structure of MBP liganded with maltotriose (J. C. Spurlino and F. A. Quiocho, unpublished data) as the radius of curvature of maltotriose approximates that of β-cyclodextrin more than any of the other linear maltose sugars for which MBP structures are available. Superimposition of the C-domains of β-cyclodextrin-bound and maltotriose-bound structures showed that several of the key nonpolar stacking interactions between aromatic residues of the protein and the g1–g3 sugars are conserved. In particular, both MTR and BCYCLO are cradled in a "cup" formed by residues Tyr 155, Trp 230, and Trp 340; see Figure 1b.

A number of the residues making key interactions in the binding of MTR to MBP, predominately C-domain residues, are observed making the same or similar interactions with the g1–g3 sugars of BCYCLO, in particular, Asp 65, Glu 111, Glu 153, Tyr 155, Tyr 210, and Trp 340; however, the extremely low number of direct sugar–protein hydrogen bond interactions is surprising. Table IV lists all of the hydrogen bond (≤3.4 Å) and van der Waals (≤4.0 Å) interactions between the ligand and the protein and bound solvent. Out of a total of 94 interactions there are only 4 direct protein–sugar hydrogen bonds and 4 mediated through (up to two)

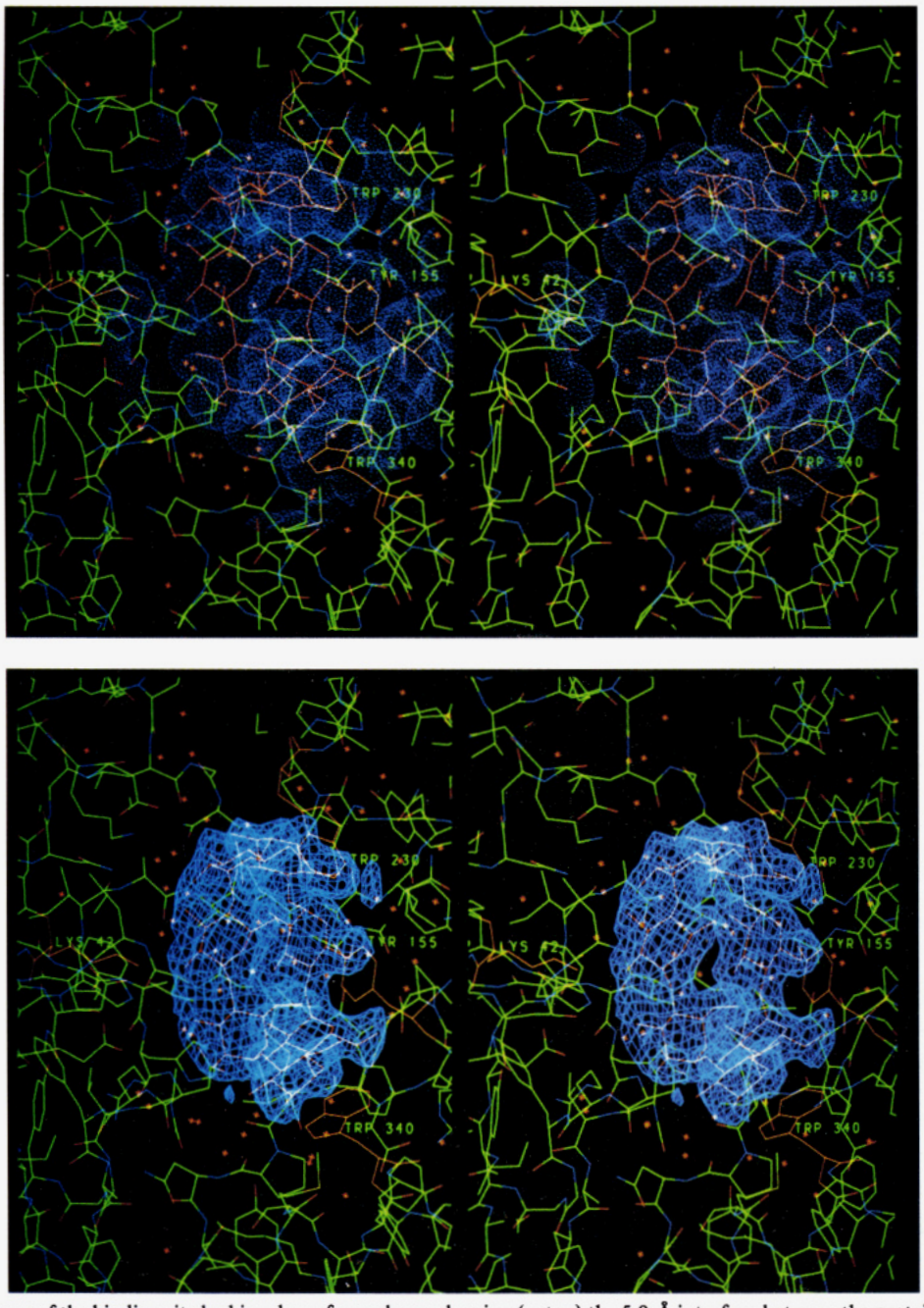

FIGURE 4: Stereoviews of the binding site looking down from above, showing (a, top) the 5.0-Å interface between the protein and β-cyclodextrin (in blue) and (b, bottom) the ($|F_o| - |F_c|$, α_c) density (excluding the sugar from calculation of F_c) for β-cyclodextrin contoured at 1.5σ (also in blue). The three aromatic residues making key stacking interactions with the ligand at the base of the cleft and Lys 42 at the top of the cleft are shown in orange.

bound water molecules, involing 5 protein residues. It should be noted, however, that this number could potentially increase to 7 direct and 15 water-mediated hydrogen bond interactions involving 14 protein residues if 4.0-Å length interactions are considered, see Figure 3.

There is a marked concentration of the protein–sugar interactions with the g1–g3 sugars at the base of the cleft. Of the 8 hydrogen bonds 5 are with g1–g3. Indeed, of the potential 22 (up to 4.0 Å) hydrogen bond interactions, all but 3 are with the g1–g3 sugars. This pattern is also true of the van der Waals contacts; of the 79 van der Waals interactions 58 are with the g1–g3 sugars.

It is quite clear that the mode of binding of β-cyclodextrin is largely dictated by the interaction of the g1–g3 sugars with the C-domain at the base of the cleft, the site of binding of linear maltodextrins. Figure 4a shows the 5.0-Å interface between the protein and sugar, clearly illustrating the high concentration of protein–sugar interactions at the base of the C-domain face of the cleft. Lateral motion of the sugar in the cleft is restricted by the sole protein–sugar hydrogen bond interaction with the N-domain at the top edge of the cleft, between (g6)O2 and the side-chain nitrogen of Lys 42, also indicated in Figure 4a. Two van der Waals contacts to the same atom further stabilize the position of the ring. In addition, Asp 14 OD1 of the N-domain makes a long-range (>4.0 Å) interaction with (g7)O3 and (g1)O2. Analysis of the *B*-factors and electron density of the ligand provides further evidence in support of this, showing that sugars g1–g3 have the lowest *B*-factors (≈37) and best electron density; see Figure 4b. The g6 unit follows after, with g4, g5, and g7 having the highest *B*-factors (≈53) and poorer, less well defined electron density.

That the protein remains in the fully open conformation upon binding of the sugar is unusual. This may initially seem unsatisfactory when considered with the tilt of the ring in the binding cleft, slanted over toward the top edge of the N-domain, and the low number of observed protein–sugar interactions. This begs the question as to whether the sugar could be tilted back slightly toward the C-domain, thus allowing the N-domain to close up further and maximize interaction between the protein and ligand. Closer analysis, however, shows this to be unlikely. Space-filling models of the binding cleft show that the sugar is quite tightly wedged into the cleft and, despite only three significant contacts, the top edge of the B face of the ring is in very close proximity to the upper edge of the cleft on the N-domain (Figure 4a), with solvent filling the gap lower down. The bottom edge of the ring and lower edges of the A face make somewhat more extensive contacts with the base and sides of the cleft on the C-domain side, again with water filling the gap further up the cleft. A slight rotation of the ring to bring the A face into closer contact with the C-domain by a few degrees may be possible by exclusion of some of the water, although this would only allow a similarly small closure of the N-domain. The increase in binding energy afforded by such a small change is likely to be small. The large number of ordered water molecules around the ligand is noteworthy, and they clearly play an important role in stabilizing the binding of the ligand to the protein.

As β-cyclodextrin is unable to cross the outer membrane of *E. coli*, it is not known whether binding to MBP would allow a productive interaction of MBP with the membrane-bound components of either the maltose transport or chemotaxis (Tar) receptors, although this would seem to be ruled out given the observed structure (Sharff *et al.*, 1992).

As to why the affinity of MBP for β-cyclodextrin is as high as for its normal ligands, the conclusion that best seems to fit the observation is that the size and structure of β-cyclodextrin are such that it "fits" snugly into the open cleft such that it can satisfy most of the C-domain interactions involved in binding linear maltodextrins while also contacting the top edge of the N-domain, preventing domain closure. Entropic effects due to partial exclusion of water from the cleft may also make a significant contribution to the binding energy.

Given that the protein remains in the open conformation and the lack of any substantive protein–sugar interactions other than with sugars g1–g3, there is little, if any, evidence of additional sugar binding sites other than those identified from the structures complexed with linear maltodextrins. Most likely, the reduced flexibility of the cyclodextrin together with its decreased radius of curvature relative to linear maltodextrins of four or more glucosyl units prevents sugars locating in binding sites other than for g1–g3.

That MBP is able to bind β-cyclodextrin, a sugar whose size and gross structure are very different from its physiological ligands, and not only manages to accommodate it in the binding cleft but also preserves many of the key interactions made with linear maltodextrins, shows the value of hinge bending as a means of allowing a protein to recognize and differentiate between a range of different ligands. This phenomenon has, to our knowledge, heretofore not been observed in a protein.

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