Prediction of the Three-Dimensional Structures of Complexes of Lysozyme with Cell Wall Substrates†

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ABSTRACT: The conformational energies of complexes of alternating copolymers of *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) with hen egg white lysozyme have been computed. This involved a complete search of the conformational space at the active site of the enzyme available to these substrates and minimization of the conformational energies of the noncovalent complexes. As with the homopolymer $(GlcNAc)_6$, the hexasaccharide $(GlcNAc\text{-}MurNAc)_2\text{-}(GlcNAc)_2$ binds preferentially on the "left" side of the active-site cleft, involving residues such as Arg-45, Asn-46, and Thr-47. The alternating copolymer $(GlcNAc\text{-}MurNAc)_3$, however, binds with its F-site residue preferentially on the "right" side of the active-site cleft, involving residues such as Phe-34 and Arg-114. The lactic acid side chain prevents good binding to the F site on the left side. This result can explain the higher rate of catalysis for the cell wall substrate (the alternating copolymer). The relative affinities of the disaccharide GlcNAc-MurNAc for all sequential pairs of sites A–F (including E and F sites on *both* sides of the cleft) are determined. It is found that the highest affinity of this disaccharide is for sites C and D and "right-side" sites E and F, in good agreement with experimental results of Sarma & Bott [Sarma, R., & Bott, R. (1977) *J. Mol. Biol. 113*, 555]. The energy of the recently determined X-ray crystallographic structure of MurNAc-GlcNAc-MurNAc bound to the B, C, and D sites of hen egg white lysozyme has been minimized and found to lead to a conformation quite similar to one which we predicted previously for the trisaccharide $(GlcNAc)_3$. The D ring is undistorted and binds close to the surface of the active-site cleft. The structure can be extended into sites E and F by addition of two GlcNAc residues, but only on the left side of the active-site cleft. This indicates that polymers bound with their D-site residues near the surface of the cleft must bind to sites E and F on the left side of the cleft, as we also predicted previously.

In a recent series of papers (Pincus et al., 1976a,b, 1977; Pincus & Scheraga, 1979), we have calculated the energies of all of the allowed conformations of oligomers of GlcNAc[1] at the active site of hen egg white lysozyme. We demonstrated that there are two lowest energy structures of complexes of the enzyme with the hexasaccharide $(GlcNAc)_6$, which involve no distortion of the geometry of the substrate. The structure of lowest energy is the one in which the substrate binds with its fourth residue from the nonreducing end in the chair form and in a D site which lies close to the surface of the active-site cleft and somewhat removed from the two acid residues, Glu-35 and Asp-52. The fifth and sixth residues of $(GlcNAc)_6$ bind to E and F sites that involve such residues as Arg-45, Asn-46, and Thr-47 on the "left" side of the active-site cleft. The second structure (of somewhat higher energy) is one in which the fourth residue of the substrate is located *deeply* in the cleft near Glu-35 and Asp-52. Its fifth and sixth residues bind to E and F sites that involve such residues as Phe-34 and Arg-114 on the "right" side of the active-site cleft. A third structure, with a distorted (half-chair) conformation for the fourth residue from the nonreducing end, was also found. This structure had the best contacts (of all three structures) in sites E and F on the "right" side of the cleft, but also had a large distortion energy. Several of these structures are shown in color stereo photographs of space-filling models in Figure 3 of the previous paper by Pincus & Scheraga (1979).

It is now of interest to consider alternating cooligomers of GlcNAc and MurNAc,[1] which are the natural cell wall substrates of lysozyme, to determine whether the lactic acid side chain (present in MurNAc but not in GlcNAc) affects the mode of binding of these substrates to lysozyme. Such a study is of special interest because it is known that a complex of lysozyme with $(GlcNAc\text{-}MurNAc)_3$ has a somewhat higher value of k_{cat} than one with $(GlcNAc)_6$ (Imoto et al., 1972). As a consequence of these calculations, we can account for the higher value of k_{cat} of alternating copolymers of GlcNAc and MurNAc and suggest an experiment to test our hypothesis.

Such calculations are also of interest for another reason. In previous papers (Pincus et al., 1976b, 1977; Pincus & Scheraga, 1979), we had predicted that oligosaccharides binding to sites B, C, and D of the enzyme would do so with their glucopyranose rings in the *undistorted* chair form and that the D site occurs near the surface of the cleft rather than deeply within it. Similar conclusions had been reached by Schindler et al. (1977) on the basis of a series of experiments. This prediction has recently been verified in a determination of the crystal structure of the complex of the trisaccharide MurNAc-GlcNAc-MurNAc with lysozyme (Kelly et al., 1979). This molecule was found to bind to sites B, C, and D in the manner predicted by Pincus & Scheraga (1979) and is the first reported structure with 100% occupancy of the D site (Kelly et al., 1979). Since the prediction had been based on $(GlcNAc)_3$ as a substrate (Pincus & Scheraga, 1979), whereas the crystal structure determination was carried out with the copolymer, it is of interest to repeat the calculations here with the copolymer, not only with the trisaccharide but also with $(GlcNAc\text{-}MurNAc)_3$ and other copolymers of

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[1] Abbreviations used: GlcNAc (or sometimes G), *N*-acetylglucosamine; MurNAc (or sometimes M), *N*-acetylmuramic acid.

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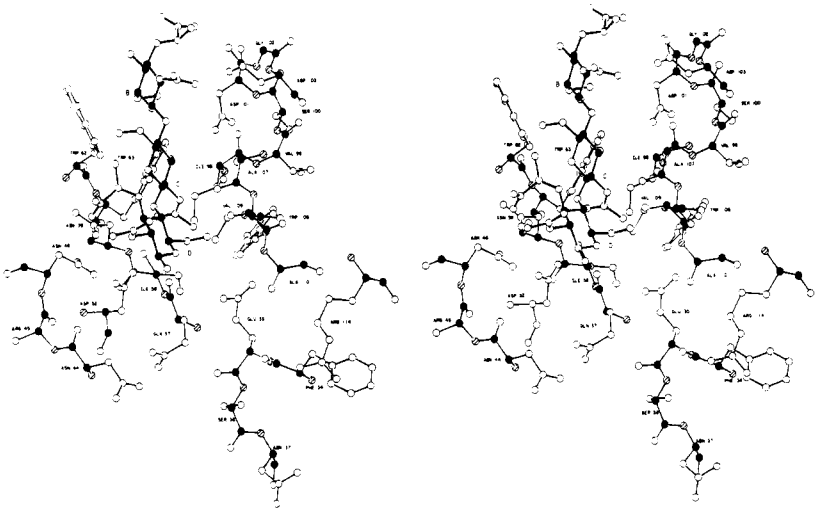

FIGURE 1: Stereo ORTEP view of energy-minimized X-ray structure (conformer 2 of Table I).

GlcNAc and MurNAc. Further, we examine here the nature of the complex whose (trisaccharide) structure was determined crystallographically with regard to the contacts that it makes with the enzyme and to the regions to which it would bind if its length were extended from a tri- to a hexasaccharide.

Methods

Conformational energy calculations were carried out as described previously (Pincus & Scheraga, 1979; Dunfield et al., 1978). Since all of the low-energy binding modes of oligomers of GlcNAc had been determined (Pincus & Scheraga, 1979), these conformations served as starting points for minimization of the energy of complexes between the enzyme and oligomers containing MurNAc residues. For any given starting conformation, the lactic acid side chain was initially placed in all of its calculated minimum energy conformations (Pincus et al., 1976a).

Minimum energy conformations of complexes involving $(GlcNAc\text{-}MurNAc)_3$ were calculated in two ways. In the first, all favorable conformations of complexes involving the disaccharide GlcNAc-MurNAc were computed, and each of these was extended residue by residue to a hexasaccharide containing alternating GlcNAc and MurNAc residues. In all of these calculations, the enzyme was held fixed in its computed lowest energy conformation, as calculated for the complex involving the corresponding GlcNAc homooligomer (Pincus & Scheraga, 1979). The lactic acid side chains were allowed to move first during energy minimization in order to relieve unfavorable contacts with the enzyme. The remaining internal and external degrees of freedom of the substrate were then varied. The structures obtained by this procedure were then subjected to a subsequent (nested) energy minimization in which all of the side chains at the active site of the enzyme and all of the atoms of the substrate were allowed to move.

In the second procedure, the hexamers $(GlcNAc\text{-}MurNAc)_3$ and $(GlcNAc\text{-}MurNAc)_2\text{-}(GlcNAc)_2$ were attached (noncovalently) to the enzyme in the lowest energy conformations obtained previously for $(GlcNAc)_6$ (Pincus & Scheraga, 1979). The energies of such complexes were then minimized, allowing for simultaneous movement of both the atoms of the substrate and of those side chains of the enzyme with which the lactic acid side chain made contact. The structures obtained by this procedure were then subjected to a further energy minimization, as described in the last sentence of the previous paragraph.

The complex of MurNAc-GlcNAc-MurNAc with lysozyme was also examined by these methods. The X-ray coordinates of this trisaccharide bound to lysozyme were obtained from the Brookhaven Protein Data Bank. Because this structure has nonstandard geometry (bond lengths and bond angles), a trisaccharide structure with standard geometry was superimposed on the original X-ray coordinates by obtaining a best least-squares fit (Pincus & Scheraga, 1979). The resulting fit had a root-mean-square deviation of 0.2 Å from the original X-ray coordinates. Since there were a number of bad contacts both between the enzyme and this standard-geometry inhibitor and within the inhibitor itself, the energy of the complex was minimized, allowing for movement of all atoms of the substrate and of the side chains in the active site of the enzyme.

Results and Discussion

Consideration of X-ray Structure. We consider first the computations based on the X-ray crystal structure of MGM bound to sites B, C, and D. The standard-geometry version of this structure is designated as conformer 1, and its conformation and energy are given in the first line of Table I. This trisaccharide is bound to the enzyme with the MurNAc residue in the D site in an undistorted chair form. Also, this residue points outward from the cleft of the active site flanked by Glu-35 and Asp-52. There are, however, a number of unfavorable contacts between the enzyme and the trisaccharide and between different atoms within the trisaccharide, as indicated by the high values of E_{SUB} and E_{INT}. The energy of the complex was therefore minimized, allowing for simultaneous movement of all atoms of MGM and of the side chains in the active site of the enzyme. The results (conformer 2) is given in the second line of Table I and illustrated in Figure 1. The energy-minimized X-ray structure has a low value of E_{INT} but a somewhat higher value of E_{SUB}. The latter is due to unfavorable contacts between the lactic acid side chain of the MurNAc residue in site B and the CH_2OH group of the GlcNAc residue in site C. A much lower energy structure could be obtained by rotating this lactic acid side chain by 180° to its alternate minimum energy position (Pincus et al., 1976a), as shown for conformer 3 of Table I. It is presumed, however, that there was not sufficient electron density in the crystallographic map of Kelly et al. (1979) for the lactic acid side chain to be placed in this position.

The disposition of the energy-minimized X-ray structure (Figure 1; conformer 2 of Table I) is quite similar to that predicted earlier for GlcNAc residues of $(GlcNAc)_6$ bound to sites B, C, and D (Pincus & Scheraga, 1979), even though this latter structure has no lactic acid side chains. It is included as conformer 5 in Table I for comparison with the other

Table I: Conformers Related to X-ray Structure of Complex with MurNAc-GlcNAc-MurNAc[a]

saccharide	rigid body variables[b]						inter-ring dihedral angles[c]		binding sites	E_{SUB}[d]	E_{INT}[d]	E_{ENZ}[d]	E_{TOT}[d]	E_{CONF}[d]
	X	Y	Z	α	β	γ	ϕ	ψ						
(1) MGM X-ray structure (conformer 1)	8.09	5.66	5.45	48.4	−21.8	10.3	−95.4 −106.0	130.6 101.3	B–C C–D	967.8	8335.6	−1307.8	7995.6	9303.4
(2) energy-minimized conformer 1 (conformer 2)	7.46	6.16	5.39	46.0	−22.9	−0.7	−85.9 −97.5	127.6 111.6	B–C C–D	−9.2	−65.3	−1326.1	−1400.6	−74.4
(3) conformer 2 with alternate lactic acid side-chain conformation (conformer 3)	7.64	6.26	5.38	47.5	−29.0	−0.8	−75.5 −93.5	139.0 116.9	B–C C–D	−19.3	−73.3	−1329.0	−1421.5	−92.5
(4) conformer 3 extended to a hexasaccharide, GMGMGG (conformer 4)	7.58	6.08	5.18	49.2	−30.2	−2.4	−55.6 −76.8 −90.4 −102.1 −40.7	118.7 140.5 117.2 99.0 106.7	A–B B–C C–D D–E E–F	−43.6	−97.3	−1324.1	−1465.0	−140.9
(5) $(GlcNAc)_6$, lowest energy "left"-side structure, from Pincus & Scheraga (1979)	7.60	6.52	6.00	50.6	−24.7	2.7	−75.8 −81.5 −98.0 −90.5 −43.0	112.5 131.4 83.0 109.9 116.3	A–B B–C C–D D–E E–F	−52.0	−105.2	−1327.4	−1484.6	−157.2

[a] All symbols and terms are explained in Pincus & Scheraga (1979). $E_{CONF} = E_{SUB} + E_{INT}$ and $E_{TOT} = E_{CONF} + E_{ENZ}$. The reference residue was always the one bound to the C site [the X-ray structure was determined by Kelly et al. (1979)]. [b] X, Y, and Z in Å; α, β, and γ in degrees. [c] ϕ and ψ in degrees. [d] In kcal/mol.

structures. The only differences between conformer 2 and the B-, C-, and D-site residues of conformer 5 of Table I are that the former is displaced by ~0.5 Å down the Z axis and the interring dihedral angle ψ between the residues in the C and D sites differs somewhat.

As may be seen in Figure 1, the lactic acid side chains of the MurNAc residues in sites B and D point outward, away from the active site, and are thus easily accommodated with only small deviations from the structures of the corresponding homooligomers of GlcNAc. As was observed in the energy computations for the lysozyme–$(GlcNAc)_6$ complex (Pincus & Scheraga, 1979) and in the X-ray crystal structure of the lysozyme–MGM complex (Kelly et al., 1979), both tryptophan residues 62 and 63 move toward the substrate, and a hydrogen bond forms between the NH of the indole ring of Trp-63 and the C=O of the *N*-acetyl group of the GlcNAc residue in site C.

In view of the striking similarities in the overall disposition and conformation between our predicted structure and the energy-minimized X-ray structure, we extended the latter (using the alternate lactic acid side-chain position for the MurNAc residue in site B; conformer 3 of Table I) by adding three GlcNAc residues to it, for binding to sites A, E, and F. Of the allowed conformations for these residues, only those that lead to binding in the "left" side region, i.e., near Arg-45, Asn-46, and Thr-47, have low energy. The result obtained for the lowest energy conformer is listed in Table I (conformer 4).

The total conformational energy (E_{TOT}) of this conformer is higher (>15 kcal/mol) than that for $(GlcNAc)_6$ (conformer 5 of Table I) because the residues in sites E and F are displaced downward, preventing optimal contacts with the residues of the left-side F site. Nevertheless, the energy-minimized X-ray structure is quite similar in conformation to that predicted for trisaccharides bound to sites B, C, and D and can be extended by addition of two GlcNAc residues only to *left-side* sites E and F.

Other Possible Binding Regions for MurNAc-GlcNAc-MurNAc. Because the X-ray structure has a conformation similar to that predicted for homopolymers of GlcNAc in sites B, C, and D, we explored the conformational space available to the alternating copolymer MGM. Previously, we found a number of stable binding sites for $(GlcNAc)_3$ (Pincus & Scheraga, 1979). The best binding region was in sites B, C, and D, with the D site close to the surface of the cleft. These conformations were used as starting points for energy minimization of complexes of the copolymer with the enzyme.

We also explored the conformational space available to the disaccharide GlcNAc-MurNAc in all of the allowed binding sites found previously for $(GlcNAc)_2$. From the lowest energy conformations found for GlcNAc-MurNAc (to be discussed in the next section), we added another MurNAc residue to the nonreducing end in all of its allowed conformations in the manner previously described (Pincus & Scheraga, 1979). This provided additional binding dispositions of the trisaccharide.

The two best trisaccharide structures obtained by the methods just described are listed in Table II, conformers 1 and 2. The first of these corresponds to a structure with its D ring turned out toward the surface of the cleft, viz., the B, C, and D sites of conformer 6 of Table I of Pincus et al. (1976b). The second corresponds to a structure whose D ring is buried more deeply in the active-site cleft and one which gives rise to a "right"-side complex. The total conformational energy (E_{TOT}) of conformer 1 is significantly lower than that of conformer 2.

Table II: Lowest Energy Minima for Copolymers of GlcNAc and MurNAc Bound to Lysozyme[a]

saccharide	rigid body variables[b]						inter-ring dihedral angles[c]		binding sites	E_{SUB}[d]	E_{INT}[d]	E_{ENZ}[d]	E_{TOT}[d]	E_{CONF}[d]
	X	Y	Z	α	β	γ	ϕ	ψ						
(1) MGM, D ring out of cleft ("left" side)	7.50	6.45	6.09	47.9	−24.6	−2.3	−76.2	124.2	B–C	−26.8	−68.4	−1334.4	−1429.6	−95.2
							−66.4	110.0	C–D					
(2) MGM, D ring deeply in cleft ("right" side)	7.39	5.07	5.17	24.8	−14.2	−22.1	−76.7	106.5	B–C	−24.3	−66.2	−1331.6	−1422.1	−90.5
							−91.7	115.8	C–D					
(3) $(GM)_3$, "left" side	7.52	6.34	6.13	48.5	−25.5	−1.9	−70.6	119.4	A–B	−55.7	−92.7	−1332.7	−1481.2	−148.5
							−76.5	121.8	B–C					
							−69.7	112.6	C–D					
							−110.7	74.5	D–E					
							−87.1	113.6	E–F					
(4) $(GM)_3$, "right" side	7.27	5.56	4.91	38.9	−12.4	−8.4	−86.4	115.6	A–B	−51.3	−107.2	−1330.3	−1488.9	−158.5
							−96.8	99.7	B–C					
							−101.8	109.3	C–D					
							−139.6	79.2	D–E					
							−71.5	141.4	E–F					
(5) $(GM)_2GG$, "left" side	7.58	6.50	6.19	47.4	−25.6	−1.2	−68.1	121.2	A–B	−55.3	−104.9	−1331.0	−1491.2	−160.2
							−76.5	119.7	B–C					
							−69.3	111.5	C–D					
							−110.4	81.1	D–E					
							−48.9	111.5	E–F					
(6) $(GM)_2GG$, "right" side	7.28	5.55	4.91	38.7	−12.2	−8.2	−82.4	122.0	A–B	−50.4	−103.9	−1330.6	−1484.9	−154.3
							−98.7	96.3	B–C					
							−101.2	109.1	C–D					
							−139.1	79.0	D–E					
							−74.6	142.5	E–F					

[a] All symbols and terms are explained in Pincus & Scheraga (1979). The reference residue was always the one bound to the C site. [b] X, Y, and Z in Å; α, β, and γ in degrees. [c] ϕ and ψ in degrees. [d] In kcal/mol.

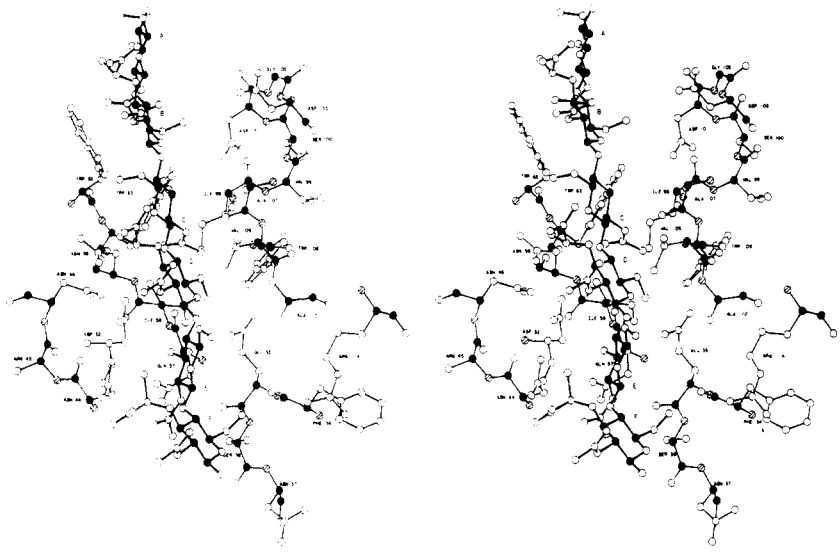

FIGURE 2: Stereo ORTEP view of lowest energy "right"-side binding conformation for $(GlcNAc\text{-}MurNAc)_3$ (conformer 4 of Table II).

For the first structure (conformer 1 of Table II), the conformations of the residues in sites B and C are quite close to those in the energy-minimized X-ray structure (conformer 2 in Table I) while the D residue of the calculated structure is somewhat further removed from the cleft. Another low-energy conformer, not shown in Table II, was found; it, too, would give rise to a left-side complex. This structure was essentially identical with that of the B-, C-, and D-site residues of conformer 5 in Table I and, hence, close to that of the energy-minimized X-ray structure. The value of E_{TOT} for this conformer was about 7 kcal/mol higher in energy than that for conformer 1 in Table II.

Binding Conformations for GlcNAc-MurNAc. From the search of the disaccharide space just described, we found two lowest energy binding modes for GlcNAc-MurNAc, the lower energy one occurring in sites C and D (for both left- and right-side binding conformations) and one slightly higher in energy occurring in the right-side sites E and F. Binding of GlcNAc-MurNAc in B–C and D–E modes was highly unfavorable, the energies of these conformations being at least 15 kcal/mol higher than that for the C–D mode. There are no favorable binding conformations in the left-side E and F sites for reasons that are presented below. The finding that there are two most favored binding modes (C–D and E–F) is in good agreement with experimental binding energies (Imoto et al., 1972) and with a crystallographic study of the binding of GlcNAc-MurNAc to turkey egg white lysozyme, in which two sharp peaks were seen in the Fourier difference map, corresponding to occupation of the C–D and E–F sites (Sarma & Bott, 1977).

Binding of Higher Oligomers Containing GlcNAc and MurNAc. Upon addition of more residues of GlcNAc and MurNAc sequentially to conformers 1 and 2 of Table II, two hexamers (one on the "left" and the other on the "right" side) were obtained, both being of significantly higher energy than those of the two lowest energy complexes formed between $(GlcNAc)_6$ and lysozyme [conformers 3 and 7 of Tables II and III, respectively, of Pincus & Scheraga (1979)]. One of these conformations for $(GlcNAc)_6$ is shown as conformer 5 in Table I. If, however, all of the six residues were added together (i.e., without being influenced by the previously located positions of MGM) so that each trisaccharide was extended to a hexasaccharide at the outset and both the substrate and active-site side chains were allowed to move, two low-energy conformations of $(GlcNAc\text{-}MurNAc)_3$ were obtained (conformers 3 and 4 of Table II). Conformer 3 is a "left"-side complex while conformer 4 is a "right"-side one. The latter is shown in Figure 2.

It should be noted that conformer 4 is significantly more stable than conformer 3. The difference in stability between the two conformers is due to the loss of a crucial hydrogen bond in the left-side F site between the 3-OH group (now replaced by the lactic acid side chain) and the C=O group of Arg-45. This favorable hydrogen bond is replaced by repulsive interactions between the lactic acid side chain and the enzyme residue, causing the reducing sugar unit to be pushed slightly away from the site. The lactic acid side chain on the right side, however, can be well accommodated without disruption of the stabilizing interactions in the right-side F site. Thus, in contrast to the results found for the relative stabilities of $(GlcNAc)_6$ bound to the left and right sides of the active site, i.e., the left-sided complex is of lower energy, the preference of $(GlcNAc\text{-}MurNAc)_3$ is clearly for the right side of the cleft.

That the reason for the reversal of specificity of binding [in that $(GlcNAc)_6$ and $(GlcNAc\text{-}MurNAc)_3$ prefer the left and right sides, respectively] is due to the unfavorable contacts of the lactic acid side chain with the left-side F site can be seen by extending both low-energy trisaccharides into the E and F sites with two GlcNAc residues rather than with GlcNAc-MurNAc (and with a GlcNAc residue in the A site). Conformer 5 is the "left"-side complex formed between $(GlcNAc\text{-}MurNAc)_2$-GlcNAc-GlcNAc and lysozyme while conformer 6 is the corresponding "right"-side complex. Here, it may be seen that the left-side complex is of lower energy, with a GlcNAc residue in site F. Since the extension of MGM in sites B, C, and D with GlcNAc-GlcNAc binds preferentially to the left side whereas its extension with GlcNAc-MurNAc results in preferential binding to the right side, the X-ray structure of the complex with MGM (Kelly et al., 1979) may not be a good model for the binding of $(GlcNAc\text{-}MurNAc)_3$.

Physical Significance of the Calculated Structures. Because the residues of oligomers binding to the D site in the "right-side" mode are positioned deeply in the cleft between the two catalytic residues, Glu-35 and Asp-52, it is tempting to speculate that this mode is the catalytic one. Since the residues of oligomers binding to the D site in the "left-side" modes are removed from this cleft region, these conformers may be bound in productive but not directly catalytic conformations. It is known that $(GlcNAc\text{-}MurNAc)_3$, which has

a higher affinity for the right side of the active site (compare E_{TOT} for conformers 3 and 4 of Table II), is a better substrate for lysozyme than is $(GlcNAc)_6$ (Imoto et al., 1972), which has a higher affinity for the left side of the active site cleft (Pincus & Scheraga, 1979).

Those oligomers, therefore, that bind preferentially to the right side of the cleft may be better substrates. Our studies suggest that substrates with a blocked 3-OH group of the sugar residue in site F (as occurs with the 3-*O*-lactyl group of a MurNAc residue in this site) will bind preferentially to the right side of the cleft. Such substrates should undergo more rapid catalysis than should corresponding substrates that contain a free 3-OH group. Because $(GlcNAc\text{-}MurNAc)_2$-$(GlcNAc)_2$ has a preference for "left-side" binding (compare E_{TOT} for conformers 5 and 6 of Table II), its *apparent* specific rate of catalysis, k_{cat}, measured by classical Michaelis–Menten analysis (Imoto et al., 1972), would be expected to be lower than that for $(GlcNAc\text{-}MurNAc)_3$. Because the affinity of $(GM)_2GG$ for the left side of the cleft relative to its affinity for the right side is the same as the corresponding relative affinity of $(GlcNAc)_6$ for the same binding sites (Pincus & Scheraga, 1979), i.e., "left-side" binding is favored for both oligomers by 6 kcal/mol, the values of *apparent* k_{cat} for both of these oligomers should be the same.

Equilibrium and relaxation studies on the binding of $(GlcNAc)_6$ to lysozyme (Holler et al., 1975) have suggested three productive binding forms in which all six saccharide units contact the enzyme. One of these forms is thought to be the actual reactive form. In our prior calculations (Pincus & Scheraga, 1979), we found three low-energy structures, one left side, one right side, and one right side with a distorted D ring. If these calculated species are the same as those inferred experimentally, then one would expect that relaxation studies of the binding of $(GlcNAc\text{-}MurNAc)_3$ to lysozyme would show *two* rather than three productive binding modes. This result would follow from the inability of the alternating copolymer to bind to the left side of the active site.

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References

Dunfield, L. G., Burgess, A. W., & Scheraga, H. A. (1978) *J. Phys. Chem. 82*, 2609.

Holler, E., Rupley, J. A., & Hess, G. P. (1975) *Biochemistry 14*, 2377.

Imoto, T., Johnson, L. N., North, A. C. T., Phillips, D. C., & Rupley, J. A. (1972) *Enzymes, 3rd Ed. 7*, 665.

Kelly, J. A., Sielecki, A. R., Sykes, B. D., James, M. N. G., & Phillips, D. C. (1979) *Nature (London) 282*, 875.

Pincus, M. R., & Scheraga, H. A. (1979) *Macromolecules 12*, 633.

Pincus, M. R., Burgess, A. W., & Scheraga, H. A. (1976a) *Biopolymers 15*, 2485.

Pincus, M. R., Zimmerman, S. S., & Scheraga, H. A. (1976b) *Proc. Natl. Acad. Sci. U.S.A. 73*, 4261.

Pincus, M. R., Zimmerman, S. S., & Scheraga, H. A. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 2629.

Sarma, R., & Bott, R. (1977) *J. Mol. Biol. 113*, 555.

Scheraga, H. A. (1981) in Proceedings of the 7th Annual Katzir-Katchalsky Conference; Structural Aspects of Recognition and Assembly in Biological Macromolecules (Balaban, M., Ed.) Elsevier, Amsterdam.

Schindler, M., Assaf, Y., Sharon, N., & Chipman, D. M. (1977) *Biochemistry 16*, 423.

Preparation of a Fluorescent-Labeled Derivative of Calmodulin Which Retains Its Affinity for Calmodulin Binding Proteins†

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ABSTRACT: Calmodulin was derivatized with 5-[[[(iodoacetyl)amino]ethyl]amino]-1-naphthalenesulfonic acid to fluorescently label the protein. This derivative (AEDANS·CaM) stimulated the Ca^{2+}-sensitive cyclic nucleotide phosphodiesterase and formed Ca^{2+}-dependent complexes with troponin I and the phosphodiesterase. Association between AEDANS·CaM and these proteins was directly monitored by changes in fluorescence anisotropy. The dissociation constants for the AEDANS·CaM–troponin I and AEDANS·CaM–phosphodiesterase complexes were 60 nM and 4 nM, respectively. This fluorescent derivative of calmodulin appears suitable for direct monitoring of the complexes between calmodulin and calmodulin binding proteins. Rotational diffusion of AEDANS·CaM was also measured with fluorescence anisotropy. These measurements indicated that the shape of calmodulin in solution is best approximated by a prolate ellipsoid.

Since the discovery of calmodulin (CaM)[1] by (Cheung, 1970), it has been determined that this regulatory protein mediates Ca^{2+} stimulation of several enzymes including the Ca^{2+}-sensitive phosphodiesterase (Cheung, 1970; Kakiuchi et al., 1970; Wang et al., 1975), brain adenylate cyclase

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[1] Abbreviations used: CaM, calmodulin; PDE, 3′:5′-cyclic nucleotide phosphodiesterase; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Mops, 4-morpholinopropanesulfonic acid; R_S, Stokes radius; R_{app}, apparent Stokes radius; $\langle\rho\rangle$, average rotational relaxation time; AEDANS·CaM, 5-[[(acetylamino)ethyl]amino]-1-naphthalenesulfonic acid labeled CaM; $PhCH_2SO_2F$, phenylmethanesulfonyl fluoride; DTT, dithiothreitol.

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