Effect of Amino Acid Composition on the Twist and the Relative Stability of Parallel and Antiparallel β-Sheets†

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ABSTRACT: The stable conformations of antiparallel and parallel β-sheets, composed of three identical chains, were determined for eleven different homopoly(amino acids) by minimizing their conformational energies. Each chain had the structure CH_3CO–$(X)_6$–$NHCH_3$, where X was Gly, Ala, Val, Ile, Leu, Lys, Ser, Thr, Phe, Tyr, or α-aminobutyric acid (Abu), a prototype residue for unbranched side chains. The first ten residues listed are those that occur most frequently in the β-sheets of globular proteins. All residues except Gly were in the L configuration. The computed twist of most of the β-sheets is right-handed, in agreement with the observed handedness of the twist in globular proteins. Only the poly(Ser) and the antiparallel poly(Leu) β-sheets have a left-handed twist. These two residues often have backbone conformations in β-sheets in proteins that correspond to *local* left-handed twist, but the net twist of the β-sheets is right-handed, because other residues present dominate the net twist. The stable conformation was also determined for β-sheets composed of two sequential copolymers, viz., CH_3CO–$(Y\text{-}Z)_3$–$NHCH_3$, where Y-Z was Val-Leu or Thr-Ser. Pairs of these residues occur with high frequency on adjacent chains in β-sheets of globular proteins. The twist of these copolymeric β-sheets is right-handed, in spite of the high Leu or Ser content, because Val···Leu and Thr···Ser neighbor interactions force the twist to be right-handed instead of left-handed as determined by Leu···Leu and Ser···Ser interactions, respectively. Thus, the twist is a function of both the overall amino acid composition and of frequency of occurrence of neighboring residue pair interactions in adjacent chains. Parallel β-sheets are more stable for Val, Ile, Lys, Ser, Thr, Phe, and Tyr, while antiparallel β-sheets are more stable for Gly, Ala, Leu, Abu, and the two copolymers, in agreement with the available experimental observations on poly(amino acid) β-sheets.

The arrangement of polypeptide chains in regularly folded structures is among the important structural patterns of the native conformation of globular proteins (Richardson, 1981; Scheraga et al., 1982). Various forms of β-sheets constitute one of the most frequent structural arrangements in globular proteins (Richardson, 1981). All observed β-sheets in globular proteins have a right-handed twist (Chothia, 1973; Salemme & Weatherford, 1981a,b; Richardson, 1981), in contrast to the ideal models (Pauling & Corey, 1953) in which the pleated sheets are not twisted. We have recently shown that the preferred direction of twist of β-sheets can be explained in terms of interatomic interactions. Conformational energy calculations on poly(L-Ala) β-sheets indicated that intrachain interactions involving the backbone and the $C^\beta H_3$ group give rise to a small tendency of the polypeptide chain to adopt a right-handed twist (Chou et al., 1982). The twist is enhanced strongly in poly(L-Val) and poly(L-Ile), as a result of intra- and interchain interactions between the bulky side chains (Chou & Scheraga, 1982; Chou et al., 1983).

There are large variations in the frequency of occurrence of various amino acid residues in β-sheets. It is to be expected that the observed properties of β-sheets in proteins, such as the sign and magnitude of the twist, are dominated by the contributions of the residues that occur frequently.[1] Therefore, we consider here β-sheets formed by polymers of the ten most frequently occurring amino acids in proteins. According to the survey of proteins by Lifson & Sander (1979, 1980a),[2] these residues are as follows, in decreasing order of frequency: Val > Leu > Ile > Ala > Thr > Ser > Tyr > Gly > Lys > Phe. We did not consider here residues that do not occur frequently in proteins, because this paper deals with the dominant energies that affect the twist and arrangement of β-sheets, and we do not analyze the factors that make residues prefer either extended (β-sheet-like) or other (non-β-sheet-like) conformational states.

Computations were carried out not only on homopolymers but also on β-sheets of two alternating copolymers, viz., poly(Val-Leu) and poly(Thr-Ser), respectively, because the frequency of occurrence of Val···Leu and Thr···Ser pairs on adjacent strands is very high in proteins (Lifson & Sander, 1980b). Interactions between such pairs were studied by considering the antiparallel β-sheets formed by the two respective copolymers (Figure 1).

The effect of increasing the size and bulkiness of side chains on the properties of β-sheets will be assessed by a comparison of β-sheets composed of alanine, α-aminobutyric acid (Abu),[3]

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[1] In statistical analyses of conformational preferences [e.g., Chou & Fasman (1974), Tanaka & Scheraga (1976), and Isogai et al. (1980)], normalized relative frequencies are used, in order to take into account the uneven distribution of amino acids in the total sample of proteins considered. In the present context, we are interested in properties that depend only on the relative frequencies of amino acids as they occur in β-sheets in proteins [as given by Lifson & Sander (1979)], irrespective of their frequency of occurrence in other conformations.

[2] Lifson & Sander (1979) defined frequencies of occurrence of residues in terms of their contacts with neighbors, rather than in terms of occurrence of single residues. Therefore, they give twice as much weight to residues occurring in interior strands of β-sheets than to those occurring in strands at the edges of β-sheets. This weighting is appropriate for the discussion in this paper, because the effect of residues on the twist depends not only on their overall frequency of occurrence in the β-sheets but also on their interactions with neighbors (see Discussion). Hence, the ranking given in the text follows the tabulation by Lifson & Sander (1979). Rankings based on single-residue frequencies (Chou & Fasman, 1974; Tanaka & Scheraga, 1976) are very similar to that of Lifson & Sander (1979). Only a few changes occur in the order of ranking of individual amino acids, but the general trends are similar in all rankings.

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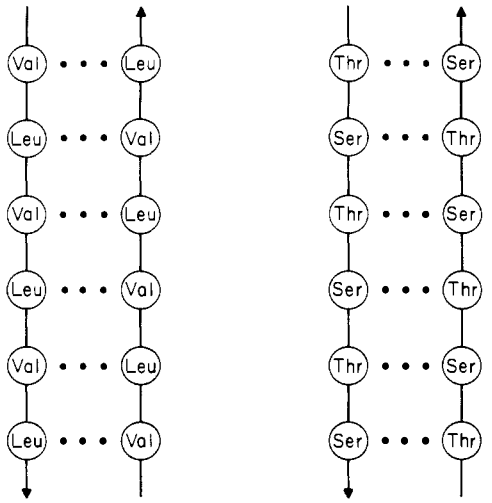

FIGURE 1: Arrangement of neighboring pairs of residues on adjacent chains in antiparallel β-sheets formed by sequential Val–Leu and Thr–Ser copolymers.

and valine, respectively. While Abu is not one of the naturally occurring amino acids in proteins, it serves as a useful prototype for amino acids with unbranched side chains (Vásquez et al., 1983).

The relative stabilities of parallel and antiparallel β-sheets formed by a given polypeptide are obtained from a comparison of their energies. They will be compared with experimental observations on preferences of various oligopeptides for the two forms (Balcerski et al., 1976; Toniolo & Palumbo, 1977; Toniolo et al., 1981).

Computational Methods

The methods used are those described earlier (Chou & Scheraga, 1982; Chou et al., 1982). Computations were carried out on β-sheets consisting of three polypeptide chains. Each chain contained six residues and was terminated by the CH_3CO- and $-NHCH_3$ end groups. The energy of each β-sheet was computed and optimized with respect to variation of the backbone and side-chain dihedral angles (ϕ, ψ, ω, and the appropriate χ^j's) and the external variables, α, β, γ, t_1, t_2, and t_3, that describe the relative position and orientation of chains in the sheet, as defined earlier (Chou & Scheraga, 1982; Chou et al., 1983). All chains of a given β-sheet were considered equivalent; i.e., corresponding dihedral angles of all chains had the same value. In the homopolymeric polypeptides, only regular chains were considered; i.e., the dihedral angles were maintained the same in each residue along the chain.[4] In the copolymers, all dihedral angles along the chain were allowed to vary independently.

The bond lengths and bond angles for each residue and the energy parameters were those of the ECEPP (Empirical Conformation Energy Program for Peptides) algorithm (Momany et al., 1975; Némethy et al., 1980). The energy was calculated with the ECEPP algorithm (Momany et al., 1975) and minimized by alternating between the two function optimizer algorithms, MINOP (Dennis & Mei, 1975) and POWELL (Powell, 1964), until the energy converged to a minimum value, within a convergence limit of 0.0001 kcal/mol. Calculations were performed on a Prime 350 minicomputer with an attached Floating Point Systems AP-120B array processor (Pottle et al., 1980).

The standard conventions of the nomenclature of polypeptide conformations are followed (IUPAC–IUB Commission on Biochemical Nomenclature, 1970). Side-chain conformational rotamers are indicated by using g^+, t, and g^- to denote χ^j = 60, 180, and −60°, respectively (Flory, 1967).

Starting conformations for the backbone were chosen on the line of the (ϕ, ψ) map along which n, the number of residues per turn, equals 2.0 (Chou et al., 1982), at 10° intervals of ϕ in the range $-160° \leq \phi \leq -80°$, with $\omega = 180°$. All of the staggered side-chain conformations were used as starting points, i.e., all combinations of side-chain dihedral angles χ^j = 60, 180, and −60° for the applicable values of j of a given residue, with one exception: for χ^2 and χ^3 in lysine, only 180° was used as the starting point, because this is the only rotamer that occurs in low-energy conformations (Zimmerman et al., 1977; Vásquez et al., 1983).

The initial values of the six external variables were chosen so that adjacent chains were longitudinally aligned with respect to each other (Figure 1). The method used to define the initial values has been described earlier (Chou et al., 1982).

The twist of the β-sheet is described here in terms of δ, the twist of the individual polypeptide chains. For the latter, δ has been defined [Figure 1 of Chou et al. (1982)] as the dihedral angle formed by atoms O_i–C'_i–C'_{i+2}–O_{i+2}, and it is a function of the helical parameter n, which itself is a function of bond lengths, bond angles, and dihedral angles in the backbone (Ramachandran & Sasisekharan, 1968; Chou & Scheraga, 1982; Chou et al., 1982). For a regular polypeptide chain, the amount of twist per two residues is defined as

$$\delta = 360°(2 - |n|)/n \quad (1)$$

and a corresponding average twist is defined for nonregular chains (Chou et al., 1982; Chou & Scheraga, 1982) as

$$\langle\delta\rangle = (360°/n_r)\sum_{i=1}^{n_r}(2 - |n_i|)/n_i \quad (2)$$

where n_i is computed for each residue i. When $n = \pm 2$, i.e., $\delta = 0$, the β-sheet is flat. Right-twisted β-sheets are characterized by $n < -2$ and $\delta > 0$ and left-twisted β-sheets by $n > 2$ and $\delta < 0$.

Results

Mimimum-energy structures were computed for β-sheets consisting of three polypeptide chains. For homopolymers, each chain had the composition $CH_3CO-(X)_6-NHCH_3$, where X = Gly, Ala, Abu, Val, Leu, Ile, Lys, Ser, Thr, Phe, or Tyr. For copolymers, the composition was $CH_3CO-(Y\text{-}Z)_3-NHCH_3$, where Y-Z = Val-Leu or Thr-Ser. Only L-amino acids were considered. More detailed comparisons of β-sheets with variable numbers of chains and of residues per chain were described earlier for Ala (Chou et al., 1982) and Val and Ile (Chou & Scheraga, 1982; Chou et al., 1983).

The results of energy minimization are presented in Tables I–III. The tables contain the dihedral angles, the twist, the unit height, the rotational and translational parameters describing the disposition of neighboring chains, and the energies for the lowest energy structures. The energies of all other structures obtained by energy minimization (including structures with other side-chain conformations) are at least 5 kcal/mol higher, and therefore, they are not considered here. Stereoscopic drawings of the antiparallel and parallel β-sheets formed by the Val-Leu copolymer are shown in Figures 2 and 3, respectively.

[3] Abbreviation: Abu, α-aminobutyric acid.

[4] It has been shown earlier (Chou et al., 1982, 1983) that there is very little difference between the twist and the energy of those β-sheets that are regular and those in which all dihedral angles are varied independently.

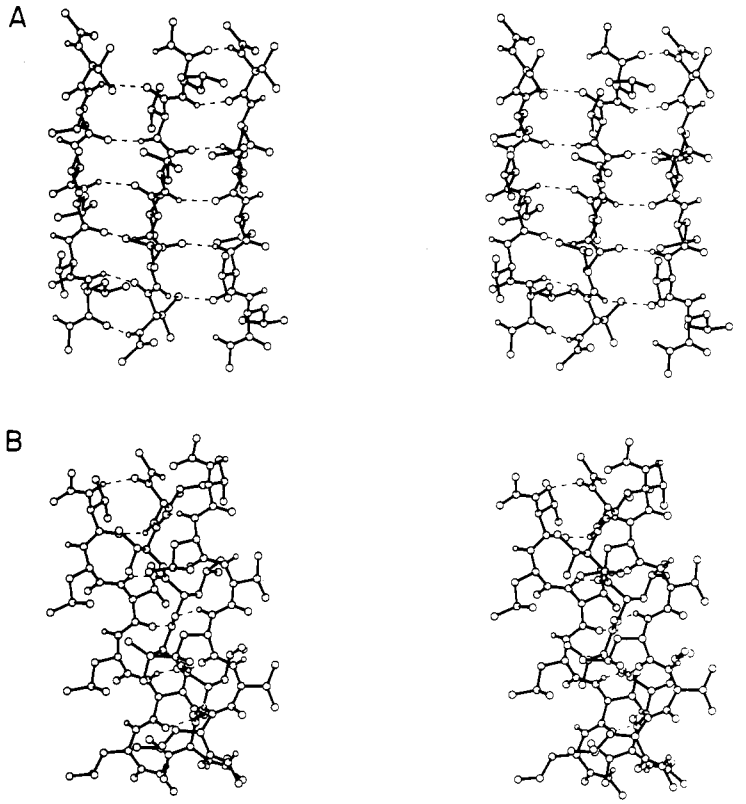

FIGURE 2: Stereoscopic drawing of computed lowest energy conformation of an antiparallel three-chain β-structure composed of CH_3CO–(L-Val-L-Leu)$_3$–$NHCH_3$ chains: (A) viewed from a direction perpendicular to the surface of the sheet; (B) viewed from the edge of the sheet. Hydrogen atoms of the side chains and of the N- and C-terminal CH_3 groups have been omitted. In part A, hydrogen bonds between adjacent chains are indicated by broken lines.

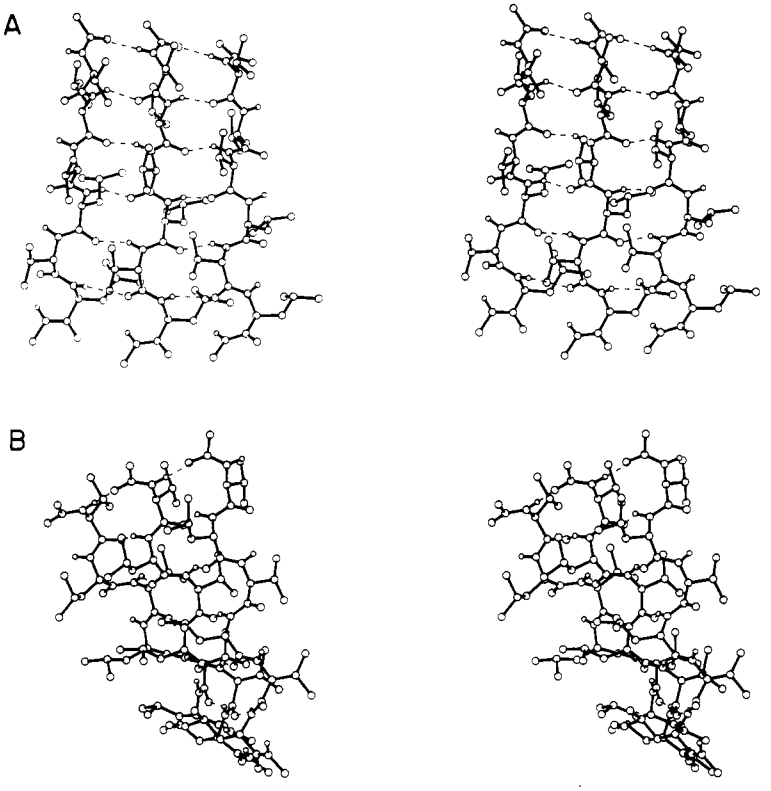

FIGURE 3: Stereoscopic drawing of computed lowest energy conformation of a parallel three-chain β-structure composed of CH_3CO–(L-Val-L-Leu)$_3$–$NHCH_3$ chains: (A) viewed from a direction perpendicular to the surface of the sheet; (B) viewed from the edge of the sheet. Hydrogen atoms of the side chains and of the N- and C-terminal CH_3 groups have been omitted. In part A, hydrogen bonds between adjacent chains are indicated by broken lines.

Backbone Conformations. The most favorable minimum-energy β-sheet conformations of the peptides studied here fall into two classes on a (ϕ, ψ) map, as shown in Figure 4. In the first class, consisting of Ala, Ser, Thr, Phe, Tyr, and the Thr-Ser copolymer, the polypeptide backbone is nearly fully extended, with dihedral angles near $(\phi, \psi) = (-150°, 150°)$. The conformations in the second class are somewhat less extended, as indicated by a lower value of the unit height h, viz.,

Table I: Parameters Characterizing Minimum-Energy Regular β-Sheets Consisting of Three CH_3CO-$(X)_6$-$NHCH_3$ Chains

Residue X		Gly		Ala		Abu		Val[b]	
Structural Arrangement[a]		A	P	A	P	A	P	A	P
Dihedral angles (deg)	φ	180	180	-150	153	-87	-82	-90	-91
	ψ	180	180	152	153	89	85	105	103
	ω	180	180	180	179	180	180	-179	-178
	χ^1			60	59	-174	-174	179	-179
	χ^2					57	57	57	58
	$\chi^{2,1}$								67
	$\chi^{2,2}$							66	
Twist (deg)	δ	0.0	0.0	6.2	1.6	10.5	11.0	32.2	29.8
Unit height (Å)	h	3.63	3.62	3.53	3.54	2.91	2.87	3.02	3.03
Euler angles (deg)	α	-158.6	26.6	-88.4	1.9	-140.1	124.5	-103.2	139.9
	β	180.0	0.0	176.7	-2.6	97.1	6.5	74.8	11.4
	γ	-41.6	-26.4	33.2	-3.3	-28.6	-118.4	-57.5	-129.6
Translational Displacements (Å)	t_1	19.6	2.6	18.9	1.2	16.1	1.2	16.9	1.9
	t_2	6.4	-4.4	4.9	-3.7	-3.9	-3.8	0.4	-4.1
	t_3	0.0	0.0	5.1	2.5	7.0	-2.4	6.0	-2.3
Energy (kcal/mol)	E_{total}[c]	-19.17	2.48	-3.46	8.90	-23.69	-22.04	-12.07	-13.46
	E_{chain}[d]	10.83	10.82	17.52	17.35	12.56	11.80	19.07	19.65
	E_{inter}[e]	-51.66	-29.98	-56.02	-43.15	-61.37	-57.44	-69.28	-72.41

Residue X		Ile[b]		Leu		Lys		Ser	
Structural Arrangement[a]		A	P	A	P	A	P	A	P
Dihedral angles (deg)	φ	-93	-94	-100	-91	-90	-86	-155	-157
	ψ	105	103	95	96	95	94	146	150
	ω	180	-177	173	-179	180	-179	-178	-179
	χ^1	-54	-52	171	-64	-62	-65	62	179
	$\chi^{2,1}$	180	-179	59	116	-169	-175	-52	65
	$\chi^{2,2}$	72	73			-178	-180		
	$\chi^{3,1}$	73	74	55	64	67	66		
	$\chi^{3,2}$			60	60	60	59		
Twist (deg)	δ	26.7	24.1	-6.4	15.4	15.0	20.6	-8.0	-7.2
Unit height (Å)	h	3.05	3.04	3.01	2.98	2.96	2.95	3.53	3.54
Euler angles (deg)	α	-117.9	-46.8	7.7	-56.8	46.0	121.3	-154.8	-57.1
	β	83.6	-9.9	-148.9	-10.1	-92.0	11.5	-121.4	4.8
	γ	-45.3	55.3	166.5	62.9	143.6	-113.3	-14.9	57.2
Translational Displacements (Å)	t_1	16.7	1.8	14.9	1.2	16.1	1.2	16.5	2.6
	t_2	-0.4	-4.1	-4.6	-4.7	-3.1	-4.6	6.3	-4.5
	t_3	6.0	-2.1	8.7	-1.5	6.7	-1.8	9.7	-0.3
Energy (kcal/mol)	E_{total}[c]	24.95	22.98	-44.22	-11.25	-94.10	-99.72	-70.84	-87.32
	E_{chain}[d]	35.33	36.01	14.07	25.19	-3.05	-5.03	10.24	9.75
	E_{inter}[e]	-81.04	-85.05	-86.43	-86.82	-84.95	-84.63	-101.56	-116.57

Residue X		Thr		Phe		Tyr	
Structural Arrangement[a]		A	P	A	P	A	P
Dihedral angles (deg)	φ	-154	-155	-149	-138	-148	-140
	ψ	163	153	152	152	153	152
	ω	176	180	179	176	178	175
	χ^1	-178	180	178	174	180	173
	$\chi^{2,1}$	67	73	-96	-117	-98	-118
	$\chi^{2,2}$	62	63			2	0
	χ^6						
Twist (deg)	δ	11.4	0.0	7.0	18.2	7.8	16.4
Unit height (Å)	h	3.57	3.55	3.54	3.51	3.54	3.51
Euler angles (deg)	α	-124.6	2.2	-152.6	-53.4	-136.1	-54.3
	β	159.1	0.1	-138.3	-13.4	-136.6	-12.4
	γ	-7.4	-2.5	-14.6	59.7	0.5	59.2
Translational Displacements (Å)	t_1	19.1	2.6	19.2	2.9	17.3	2.8
	t_2	5.4	-4.6	4.5	-5.5	4.8	-5.5
	t_3	1.9	-0.3	8.2	0.3	10.6	0.4
Energy (kcal/mol)	E_{total}[c]	-108.67	-116.11	-103.52	-105.50	-110.22	-117.08
	E_{chain}[d]	5.78	2.90	-12.62	-9.38	-14.57	-11.22
	E_{inter}[e]	-126.01	-124.81	-65.66	-77.36	-66.51	-83.42

[a] A = antiparallel, P = parallel. [b] Chou et al., 1983. [c] Total energy of the β-sheet. $E_{total} = 3E_{chain} + E_{inter}$. [d] Total intramolecular energy of each constituent chain. [e] Sum of all interchain energies in the β-sheet.

Table II: Dihedral Angles Characterizing Minimum-Energy Nonregular β-Sheets of Alternating Copolymers Consisting of Three CH_3CO-(Y-Z)$_3$-$NHCH_3$ Chains

Repeating Unit Y-Z		Val-Leu													
Structural Arrangement[a]		A						P							
	Val	φ	ψ	ω	χ^1	$\chi^{2,1}$	$\chi^{2,2}$	φ	ψ	ω	χ^1	$\chi^{2,1}$	$\chi^{2,2}$		
Dihedral angles (deg)		-90	102	-179	178	57	65	-99	102	-175	-178	60	67		
		-93	83	-178	-175	60	71	-85	102	-177	179	57	66		
		-88	91	-176	-177	58	70	-82	103	-175	177	55	66		
	Leu	φ	ψ	ω	χ^1	χ^2	$\chi^{3,1}$	$\chi^{3,2}$	φ	ψ	ω	χ^1	χ^2	$\chi^{3,1}$	$\chi^{3,2}$
		-79	104	173	174	63	54	59	-87	97	179	-83	65	50	59
		-89	104	177	171	62	55	59	-86	101	179	-69	153	60	58
		-83	86	178	-177	67	52	60	-84	79	179	-53	-179	66	72
Repeating Unit Y-Z		Thr-Ser													
Structural Arrangement[a]		A						P							
	Thr	φ	ψ	ω	χ^1	$\chi^{2,1}$	$\chi^{2,2}$	φ	ψ	ω	χ^1	$\chi^{2,1}$	$\chi^{2,2}$		
Dihedral angles (deg)		-150	155	179	177	68	59	-155	155	-178	-179	78	63		
		-152	154	178	176	68	58	-155	155	-178	-179	72	63		
		-153	155	177	176	67	58	-155	155	-178	-179	73	63		
	Ser	φ	ψ	ω	χ^1	χ^2		φ	ψ	ω	χ^1	χ^2			
		-154	153	179	60	-66		-156	154	180	59	-61			
		-154	154	180	59	-67		-156	154	180	59	-61			
		-153	147	180	59	-65		-156	154	180	59	-61			

[a] A = antiparallel, P = parallel.

Table III: Parameters Characterizing Minimum-Energy Nonregular β-Sheets of Alternating Copolymers Consisting of Three CH_3CO-(Y-Z)$_3$-$NHCH_3$ Chains

Repeating Unit Y-Z		Val-Leu		Thr-Ser	
Structural Arrangement[a]		A	P	A	P
Average twist (deg)	$\langle\delta\rangle$	19.0	24.8	2.0	3.0
Unit height (Å)	h	2.96	2.96	3.54	3.55
Euler angles (deg)	α	-119.0	113.7	-178.4	2.4
	β	-101.2	18.0	-175.4	-4.8
	γ	-50.5	-103.7	-53.4	-3.4
Translational Displacements (Å)	t_1	15.7	1.8	19.1	2.5
	t_2	-0.7	-4.5	4.7	-4.6
	t_3	8.6	-0.6	4.5	0.1
Energy (kcal/mol)	E_{total}[b]	-31.48	-24.83	-80.50	-66.10
	E_{chain}[c]	13.56	16.56	7.42	6.02
	E_{inter}[d]	-72.16	-74.51	-102.76	-84.16

[a,b,c,d] See footnotes a,c,d,e, respectively of Table I.

$h \approx 3.0$ Å for the second class and $h \approx 3.5$ Å for the first class. Val, Ile, Leu, Lys, Abu, and the Val-Leu copolymer belong to the second class. Their dihedral angles, occurring near (ϕ, ψ) = (−90°, 100°), are not far from those of the C_7^{eq} conformation (Zimmerman et al., 1977), but β-sheets containing this backbone conformation can also form good interchain hydrogen bonds, as was shown earlier for Val and Ile (Chou & Scheraga, 1982; Chou et al., 1983) and as can be seen in Figures 2 and 3. Glycine constitutes a special case, because of the absence of a chiral C^α atom. The most stable conformation in a poly(Gly) β-sheet is the fully extended chain, with (ϕ, ψ) = (±180°, ±180°).

The first class contains the aromatic residues (Phe and Tyr), for which the C_7^{eq} conformation is less favorable than an extended one, because of the presence of the bulky planar aromatic ring in the side chain (Zimmerman et al., 1977), and also Ser and Thr. For the latter two, conformational preferences are influenced strongly by dipole–dipole and hydrogen-bonding interactions involving the side-chain hydroxyl group (Lewis et al., 1973; Hodes et al., 1978). In the *N*-acetyl-*N'*-methylamides of these two residues, the C_7^{eq} conformation is more stable than the extended conformation, mainly because of side-chain–backbone hydrogen bonds (Lewis et al., 1973; Zimmerman et al., 1977; Vásquez et al., 1983). In β-sheets, the more extended conformation apparently is favored because it allows more favorable alignment of the O–H dipoles in neighboring chains. The second class contains the aliphatic amino acids with β- or γ-branching (Val, Ile, and Leu), as well as unbranched side chains with only aliphatic CH_2 groups near the backbone (Abu and Lys). Except for Ile, this preference is caused by intraresidue interactions that favor the C_7^{eq} over the extended conformation (Lewis et al., 1973; Zimmerman et al., 1977; Vásquez et al., 1983). In the case of Ile, we have shown that interchain interactions lead to the preference for the C_7^{eq} conformation (Chou et al., 1983).

The dihedral angles of antiparallel and parallel β-sheets containing a given residue (or a given alternating pair of residues) are similar, differing by at most 10° from each other (Figure 4). For some of the amino acids, differences in the specific interactions between side chains in either the antiparallel or the parallel structure may alter the twist and/or the relative energies considerably, as described under Discussion, but they do not cause large changes in the backbone conformation. Thus, the observed spread of (ϕ, ψ) values of β-sheets in proteins is a consequence of variations in the amino acid composition and possibly of long-range interactions with other parts of the protein molecule but not of the direction of neighboring chains.

Twist of Minimum-Energy Conformations. The computed values of the twist δ are summarized in Table IV. Most values are positive, indicating that most of the minimum-energy β-sheets formed by poly(amino acids) have a right-handed twist, in agreement with the observed sense of twist of β-sheets in proteins. The only exceptions in Table IV are poly(L-Ser) in both the antiparallel and parallel forms and poly(L-Leu)

Table IV: Computed Twist and Total Energy of Computed Minimum-Energy β-Sheets Composed of Polypeptides of Various Residues and Frequency of Occurrence of These Residues in β-Sheets of Globular Proteins

	computed twist (deg)		computed energy (kcal/mol)		obsd frequency in β-sheets in proteins[a]			
					residues		pairs in adjacent strands	
residue	A[b]	P[b]	A	P	A	P	A	P
homopolymers X	δ				X		X···X pairs	
Gly	0.0	0.0	−19.17	2.48	1.02	1.44	0.00	1.00
Ala	6.2	1.6	−3.46	8.90	1.52	1.68	0.70	1.00
Abu	10.5	11.0	−23.69	−22.04				
Val	32.2	29.8	−12.07	−13.46	2.38	4.10	1.20	1.50
Ile	26.7	24.1	24.95	22.98	1.42	2.40	1.00	1.30
Leu	−6.4	15.4	−44.22	−11.25	1.78	2.02	0.60	0.90
Lys	15.0	20.6	−94.10	−99.72	1.06	0.84	1.20	1.80
Ser	−8.0	−7.2	−70.84	−87.32	1.56	1.26	1.50	0.90
Thr	11.4	0.0	−108.67	−116.11	1.76	0.80	1.50	1.80
Phe	7.0	18.2	−103.52	−105.50	0.86	0.92	1.00	1.10
Tyr	7.8	16.4	−110.22	−117.08	1.24	0.80	2.00	1.10
copolymers Y-Z	⟨δ⟩						Y···Z pairs	
Val-Leu	19.0	24.8	−31.48	−24.83			1.50	0.80
Thr-Ser	2.0	3.0	−80.50	−66.10			1.90	1.80

[a] As defined by Lifson & Sander (1979, 1980b). A value of 1.0 corresponds to random frequency of occurrence (see footnote 1). [b] A = antiparallel; P = parallel.

in the antiparallel form[5] with negative values of δ, i.e., left-handed twist, as well as poly(L-Thr) in the parallel form with no twist. It is notable, however, that β-sheets formed by the Val-Leu and Thr-Ser copolymers have a right-handed twist, in contrast to the homopolymers. The small value of δ for the Thr-Ser copolymer results from the compensation between opposing tendencies of the Thr and Ser components.

The latter finding parallels the behavior of observed β-sheets in proteins. A survey of the dihedral angles in β-sheets found in 34 globular protein structures, listed in the Brookhaven Protein Data Bank (Bernstein et al., 1977), indicates that the (ϕ, ψ) values for most residues correspond to a right-handed twist. Occasionally, individual residues in β-sheets have values of (ϕ, ψ) that correspond to a left-handed twist. In spite of the local left-handed twist in the backbone of these residues, the overall twist of the β-sheets containing these residues is right-handed. This situation occurs frequently with Ser. For example, the β-sheet of carboxypeptidase A contains four Ser residues, three of which have local left-handed twist, even though the entire β-sheet has a strong right-handed twist (Quiocho & Lipscomb, 1971).

The Leu residue occurs frequently in β-sheets, but the frequency of Leu···Leu neighbors on neighboring chains is very low, much less than the frequency for random pairing (Lifson & Sander, 1980b). Thus, the tendency of Leu···Leu pairs for left-handed twisting in antiparallel poly(L-Leu) β-sheets does not cause a significant reduction of the average twist in proteins. The effect of neighboring pairs will be discussed in more detail under Discussion.

Comparison of poly(L-Ala), poly(L-Abu), and poly(L-Val) β-sheets (Table IV) indicates that the increase of the size of substituents on the C^β atom generally causes the twist to become more right-handed (i.e., δ changes in the positive direction). A similar correlation is seen on going from poly(L-Ser) to poly(L-Thr). As we have shown earlier, this is due to the increased interactions between neighboring bulky side chains, both within and between strands of the β-sheet (Chou et al., 1983). Comparison of poly(L-Abu) and poly(L-Lys) indicates that specific effects of the packing of long side chains can increase the twist even in the absence of bulky substitutions on the C^β atom.[6]

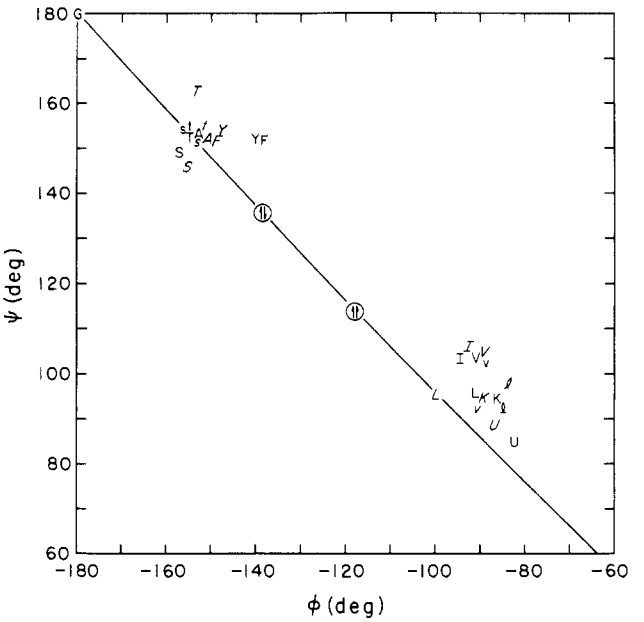

FIGURE 4: Locations of computed minimum-energy conformations of β-sheets on a (ϕ, ψ) map. The conformations of regular poly(amino acids) are denoted by capital letters, the one letter abbreviations for the residues: A, Ala; F, Phe; G, Gly; I, Ile; K, Lys; L, Leu; S, Ser; T, Thr; V, Val; Y, Tyr; U, Abu. The locations of the average dihedral angles for the two kinds of residues in the sequential copolymers of Leu-Val and Thr-Ser are indicated by the corresponding lower-case letters. Letters in Roman type denote parallel-chain structures; those in italic type denote antiparallel-chain structures. Symbols in circles indicate the dihedral angles of the ideal Pauling–Corey (1953) pleated sheets. β-Sheets with no twist are formed when $n = 2$. The diagonal line shown in the figure corresponds to the values of (ϕ, ψ) for which $n = 2$ *only* when $\omega = 180°$. The position of the line shifts slightly when the peptide group is not planar,[5] i.e., when $\omega \neq 180°$ (Chou & Scheraga, 1982). This is the case for Leu, among others. The location of point *L*, in relation to its twist and the position of the line, is discussed in footnote 5.

[5] In Figure 4, the symbol *L* for the antiparallel poly(L-Leu) β-sheet is located at the value of $(\phi, \psi) = (-100°, 95°)$ that appears to lie on the $n = 2$ line, even though this β-sheet has a left-handed twist. This happens because the twist depends not only on (ϕ, ψ) but also on ω. The $n = 2$ line in Figure 4 is drawn for the case of $\omega = 180°$. For poly(L-Leu), however, $\omega = 173°$ (Table I). This combination of (ϕ, ψ) and ω causes the twist to be left-handed (Chou & Scheraga, 1982).

Relative Stability of Antiparallel and Parallel β-Sheets. The total energy of the three-stranded β-sheets is summarized in Table IV. The antiparallel β-sheet has lower energy; i.e., it is favored over the parallel β-sheet for the residues with a small unbranched side chain or no side chain (Gly, Ala, and Abu) and for Leu, while the parallel β-sheet is favored for residues with β-branching (Val, Ile, and Thr), for residues with polar groups near the backbone (Ser and Thr), for aromatic residues (Phe, Tyr), and for the long Lys side chain. It was pointed out earlier that bulky side chains can be accommodated more easily in the parallel structure (Chou et al., 1983).

Discussion

The backbone dihedral angles, (ϕ, ψ), of residues in β-sheets in globular proteins can have a wide range of values (Chothia, 1973; Bernstein et al., 1977), because of differences in the structure of the residues and specific steric interactions within the β-sheet. For β-sheets formed from the residues investigated in this study, the dihedral angles tend to cluster in two regions, as was pointed out above (Figure 4).

The (ϕ, ψ) regions containing the two classes do not coincide with the conformations corresponding to the ideal nontwisted pleated sheets of the Pauling–Corey (1953) models, viz., $(\phi, \psi) = (-139°, 135°)$ and $(-119°, 113°)$ for antiparallel and parallel β-sheets, respectively, but they are shifted to either side of these two conformations. This indicates that the presence of specific intra- and interchain interactions can modify the conformation of a β-sheet, while its essential appearance is retained (Chou et al., 1982, 1983). The result also helps to explain the existence of a wide range of observed (ϕ, ψ) values in β-sheets in proteins. Salemme & Weatherford (1981b) pointed out that coiled-coil strands occur frequently in antiparallel β-sheets, mainly at the edges of sheets or in two-stranded sheets. Chothia (1983) recently described a condition on ϕ and ψ for the presence of strongly twisted β-sheets in which the chains are coiled as well as twisted. According to his description, coiling requires the alternation of residues with dihedral angles falling into two distinct regions of the (ϕ, ψ) map. In the examples shown by Chothia, these (ϕ, ψ) regions coincide approximately with the two classes represented in Figure 4. Thus, the presence of the coiling (possibly in an alternating Phe-Leu copolymer, with one representative of each class) is a resultant, in part, of conformational preferences of the amino acid residues present in such sheets, in addition to possible long-range effects.

The computed relative stability of the antiparallel and parallel β-sheet structures for various amino acids agrees with the available experimental observations on oligopeptides in the solid state and in solution (Balcerski et al., 1976; Toniolo & Palumbo, 1977; Toniolo et al., 1976, 1981; C. Toniolo, personal communication). While poly(L-Ala) and poly(L-Leu) have been observed to form antiparallel β-sheets, there is an observed tendency toward stabilization of the parallel structure for poly(L-Val), poly(L-Ile), poly(L-Phe), and poly(L-Tyr). The parallel structure has the lower computed energy for the latter four amino acids but not for poly(L-Ala) and poly(L-Leu) (Table IV).

The relative stability of the antiparallel and parallel β-sheets depends largely on intra- and interchain interactions involving the side chains and, hence, on the efficiency of packing of strands in the β-sheet. The total energies listed in Table IV are the resultants of the balance of all interatomic interactions, and they cannot always be attributed to individual pairwise atomic interactions (Chou et al., 1982, 1983). Interchain backbone hydrogen bonds are important in determining the relative arrangement of the chains in the β-sheet and the flexibility of the sheet (Salemme & Weatherford, 1981a,b), but the twist depends essentially on side-chain interactions (Chou et al., 1982, 1983; Chou & Scheraga, 1982).

In a β-sheet with a given amino acid sequence, the net twist depends on two factors. One of them is the amino acid composition, because each residue makes a specific contribution to the net twist. The other factor is the frequency of various intrachain and interchain neighbor pairs, as determined by the amino acid sequence and by the arrangement of neighboring chains in the sheet. The twist depends strongly on the intrachain and interchain interactions between neighboring residues, as shown in this paper and earlier (Chou & Scheraga, 1982; Chou et al., 1983). Therefore, in assessing the general contribution of a given residue to the twist and stability of β-sheets, it is not sufficient to consider only the overall frequency of this residue in β-sheets. The frequency of occurrence of pairs, involving this residue, in neighboring chains must also be taken into account [cf. Lifson & Sander (1980b)].

For example, Leu occurs frequently in β-sheets, but the frequency of Leu···Leu pairs in neighboring positions on adjacent strands is lower than average, especially in antiparallel β-sheets (Table IV). Therefore, Leu···Leu pairs make a small contribution to the twist of antiparallel β-sheets. Even though Leu···Leu pairs give rise to a left-handed twist in antiparallel poly(L-Leu) β-sheets ($\delta = -6.4°$), the presence of Leu in observed β-sheets in proteins does not reduce significantly the net twist of the latter, because Leu···Leu pairs are infrequent. On the other hand, the frequency of Val···Leu neighbor pairs in antiparallel β-sheets is very high in proteins, as shown by the high value of the pair frequency listed in Table IV (Lifson & Sander, 1980b). Such pairs tend to increase the right-handed twist, as seen from the large value ($\langle\delta\rangle = 19.0°$) computed for the antiparallel poly(L-Val-L-Leu) β-sheet.

In a similar fashion, Thr···Ser pairs are more frequent in neighboring positions on adjacent strands than Ser···Ser pairs (Lifson & Sander, 1980b). The Thr···Ser pairs contribute to a right-handed twist of the sheet, as seen from the computed values of δ for the Thr···Ser copolymer instead of the left-handed twist contribution of Ser···Ser pairs, as seen in poly(L-Ser).

On the other hand, Val and Ile not only occur frequently in β-sheets, but the number of Val···Val and Ile···Ile pairs on adjacent chains also is very high (Toniolo, 1978; Lifson & Sander, 1979, 1980a). Therefore, the contribution of such pairs to the net twist of β-sheets in proteins is important. The large values of the computed δ in poly(L-Val) and poly(L-Ile) β-sheets constitute one of the reasons for some high observed twists in β sheets in proteins.

A recent electron microscopic study (Lotz et al., 1982) of the morphology of single crystals of *Bombyx mori* silk fibroin

[6] Unfavorable interactions between bulky side chains can also be reflected in unusual values of side-chain dihedral angles rather than in possible changes in the twist. For example, in the *regular* parallel β-sheet formed by poly(L-Leu), $\chi^2 = 116°$ (Table I); i.e., it is near the value for an *eclipsed* conformation. In the regular structure, where χ^2 takes the same value for every residue along the chain, this appears to be the only favorable way to pack the side chains. By contrast, the effect of varying the dihedral angles of each residue independently is seen in the parallel-chain poly(L-Val-L-Leu) β-sheet. In this structure, side-chain conformations adjust so that, for Leu, $\chi^2 = 65°$ for the first residue and $\chi^2 = 153$ and $-179°$, respectively, for the subsequent residues (Table III); i.e., the values are much closer to those for staggered side-chain conformations. These conformations were obtained by rotations in opposite directions from the one implied by the value of χ^2 in the regular structure, and they result in a more favorable packing of the side-chain methyl groups (Figure 3).

and of alternating copolymers of Ala and Gly has shown that these crystals are helically twisted with a uniform sense of twist. In the case of silk fibroin, consisting of 86% Gly, L-Ala, and L-Ser, with small amounts of other L residues (Lucas & Rudall, 1968), and of the regular-sequence copolymers (L-Ala-Gly)$_n$ and (L-Ala-L-Ala-Gly)$_n$, the observed structure of the crystalline lamellae corresponds to a right-handed twist of the constituent β-sheets, while the sense of the twist is of opposite sign for (D-Ala-Gly)$_n$. The magnitude of the twist depends on the crystal morphology and crystallization conditions, but it is always small, less than 1° (Lotz et al., 1982). The observations agree with the results of our study. On the basis of the results presented here, very small net right-handed twists are to be expected for β-sheets composed largely or exclusively of Gly and L-Ala, with a small proportion of L-Ser. As shown in Table IV, L-Ala contributes a small right-handed twist, and Gly does not contribute at all, while the presence of a small amount of L-Ser further decreases the right-handed twist by compenstion.[7]

Conclusions

We have shown in this paper and in earlier papers (Chou et al., 1982, 1983; Chou & Scheraga, 1982; Scheraga et al., 1982) that the observed right-handed twist of β-sheets in proteins can be explained in terms of the energetics of noncovalent interactions within and between the chains forming the β-sheet. An extended polypeptide chain composed of L-amino acids has an intrinsic tendency for this handedness of the twist, as seen for poly(L-Ala) (Chou et al., 1982), but the magnitude of the twist is greatly influenced by interactions involving the side chains, so that it is a function of the composition and structure of the β-sheet. Interchain interactions may alter the magnitude and even the preferred sign of the twist (Chou et al., 1983). As a result, the magnitudes of the twist and the relative stabilities of the parallel and antiparallel β-sheets depend both on the amino acid composition and on the frequency of occurrence of various pairs of neighbor residues in adjacent strands of the β-sheet. The specific value of the twist of a given β-sheet in a protein may differ from the values reported here for poly(amino acid) model β-sheets for several reasons, e.g., long-range interactions with the rest of the protein. These reasons were discussed in detail earlier (Chou et al., 1982).

The analysis of the interactions involving the ten most frequently occurring residues in β-sheets of proteins, presented here, provides the basic data for the assessment of the twist and stability of β-sheets with amino acid sequences observed in proteins. An extension of the computations to the analysis of the properties of β-sheets with observed sequences and to the interactions between β-sheets is in progress in our laboratory.

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Registry No. CH_3CO-$(Gly)_6$-$NHCH_3$, 87597-10-2; CH_3CO-$(Ala)_6$-$NHCH_3$, 72617-38-0; CH_3CO-$(Abu)_6$-$NHCH_3$, 87597-11-3; CH_3CO-$(Val)_6$-$NHCH_3$, 84794-68-3; CH_3CO-$(Ile)_6$-$NHCH_3$, 87189-00-2; CH_3CO-$(Leu)_6$-$NHCH_3$, 87597-12-4; CH_3CO-$(Lys)_6$-$NHCH_3$, 87597-13-5; CH_3CO-$(Ser)_6$-$NHCH_3$, 87597-14-6; CH_3CO-$(Thr)_6$-$NHCH_3$, 87597-15-7; CH_3CO-$(Phe)_6$-$NHCH_3$, 87597-16-8; CH_3CO-$(Tyr)_6$-$NHCH_3$, 87597-17-9; CH_3CO-$(Val\text{-}Leu)_3$-$NHCH_3$, 87597-18-0; CH_3CO-$(Thr\text{-}Ser)_3$-$NHCH_3$, 87597-19-1.

[7] In silk, the β-sheets consist of many strands and are stacked. Such an arrangement can occur only when the twist is small (Salemme & Weatherford, 1981a,b; Chou et al., 1982). This may be one of the reasons why residues that favor a small twist (such as Gly, Ala, and Ser) occur frequently in silk.

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Binding of Saccharide to Demetalized Concanavalin A†

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ABSTRACT: Demetalized concanavalin A (apo-Con A) exists in two conformational states designated locked (PL) and unlocked (P). Brown et al. [Brown, R. D., III, Koenig, S. H., & Brewer, C. F. (1982) *Biochemistry 21*, 465–469] obtained the value of 0.14 for the equilibrium ratio of [PL]/[P] at 25 °C, pH 6.4. More recently, we have shown that in the presence of 100 mM methyl α-D-mannopyranoside (α-MDM) Mn^{2+} ions bind tightly and pairwise to the S1 and S2 sites of each monomeric unit of PL. This allows measurement of the concentration of PL by titration of a sample with Mn^{2+} ions at 5 °C in the presence of a high concentration of α-MDM, while monitoring the binding of Mn^{2+} by measuring the solvent proton magnetic relaxation rates. We show that equilibration of apo-Con A with α-MDM at 25 °C, pH 6.4, results in an increase of the concentration of locked species due to binding of α-MDM to PL, and we deduce the value 29 mM for K_{SPL}, the dissociation constant of the α-MDM–PL complex at 25 °C, pH 6.4. We find that α-MDM also binds to P, though weakly, and to its binary and ternary complexes with Mn^{2+}. Approximate values for the respective dissociation constants are 2400, 100, and 85 mM, compared to about 100 μM for fully active Con A.

Brown et al. (1977) discovered that the metalloprotein concanavalin A (Con A)[1] can exist in two conformational states, with comparable free energies, that interconvert slowly (minutes to hours) because of a relatively high activation barrier that separates the two conformations. Which of the two states is lower in energy depends on the occupancy of two metal-binding sites per Con A monomer. When both S1 (the "transition metal site") and S2 (the "calcium site") are occupied, the protein at equilibrium is essentially all in the conformation called "locked", so named because of its greater affinity for metal ions than the other, the "unlocked", conformation. Conversely, the demetalized protein at equilibrium is predominantly in the unlocked conformation, the ratio of concentrations of locked to unlocked species being 0.14 at 25 °C, pH 6.4 (Brown et al., 1982; Koenig et al., 1982). Occupancy of only S1 by Mn^{2+} results in an equilibrium ratio of locked to unlocked binary Mn^{2+}–Con A complexes of about 1:2 (Brewer et al., 1983a).

Binding of metal ions to apo-Con A in either conformation is sequential (Kalb & Levitzki, 1968; Brewer et al., 1983a); S1 must be occupied before S2. Addition of appropriate metal ions to unlocked apo-Con A produces a weakly associated ternary complex that then transforms to the locked conformation by a first-order process (Brown et al., 1977). In all cases, the locked ternary complex binds sacchardide with the specificity and strength of "native" Con A[2] (Harrington & Wilkins, 1978). When observed this way, binding of saccharide also appears sequential. The question that arises, and that has been addressed previously (Koenig et al., 1978), is whether binding of saccharide is in fact sequential, requiring the presence of metal ions, or whether it is the conformational change induced by the presence of metal ions that is mainly responsible for the saccharide affinity of Con A. The question if even more meaningful because Brown et al. (1977) also showed that apo-Con A could be produced and maintained, for hours (at least) at 5 °C, in the locked conformation, the conformation of the saccharide-binding metalloforms.

It is also known that Ca^{2+} alone can bind to Con A to produce a locked metalloform that binds saccharide as well as native Con A (Koenig et al., 1978; Harrington & Wilkins, 1978). By inference from these data, this form contains two Ca^{2+} ions per Con A monomer, a point recently reexamined and confirmed by Brewer et al. (1983a). Koenig et al. (1978) studied the kinetics of replacement of Ca^{2+} by Mn^{2+}, to form the more stable Ca^{2+}–Mn^{2+}–Con A (native) complex, as a function of saccharide concentration (α-MDM). They found that the rate saturates for α-MDM concentrations above ~30 mM, indicating that the metal ions could be exchanged with saccharide still bound, from which they inferred that the locked saccharide–apo-Con A complex (SPL) exists. The kinetic experiments of Koenig et al. (1978) only yielded a value for K_{SPL}, the saccharide dissociation constant of SPL, multiplied

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[1] Abbreviations: Con A, concanavalin A with unspecified metal ion content and conformational state; α-MDM, methyl α-D-mannopyranoside; NMRD, nuclear magnetic relaxation dispersion (more specifically, the magnetic field dependence of the spin–lattice relaxation rate of solvent protons in the Con A solutions used here).

[2] Native Con A is taken here to mean the locked Ca^{2+}–Mn^{2+}–Con A ternary complex.

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