β Structure of Aqueous Staphylococcal Enterotoxin B by Spectropolarimetry and Sequence-Based Conformational Predictions†

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ABSTRACT: Conformations of the globular protein staphylococcal enterotoxin B have been examined experimentally by ultraviolet circular dichroism (CD) and visible optical rotatory dispersion (ORD). Chen-Yang-Chau analysis (Chen, Y.-H., Yang, J. T., and Chau, K. H. (1974), *Biochemistry 13,* 3350) of the far-ultraviolet CD spectrum of native enterotoxin B revealed (assuming an average helix length of 11 residues) 9% α helix, 38% β structure, and 53% random coil. A fourfold increase in α-helix was observed for enterotoxin exposed to 0.2% sodium dodecyl sulfate, behavior typical for globular proteins of low helical content. Values of −40 to −50 for the Moffitt-Yang parameter b_0 calculated from visible ORD suggested 6–13% α helix in native enterotoxin. Application of a new predictive model (Chou, P. Y., and Fasman, G. D. (1974), *Biochemistry 13,* 222) to the amino acid sequence of enterotoxin B indicated 11% α helix, 34% β structure, and 55% coil in native enterotoxin. The excellent agreement for the amount of α and β conformation utilizing different optical and predictive methods indicates β structure as the dominant secondary structure in native enterotoxin B. Most of the β structure is predicted by Chou-Fasman analysis to reside in two large regions of antiparallel β sheet involving residues 81–148 and residues 184–217. Such highly cooperative regions of antiparallel β sheet account for the slow unfolding of enterotoxin B in concentrated guanidine hydrochloride and rapid folding of guanidine hydrochloride denatured enterotoxin B to native conformation(s) (Warren, J. R., Spero, L., and Metzger, J. F. (1974), *Biochemistry 13,* 1678). A more than twofold increase in α-helix content with a small diminution in β structure was detected by CD and ORD upon acidification of aqueous enterotoxin to pH 2.5. Thus, the β structure of enterotoxin B appears to resist isothermal denaturation and constitutes a stable interior core of structure in the enterotoxin molecule.

Staphylococcal enterotoxin B is a bacterial exoprotein which can be purified to molecular homogeneity (Schantz et al., 1965) and is composed of a single polypeptide chain of 239 amino acid residues (Huang and Bergdoll, 1970). The native SEB[1] molecule in aqueous solution shows an unusual kinetic resistance toward isothermal denaturation by Gdn·HCl or urea, yet appears to be rapidly denatured upon exposure to an acid environment (pH <3.5) in the absence of Gdn·HCl or urea (Warren et al., 1974a). To further elucidate structures of SEB in native and denatured states, a systematic CD study of SEB under a variety of solvent conditions has been performed. The CD of native SEB in 0.15 M KCl demonstrated a high content of β structure (greater than one-third of the polypeptide chain) and relatively little α helix (about one-tenth of the polypeptide chain). Application of the Chou-Fasman sequence analysis (1974a,b) to SEB indicated that most of the native β structure is composed of numerous β segments held in a repeating, antiparallel configuration by intervening β bends. This highly cooperative β-sheet configuration probably contributes to the kinetic stability of native SEB toward perturbation by Gdn·HCl or urea (Warren et al., 1974a). Circular dichroism also revealed that disruption of native SEB structure by acid pH resulted in a several-fold increase of α helix and not an increase in the amount of random structure. Thus, the products of acid and guanidine denaturation differ markedly in that SEB at equilibrium in concentrated Gdn·HCl demonstrated greatly diminished secondary structure by CD. Possible implications for biological interactions of the β structure of native SEB and of the α helices produced by protonation are discussed.

Experimental Procedure

Materials. Highly-purified SEB (Schantz et al., 1965) was a generous gift of Dr. Joseph F. Metzger of the United States Army Medical Research Institute of Infectious Diseases, Frederick, Md., and was stored as a salt-free, lyophilized powder at 4 °C. Ultra-pure guanidine hydrochloride was utilized as supplied by Schwarz/Mann. Sodium dodecyl sulfate purchased from Fisher Chemical Co. was further purified by crystallization from hot ethanol. Inorganic compounds were analytical reagent grade products. Deionized water was used in the preparation of all solutions.

Methods. The visible optical rotatory dispersion and ultraviolet circular dichroism spectra of aqueous SEB were recorded on a Durrum-Jasco ORD/UV-5 spectropolarimeter equipped with a circular dichroism accessory. Solutions were prepared for spectropolarimetry by precise volumetric dilution of concentrated stock solutions of SEB dissolved in 0.15 M KCl or water. Stock solutions and solvents were clarified by filtration through fine sintered-glass filters using positive nitrogen pressure. A 1.0-cm water-jacketed cell was utilized for optical rotatory dispersion measurements in the 300–400 nm wavelength range and for circular dichroism measurements in the 210–300 nm wavelength range. The CD accessory was calibrated using *d*-camphor-10-sulfonic acid (Cassim and Yang, 1969). Baselines were determined for each solvent mixture. Optical rotatory dispersion data in the 310 to 400 nm range were analyzed by use of the Moffitt-Yang equation, $[m']_\lambda = a_0\lambda_0^2/(\lambda^2 - \lambda_0^2) + b_0\lambda_0^4/(\lambda^2 - \lambda_0^2)^2$, by which values for the parameters a_0 and b_0 expressed in deg cm^2 dmol^{-1} were derived as previously described (Yang, 1967) from the reduced mean residue rotation, $[m']_\lambda$. The value of λ_0 was set at 212

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[1] Abbreviations used are: SEB, staphylococcal enterotoxin B; Gdn·HCl, guanidine hydrochloride; CD, circular dichroism; ORD, optical rotatory dispersion.

nm for these calculations. The circular dichroism data are presented as the reduced mean residue ellipticity, $[\theta]_\lambda$, in deg cm^2 dmol^{-1}, as calculated from the expression $[\theta]_\lambda = \theta_\lambda M_0/10(lc)$, in which θ_λ is the observed ellipticity at wavelength λ, M_0 the mean residue weight of the protein, l the path length in centimeters, and c the protein concentration in g per ml. A value of 119 was used for M_0 of SEB (Huang and Bergdoll, 1970). SEB concentrations were calculated from an extinction coefficient ($E_{277}^{1\%}$) of 14.0 (Schantz et al., 1965). All reported values for the optical rotatory dispersion or circular dichroism of SEB were constant for 16–24 h and thus represent equilibrium values.

Results

Hydrodynamic properties of SEB in unbuffered, dilute neutral salt solution and isothermal denaturation difference spectra observed for SEB exposed to concentrated Gdn·HCl or urea solution indicate that native SEB is a globular protein (Wagman et al., 1965; Warren et al., 1974a). A notable feature of the far-ultraviolet CD spectrum of native, globular SEB is a positive peak with a maximum at approximately 234 nm (Figure 1A). Peptide bond conformations of repeatable order (α helix, β sheet) are the major contributors to CD spectra of proteins below 240 nm (Beychok, 1966). The CD spectra of globular proteins with a high content of β sheet demonstrate similar positive peaks or shoulders in the far-ultraviolet region (Kartha et al., 1967; Cathou et al., 1968; Ross and Jirgensons, 1968; Pflumm and Beychok, 1969; Ghose and Jirgensons, 1971). Also, the far-ultraviolet CD spectrum computed from x-ray diffraction and CD data for the β structure of globular proteins is strongly positive in the 230–240 nm region (Saxena and Wetlaufer, 1971). However, the large number of tyrosine residues present in the SEB peptide chain (tyrosine constitutes approximately 9% of amino acids in SEB (Huang and Bergdoll, 1970)) could also contribute significantly to the positive CD peak at 234 nm. For example, major contributions are apparently made by the ^{1}La transitions of tyrosine to a discrete region of positive ellipticity observed near 240 nm in the CD spectrum of RNase S (Goux and Hooker, 1975). Thus, to more rigorously test for the presence of significant β structure in native SEB, far-ultraviolet CD curves characteristic of globular proteins with different helical, β, and unordered conformations were constructed by the Chen–Yang–Chau method (1974) and compared with the entire experimental far-ultraviolet CD curve of native SEB. As shown in Figure 1A, the predicted curve (assuming an average helix length of 11 residues) for a globular protein with 9% α helix, 38% β sheet, and 53% random coil is superposable on the experimental curve of native SEB. It appears, therefore, that SEB in its native conformation contains a high content of β sheet. Low helical content was confirmed for native SEB by visible ORD. Enterotoxin in unbuffered 0.15 M KCl solution obtained a value of −40 to −50 for the Moffitt–Yang parameter b_0. Since the predicted value of b_0 increases from −390 for a six-residue helix to −630 for a helix of infinite length (Chen et al., 1974), the observed value of b_0 for native SEB is compatible with 6–13% helix.

Globular proteins with a substantial content (30–80%) of α-helix do not demonstrate significant change in CD spectra upon treatment with sodium dodecyl sulfate (Visser and Blout, 1970). The marked shift to more negative values observed for the far-ultraviolet CD spectrum of SEB in 0.2% sodium dodecyl sulfate (Figure 1A) indicates a large increase in the α-helical content of SEB in presence of the detergent. An approximate estimate based on the observed value of −12 000 for $[\theta]_{222}$ of SEB in 0.2% sodium dodecyl sulfate reveals that

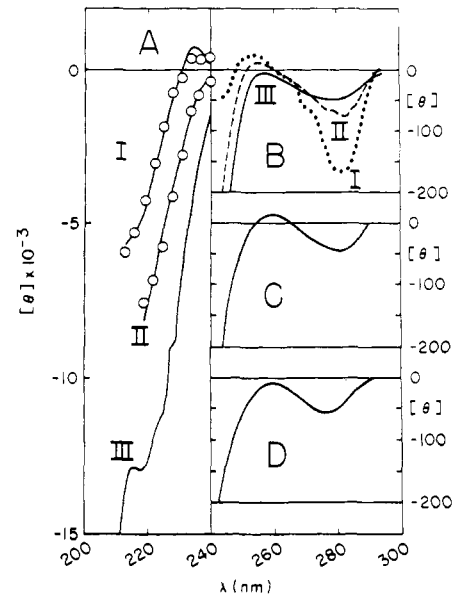

FIGURE 1: The far-ultraviolet (panel A) and near ultraviolet (panels B–D) circular dichroism spectra of SEB. The reduced mean residue ellipticity $[\theta]$ is indicated as a function of wavelength λ for: SEB in 0.15 M KCl with pH of 5.5 (curve I, panels A and B), SEB in 0.15 M KCl acidified to pH 2.5 by addition of dilute HCl (curve II, panels A and B), SEB in 0.2% sodium dodecyl sulfate (curve III, panels A and B), and SEB in 4.1 M (panel C) or 6.0 M (panel D) Gdn·HCl. Temperature was maintained at 26 ± 1 °C by a circulating water bath for CD measurements on the native protein (SEB in 0.15 M KCl, pH 5.5), but ambient room temperature (22–24 °C) was used for measurements on SEB in acidified KCl, sodium dodecyl sulfate, or Gdn·HCl solution. The CD spectra reported in panels A–D represent equilibrium values for $[\theta]$. Maximal variation in absolute values of $[\theta]$ for replicate curves was ca. ±100 in the wavelength region 219–228 nm, ±80 from 230–237 nm, and ±10 from 240–300 nm. The concentration of SEB in the far-ultraviolet spectrum was 2.2–2.4 × 10^{-6} M and in the near-ultraviolet spectrum 4.8–5.9 × 10^{-5} M. The open circles of curves I and II in panel A represent values of $[\theta]$ predicted at the indicated wavelengths by the Chen–Yang–Chau method (1974).

40% of the residues are in an α helix (Chen et al., 1974). This fourfold increase of α-helix content for SEB in sodium dodecyl sulfate solution supports the conclusion that native SEB contains very little α helix (Jirgensons and Capetillo, 1970). The procedure of Visser and Blout (1970) to increase β-sheet content by dialysis of protein in 0.2% sodium dodecyl sulfate against deionized water was attempted with SEB; however, SEB precipitated from solution during dialysis. Acidification of SEB in 0.15 M KCl was unexpectedly found to increase the helix in enterotoxin. A gradual expansion of SEB in 0.15 M KCl from pH 3.5 to 2.2 has been detected by near-ultraviolet difference spectroscopy, viscometry, and ultracentrifugation (Warren et al., 1974a). Upon decreasing the pH of SEB in 0.15 M KCl from 5.5 to 2.5 in the present study, the CD spectrum of the protein became significantly more negative in the 216–240-nm region (Figure 1A) and the Moffitt–Yang parameter calculated from visible ORD decreased from −45 to −112. The curve predicted by the Chen–Yang–Chau method (1974) for SEB at pH 2.5 (Figure 1A) is for a protein with 21% α helix (average helix length = 11 residues), 29% β sheet, and 50% random coil; the value of b_0 at pH 2.5 suggests 18 to 23% helix, depending on the average segment length of helix used for the calculation (Chen et al., 1974). The CD and ORD results thus indicate that the helical content of SEB is more than doubled during acid expansion of the protein. Titration of acidic SEB solution back to pH 5.5 resulted in slow aggregation of protein and consequently the acid-induced conformational change was irreversible.

TABLE I: Regions of α Helix, β Sheet, and Tetrapeptide Breakers in the SEB Molecule as Predicted by Chou–Fasman Protein Conformational Parameters.[a,b]

Sequence	⟨P_α⟩	⟨P_β⟩	Predicted
13 14 15 16 Lys Ser Ser Lys [b b b b]	0.93	0.73	B_{β_1}
17 18 19 20 21 Phe Thr Gly Leu Met [h h i h H]	1.00	1.24	$\beta 1$[c]
24 25 26 27 28 Met Lys Val Leu Tyr [H b H h h]	1.07	1.31	$\beta 2$[c]
29 30 31 32 Asn Asn Asp His [b b i b]	0.92	0.70	B_{β_2}
40 41 42 43 Ser Ile Asn Glu [b H b B]	1.01	0.81	B_{β_3}
44 45 46 47 48 49 Phe Phe Asp Leu Ile Tyr [b h i h H h]	0.99	1.23	$\beta 3$
50 51 52 53 Leu Tyr Ser Ile h h b H]			
54 55 56 57 Lys Asp Thr Lys [b i h b]	0.99	0.87	B_{β_3}
60 61 62 63 Asn Tyr Asp Asn (b b i b)	0.76	0.85	B_{α_1}
64 65 66 67 68 69 Val Arg Val Glu Phe Lys (h i h H h I	1.12	0.99	$\alpha 1$
70 71 72 73 74 Asn Lys Asp Leu Ala b I i H H)			
75 76 77 78 Asp Lys Tyr Lys (i I b I)	0.93	0.89	B_{α_1}
77 78 79 80 Tyr Lys Asp Lys [h b i b]	0.93	0.89	B_{β_4}
81 82 83 84 85 Tyr Val Asp Val Phe [h H i H h]	1.00	1.33	$\beta 4$[d]
89 90 91 92 93 94 Tyr Tyr Gln Cys Tyr Phe [h h h h h h]	0.82	1.28	$\beta 5$[d]
95 96 97 98 Ser Lys Lys Thr [b b b h]	0.94	0.85	B_{β_5}
107 108 109 110 Thr Lys Arg Lys [h b i b]	0.94	0.90	B_{β_6}
111 112 113 114 115 116 Thr Cys Met Tyr Gly Gly [h h H h i i	0.80	1.24	$\beta 6$
117 118 Val Thr H h]			
119 120 121 122 Glu His Gly Asn [B b i b]	1.01	0.61	B_{β_6}
120 121 122 123 His Gly Asn Asn [b i b b]	0.81	0.71	B_{β_7}
124 125 126 127 128 129 Gln Leu Asp Lys Tyr Tyr [h h i b h h	0.96	1.18	$\beta 7$
130 131 132 133 134 135 Arg Ser Ile Thr Val Arg i b H h H i			
136 137 Val Phe H h]			
138 139 140 141 Glu Asp Gly Lys [B i i b]	1.03	0.65	B_{β_7}
139 140 141 142 Asp Gly Lys Asn [i i b b]	0.83	0.75	B_{β_8}
143 144 145 146 147 148 Leu Leu Ser Phe Asp Val [h h b h i H]	1.12	1.15	$\beta 8$
149 150 151 152 Gln Thr Asn Lys [h h b b]	0.95	0.96	B_{β_8}
151 152 153 154 Asn Lys Lys Lys (b I I I)	0.99	0.72	B_{α_2}
155 156 157 158 159 160 Val Thr Ala Glu Gln Leu (h i H H h H	1.12	1.10	$\alpha 2$[e]
161 162 163 164 Asp Tyr Leu Thr i b H i)			
164 165 166 167 Thr Arg His Tyr (i i h b)	0.86	1.03	B_{α_2}
180 181 182 183 Ser Pro Tyr Glu [b b h B]	0.88	0.72	B_{β_9}
184 185 186 187 188 189 Thr Gly Tyr Ile Lys Phe [h i h H b h	0.88	1.22	$\beta 9$
190 Ile H]			
191 192 193 194 Glu Asn Glu Asn [B b B b]	1.13	0.46	B_{β_9}
192 193 194 195 Asn Glu Asn Ser [b B b b]	0.95	0.57	$B_{\beta_{10}}$
196 197 198 199 200 201 Phe Trp Tyr Asp Met Met [h h h i H H]	1.04	1.32	$\beta 10$
202 203 204 205 Pro Ala Pro Gly [b I b i]	0.79	0.76	$B_{\beta_{10}}$
209 210 211 212 Asp Gln Ser Lys [i h b b]	1.00	0.87	$B_{\beta_{11}}$
213 214 215 216 217 Tyr Leu Met Met Tyr [h h H H h]	0.99	1.43	$\beta 11$
218 219 220 221 Asn Asn Asp Lys [b b i b]	0.88	0.71	$B_{\beta_{11}}$
223 224 225 226 Val Asp Ser Lys (h i i I)	1.00	0.98	B_{α_3}
227 228 229 230 231 232 Asp Val Lys Ile Glu Val (I h I I H h)	1.14	1.12	$\alpha 3$[e]
233 234 235 236 237 Tyr Leu Thr Thr Lys [h h h h b]	0.93	1.13	$\beta 12$

[a] α = α helix, β = β sheet, and B = tetrapeptide breaker for the indicated sequence of amino acids. The particular α helix or β sheet terminated by a breaker is indicated by the subscripts to B. ⟨P_α⟩ or ⟨P_β⟩ = the average conformational parameter of a helix or sheet for each predicted helix, sheet, or tetrapeptide breaker = $\Sigma(P_\alpha)_j$ or $\Sigma(P_\beta)_j / \Sigma n_j$, where $(P_\alpha)_j$ *or* $(P_\beta)_j$ is the α or β conformational parameter for each amino acid residue j and Σn_j the total number of residues in the helix, sheet, or tetrapeptide breaker. Values for P_α and P_β were obtained from Table II of Chou and Fasman (1974a). The assignment of amino acids (based on Table I of Chou and Fasman (1974b)) as H (strong helix or β former), h (helix or β former), I (weak helix or β former), i (indifferent), or b (helix or β breaker) is enclosed in parentheses for predicted helices or tetrapeptide breakers for predicted helices; amino acid assignments for predicted sheets or tetrapeptide breakers for sheets are enclosed in brackets. α helices and β sheets are numbered (column 4) from the N-terminal amino acid residue of SEB. Amino acid sequences in the SEB molecule are as published by Huang and Bergdoll (1970). [b] The following amino acid sequences of SEB demonstrated helical potential, but because of C-terminal Asp or Glu the helices were not allowed: 17–22 (⟨P_α⟩ = 1.09), 133–138 (⟨P_α⟩ = 1.09), 143–148 (⟨P_α⟩ = 1.03), 188–193 (⟨P_α⟩ = 1.04). Also, ⟨P_α⟩ for region 172–177 is 1.12, but an α helix at these residues would place two positively charged lysines at the N-terminal helical end (strongly opposite to the usual charge directionality of a helix). [c] To avoid an internal Glu-22, β_1 was terminated at Met-21 and β_2 at Met-24. β_1 and β_2 are separated by strong β-breaker Glu-22 and β-breaker Asn-23. [d] Incorporation of β_4 and β_5 into a single β sheet would be unlikely due to the GlyAlaAsn (=11b) tripeptide at 86–88 with ⟨P_β⟩ = 0.81. [e] The value of ⟨P_α⟩ for region 155–164 is only slightly greater than the value of ⟨P_β⟩, but the presence of Glu at position 158 would indicate no β sheet, since Glu rarely occurs in a β sheet. Likewise, even though ⟨P_β⟩ for region 230–236 is 1.20, a β sheet involving these residues would contain an unfavorable internal Glu.

An intense negative band centered at 281 nm is the most prominent characteristic of the near-ultraviolet CD spectrum of native SEB (Figure 1B). Optical activity of native proteins in the near-ultraviolet is generated by the inherent asymmetry of cystine and asymmetries imposed upon tyrosine, phenylalanine, tryptophan, and cystine residues by tertiary structures (Beychok, 1966). Un-ionized tyrosyl residues of globular proteins ordinarily contribute near-ultraviolet CD bands centered near 274–280 nm; consequently, the 21 tyrosine residues of SEB are likely responsible for the near-ultraviolet negative extremum of native enterotoxin. The single disulfide bond of SEB could make a contribution of 25 deg cm^2 $dmol^{-1}$ at around 270 nm (plus or minus, depending on chirality of the disulfide) (Horwitz et al., 1970; Sears and Beychok, 1973) and perhaps contributes to the shoulder at 272–273 nm in the native spectrum (Figure 1B). Neither a discrete CD band nor shoulder was resolved at 290–292 nm for the single tryptophan of SEB. Although the 12 phenylalanine residues would contribute very little to the near-ultraviolet spectrum of native SEB above 270 nm, the weak maximum centered at 252–253 nm is possibly due to phenylalanine. The near-ultraviolet CD spectrum of SEB was markedly altered by denaturing solvents. As shown in Figure 1B, helix-promoting solvents (acidified KCl solution and aqueous sodium dodecyl sulfate) induced a significant decrease in the near-ultraviolet negative molar ellipticity of SEB. Thus, an increase in the helical content of SEB was accompanied by a change of local conformation around the chromophoric residues. Enterotoxin extensively unfolded in concentrated Gdn·HCl solution (Dalidowicz et al., 1966; Warren et al., 1974a) demonstrated near-zero values for the Moffitt–Yang parameter b_0 and an increase in the magnitude of a_0 from −80 to values of −500 and −530 for 4.1 and 6.0 M Gdn·HCl, respectively. Although strong absorbance by Gdn·HCl in the far-ultraviolet precluded precise measurement, the CD spectrum from 220 to 240 nm decreased in magnitude as expected for unfolding. A pronounced shift in the near-ultraviolet CD spectrum resulted from exposure of SEB to Gdn·HCl; the sharp negative extremum with $[\theta]_{281} = -165$ observed for native protein was replaced for SEB in 4.1 M or 6.0 M Gdn·HCl by a broad negative band centered at 278–282 nm with a molar ellipticity of −45 to −55 (Figures 1C and 1D). Ellipticity from the single disulfide bond of SEB would not yield a curve with an extremum around 280 nm, but would instead yield a curve decreasing monotonically in magnitude as wavelength increased from 270 to 300 nm (Horwitz et al., 1970). Thus, despite the considerable loss of native structure in concentrated Gdn·HCl, the negative CD bands observed in the spectra of Figures 1C and 1D indicate that residual folded SEB might be present in concentrated Gdn·HCl solution. Alternatively, the near-ultraviolet CD bands observed for SEB in concentrated Gdn·HCl could also be associated with the rotatory strength of the many tyrosine residues in a completely unfolded SEB molecule; indeed, this interpretation is compatible with our earlier conclusion based on intrinsic viscosity values that SEB in 6.0 M Gdn·HCl is completely devoid of noncovalent structure (Warren et al., 1974a). Reversible unfolding of SEB in Gdn·HCl (Warren et al., 1974a) was confirmed by observed native values for the near-ultraviolet $[\theta]$ and Moffitt–Yang parameters a_0 and b_0 after removal of 4.1 M Gdn·HCl by dialysis. The slow kinetics of SEB unfolding in aqueous Gdn·HCl (Warren et al., 1974a) was also confirmed in the present study. Thus, unfolding of SEB as followed by change in $[\theta]$ from 240 to 300 nm did not reach equilibrium for 2 h in 6.0 M Gdn·HCl, for 2–3 days in 4.1 M Gdn·HCl.

TABLE II. Regions of β Turn in the SEB Molecule as Predicted by Chou–Fasman Protein Conformational Parameters.[a]

Sequence	Tetrapeptide	$P_t \times 10^4$	$\langle P_t \rangle$[b]
5–8	Asp Pro Lys Pro	1.55 3.49	1.25
12–15	His Lys Ser Ser	0.66 3.48	1.17
28–31	Tyr Asn Asn Asp	1.49 2.07	1.39
58–61	Leu Gly Asn Tyr	0.75 1.11	1.17
75–78	Asp Lys Tyr Lys	0.88 2.57	1.08
86–89	Gly Ala Asn Tyr	1.12 3.09	1.23
97–100	Lys Thr Asn Asn	0.78 1.57	1.29
106–109	Asn Thr Lys Arg	0.64 1.92	1.14
120–123	His Gly Asn Asn	1.05 4.14	1.36
140–143	Gly Lys Asn Leu	0.92 2.43	1.14
149–152	Gln Thr Asn Lys	0.73 2.50	1.13
180–183	Ser Pro Tyr Glu	0.63 2.32	1.15
192–195	Asn Glu Asn Ser	0.73 2.74	1.32
203–206	Ala Pro Gly Asn	1.37 4.07	1.30
209–212	Asp Gln Ser Lys	0.85 2.96	1.18
217–220	Tyr Asn Asn Asp	1.49 2.07	1.39

[a] P_t = the probability of β turn occurrence at the indicated sequence of amino acids = $(f_1)(f_2)(f_3)(f_4)$ = the product of the frequencies of occurrence of amino acid residues at the 1st, 2nd, 3rd, and 4th position in β turns of proteins. The first column of values for P_t of each sequence was calculated using the β turn frequencies of the 20 amino acids in 12 globular proteins reported in Table VII of Chou and Fasman (1974b); the second column of values for P_t was calculated using the β turn frequencies in 17 non-heme proteins reported in Table I of Chou, Adler, and Fasman (1975). [b] $\langle P_t \rangle$ = the average β turn conformational parameter for each tetrapeptide = $\Sigma(P_t)_i/4$, where $(P_t)_i$ is the β turn conformational parameter for each amino acid residue i in the tetrapeptide. Values for P_t are based on x-ray crystallography data of 17 non-heme proteins and were obtained from Table I of Chou, Adler, and Fasman (1975).

Discussion

A comprehensive technique for the prediction of secondary structure in globular proteins from a consideration of amino acid sequence has recently been formulated by Chou and Fasman (1974a,b). Conformational parameters P_α and P_β were determined for each of the 20 amino acids by the expressions $f_\alpha/\langle f_\alpha \rangle$ and $f_\beta/\langle f_\beta \rangle$, in which f_α or f_β represents the frequency as determined by x-ray crystallography of each amino acid in helical or β regions of 15 globular proteins and $\langle f_\alpha \rangle$ and $\langle f_\beta \rangle$ the average frequency of all residues in helices and β regions (Chou and Fasman, 1974a). When four helix formers out of six contiguous residues or three β-sheet formers out of five contiguous residues are detected in a primary sequence of amino acids, nucleation of a helix or β sheet is predicted which continues in both directions until terminated by a tetrapeptide with 50% or more breakers or indifferent residues (Chou and Fasman, 1974b). The primary structure of SEB has been published (Huang and Bergdoll, 1970) and we have applied Chou–Fasman analysis to the SEB sequence to predict specific regions of secondary structure. As shown in Table I, SEB is predicted to contain 12 strands of β structure and 3 α helices. The secondary structures defined for SEB by Chou–Fasman analysis demonstrate 82 amino acid residues in β structures and 27 residues in α helices; since SEB contains 239 amino acid residues (Huang and Bergdoll, 1970), this is equivalent to 11% α helix, 34% β structure, and 55% coil in the native SEB molecule. The amount of α-, β-, and coil conformation calculated for native SEB by Chou–Fasman analysis is in excellent agreement with the experimental values (9% α, 38% β, and 53% coil conformation) (Figure 1A) determined from the far-ultraviolet CD spectrum of SEB in 0.15 M KCl (pH 5.5) by the Chen–Yang–Chau method (1974). The importance of β bends in "directing" regular structures of proteins

into native orientation has recently been recognized (Lewis et al., 1971). Although diagnostic ORD or CD parameters have not been developed for β bends, β bends can be located in native proteins from x-ray crystallography models (Lewis et al., 1971). Using the x-ray crystallography structure of 12 different proteins, Chou and Fasman (1974b) have calculated the frequency of occurrence in β turns of the 20 amino acids; the Chou–Fasman values for the β-turn frequency of amino acids have been applied to the SEB sequence to define where 180° chain reversals most probably occur (Table II). Sixteen β bends have a high probability of occurrence in the SEB molecule. Utilizing the revised set of β-turn frequencies of amino acids based on the x-ray crystallography structure of 17 nonheme proteins (Chou et al., 1975), 15 of the 16 tetrapeptides shown in Table II retained a high probability of being in a β bend (Table II). Even though the probability of β-turn 58–61 was less than the proposed cut-off value of 1.6×10^{-4}, it still exceeded the average probability of 0.85×10^{-4} for tetrapeptides to be in the β turns of the 17 nonheme proteins (Chou et al., 1975). Also, values of $\langle P_t \rangle$ for each of the 16 predicted β turns calculated from amino acid values of P_t based on x-ray crystallography data of the 17 nonheme proteins (Chou et al., 1975) were significantly larger than unity (Table II). Therefore, no serious discrepancies were observed for locations of 180° reversals of chain direction in SEB as predicted by β-turn frequencies of amino acids in 12 (Chou and Fasman, 1974b) or 17 (Chou et al., 1975) native globular proteins. The most important feature of the predicted β bends in SEB is their close proximity to predicted β sheets and α helices. Seven of the β turns bring five strands of β sheet into an antiparallel configuration from residues 81 to 148 and constrain the α helices at residues 64–68 and 155–164 in close proximity to hydrophobic β sheets at 44–53 and 143–148, respectively. The three strands of β sheet between residues 184 and 217 are likewise directed by interposed β bends into an antiparallel pleated-sheet configuration. These two large regions of antiparallel β sheet are indicated in Table I by vertical rules. A notable feature of the antiparallel β-sheet region predicted for residues 81–148 is the presence of a disulfide bond between half-cystines 92 and 112, which forms a covalent cross-link between the β sheets at residues 89–94 and 111–116 (designated β5 and β6, respectively, in Table I). We have previously observed that reduction and alkylation in 8 M urea of this disulfide followed by removal of urea by dialysis resulted in refolding of SEB to a compact form lacking the isothermal kinetic stability of untreated native SEB (Warren et al., 1974b). It is suggested, therefore, that folding of SEB with an interior core of repeating antiparallel β sheet around an intact 92–112 disulfide bond is critical for the kinetic stability of native SEB. The extensive antiparallel β sheet from residues 184–217 would also undoubtedly contribute to such an interior core of structure.

Finally, some implications of the large amount of β structure for biological activity of SEB as an enterotoxin and a lymphocyte mitogen will be briefly reviewed. Gastric acid pH serves as an effective barrier for the entry of many infectious microorganisms and toxic microbial products into the small intestine (Gionella et al., 1973). However, the enterotoxic action of SEB is neither neutralized nor activated by in vivo transit through gastric acid (Bergdoll, 1970) and thus the enterotoxic site(s) must be relatively acid stable. Recent work with isolated peptide fragments of SEB suggests a structurally complex enterotoxic site(s) which requires the cooperative interaction of the 1–97 and 98–239 peptide chains (Spero et al., 1975). Since our in vitro data indicate that β structure is largely conserved at acid pH, it is a good possibility that discrete areas in regions of β structure (β sheets and/or β turns) contain the enterotoxic site(s) of the SEB molecule. In addition, SEB is a potent in vitro lymphocyte mitogen (Peavy et al., 1970) and evidence has been obtained that SEB resembles mitogenic plant lectins in that interaction with specific cell receptors is required for expression of transforming activity (Powell and Leon, 1970; Novogrodsky and Katchalski, 1971; Sharon and Lis, 1972; Allan and Crumpton, 1973; Warren et al., 1975). The x-ray crystallography data and/or CD spectra reported to date indicate a predominance of β structure for mitogenic and/or cytoagglutinating lectins (Pflumm et al., 1971; Edelman et al., 1972; Pére et al., 1975; Shimazaki et al., 1975). The important possibility arises that the unusually high content of β structure in both SEB and the plant lectins facilitates attachment of the proteins to cell surface receptors. An additional feature of lectin structure recently identified is the alteration of lectin conformation which occurs upon the binding of haptenic monosaccharides (Pflumm et al., 1971; Pére et al., 1975; Shimazaki et al., 1975). Efforts so far to identify a specific monosaccharide which is inhibitory for the mitogenicity of SEB have been unsuccessful (Warren et al., 1975). However, the behavior of SEB in the presence of detergent or dilute acid can be regarded as a model for interaction of the protein with hydrophobic or charged regions of membrane receptors. The increase in α helix content observed in detergent or acid solution by CD measurements perhaps also occurs when SEB is complexed to lymphocyte receptors. Several α helices were not allowed during calculation of secondary structures in SEB because of unfavorably placed, charged amino acid side chains (footnote *b*, Table I). Shielding of these critically placed amino acid side chains by charged or hydrophobic regions of receptor sites could favor formation of helices and thereby induce mitogenic conformations. The far-ultraviolet CD spectrum of the mitogenic lectin concanavalin A in aqueous solution demonstrates a similar intensification at alkaline pH (Pflumm and Beychok, 1974), suggesting that specific titratable amino acid groups also play an important role in secondary structures assumed by this protein mitogen.

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