Effect of Sodium Dodecyl Sulfate and Its Homologs on Circular Dichroism of α-Chymotrypsin†

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ABSTRACT: The effect of sodium dodecyl sulfate on the solubility, circular dichroism spectra, and active-site retention of α-chymotrypsin at two pH levels has been examined. At pH 4.6 an insoluble detergent–protein complex is formed which dissolves at detergent-to-protein molar ratios of 65 or greater; at pH 5.6 no precipitation occurs. At pH 4.6 the far-ultraviolet circular dichroism spectra indicate the presence of two protein conformations in the detergent solutions which are distinct from each other and from the native conformation; at pH 5.6 only one of these conformations is indicated. In the presence of saturating amounts of dodecyl sulfate at both pH's the protein adopts a conformation of a higher helix content than the native protein. Lower detergent concentrations at pH 4.6 but not at pH 5.6 produce a protein far-uv circular dichroism spectrum suggestive of predominantly β structure. The conformations are interpreted in terms of two levels of nonspecific detergent–protein interactions. The near-uv spectra of the complexes indicate exposure of the tyrosine and tryptophan residues of the protein to the aqueous environment at high detergent concentrations. Near-uv spectra arising primarily from disulfide transitions are produced when detergent is partially removed from the saturated complex by dialysis; the far-uv spectrum indicates that the polypeptide backbone is enriched in β form as dodecyl sulfate is removed. Sodium octyl and decyl sulfates are inactive in producing chymotrypsin circular dichroism changes at low concentrations (1100 molecules of decyl sulfate/molecule of chymotrypsin produced approximately the same conformational change as 67 molecules of dodecyl sulfate). Sodium dodecyl and tetradecyl sulfates at molar ratios of $\lesssim 30$ both cause enhancement of tryptophan near-uv ellipticity; this effect is attributed to minor changes in the environments of the buried residues. At pH 5.6 the chymotrypsin active site is too disrupted to react with *N-trans*-cinnamoylimidazole when the molar ratio of dodecyl sulfate to protein is $\gtrsim 50$.

Knowledge of the conformation of chymotrypsin in solution under various conditions has been the target of many investigations, with frequent attempts to relate changes in conformation to variations in enzymic activity or to various crystallographic features of the enzyme or the zymogen. One of the most popular tools in these conformational studies has been circular dichroism (CD) spectroscopy (Fasman *et al.*, 1966; McConn *et al.*, 1969; Volini and Tobias, 1969; Hess *et al.*, 1970; Cuppett *et al.*, 1971; Morrisett and Broomfield, 1971). Visser and Blout (1971) have described pronounced changes in the far-ultraviolet CD spectrum of chymotrypsin and other proteases in the presence of high concentrations of sodium dodecyl sulfate; chymotrypsin, elastase, trypsin, and pepsin were all reported to be inactivated by the treatment with sodium dodecyl sulfate. Gaudin and Viswanatha (1972) have examined the effect of sodium dodecyl sulfate on the catalytic behavior of chymotrypsin; they report that (1) total loss of activity after a 10-min incubation time required detergent-to-protein ratios as high as 15 by weight, and (2) addition of the enzyme directly to a substrate–detergent mixture resulted in enhanced activity at high detergent concentrations (10^{-2} M or higher). However, equilibrium dialysis measurements (Pitt-Rivers and Impiombato, 1968; Reynolds and Tanford, 1970a) have indicated that the maximum ratio of detergent bound to protein in a sodium dodecyl sulfate–protein complex is 1.4 (w/w). In addition, Rossi and Bernhard (1971) have reported that in 2×10^{-2} M sodium dodecyl sulfate the slowest rate of denaturation of α-chymotrypsin between pH's 4 and 10 was of the order of 1 min^{-1}.

In view of the apparent inconsistencies described in the preceding paragraph, it was of interest to examine the effects of various levels of sodium dodecyl sulfate on the circular dichroism spectrum of chymotrypsin and on the integrity of its active site. The results of such studies at two pH levels are reported here. Also, the effects of three dodecyl sulfate homologs on the CD spectrum of the enzyme are described. This work is an extension of earlier studies from this laboratory on the effects of amphiphilic substances, chiefly sodium dodecyl sulfate, on several other proteins as tested by optical rotatory dispersion and circular dichroism (*e.g.*, Jirgensons, 1961, 1966; Jirgensons and Capetillo, 1970; Lee and Jirgensons, 1971; Nakagawa and Jirgensons, 1973).

Experimental Section

Materials. Highly purified sodium octyl, decyl, dodecyl, and tetradecyl sulfates were purchased from Mann Research Laboratories. Recrystallization of the dodecyl sulfate salt from boiling ethanol produced no observable difference in the interactions of the detergent with protein when compared with the unrecrystallized material. The octyl, decyl, and tetradecyl sulfates (and in most cases the dodecyl sulfate) were used as received. Bovine α-chymotrypsin (three-times crystallized) and bovine chymotrypsinogen (five-times crystallized) were obtained from Worthington Biochemical Corp.; bovine albumin was supplied by Pentex Inc.

Procedures. Circular dichroism was measured on a modified Durrum-Jasco recorder and spectrophotometer, Model CD-SP. The circular dichroic intensity was calibrated using *d*-10-camphorsulfonic acid (DeTar, 1969). All spectra were recorded at a speed of 5 nm/min, using a pen period of 5 sec. Cell thicknesses were 0.5 or 0.1 mm for wavelengths shorter

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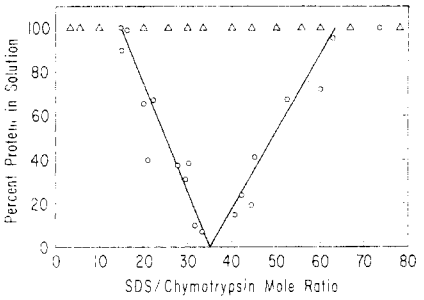

FIGURE 1: Relative solubility of α-chymotrypsin in sodium dodecyl sulfate solutions at pH 4.6 (○) and 5.6 (△). Initial protein concentration $\sim 2 \times 10^{-5}$ M; percentage protein remaining unprecipitated at pH 4.6 estimated from A_{280} of the supernatant liquids. Total detergent concentrations in the mixtures are expressed as the molar ratios [sodium dodecyl sulfate]/[chymotrypsin]; temperature = 25°.

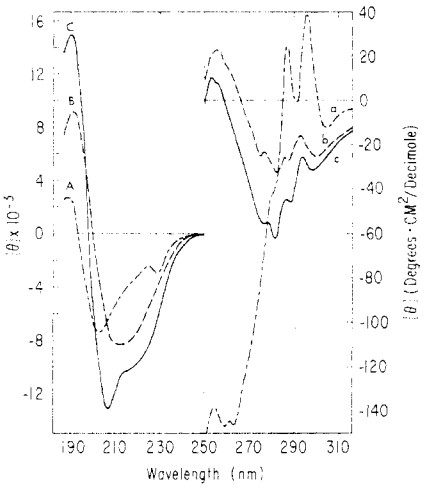

FIGURE 2: Circular dichroism spectra of chymotrypsin at pH 4.6. Protein concentrations = 2×10^{-5} M (near uv) and 1×10^{-6} to 3×10^{-6} M (far uv). Detergent ratios = zero (A,a); 74 (B,b); 1800 (C,c). Recording of spectrum (C,c) was started 2 hr after mixing; (A,a) and (B,b) were recorded 1 day after mixing the solutions.

than 250 nm; between 320- and 250-nm cells of 1- or 2-cm path length were used. Temperatures in the spectrophotometer sample compartment ranged from 25 to 30°. Mean residue ellipticities were calculated using the mean residue mol wt 104 for chymotrypsin, 105 for chymotrypsinogen, and 110 for albumin.

Protein concentration determinations, activity measurements, and detergent analyses were carried out on a Beckman DU spectrophotometer equipped with a Gilford Model 222B photometer and a Gilford Model 6040 recorder. Wavelength settings were calibrated using neodymium and holmium oxide glasses. Protein concentrations were calculated using molar absorbancy values of 5.0×10^4 at 280 nm for chymotrypsin (Dixon and Neurath, 1957) and 5.02×10^4 at 282 nm for chymotrypsinogen (Wetlaufer, 1962). For bovine albumin the molar concentration was calculated using $E_{1\%}^{1\,cm} = 6.67$ at 278 nm (Reynolds *et al.*, 1967) and a molecular weight of 65,000 (Steinhardt *et al.*, 1972). Operational normalities of chymotrypsin solutions were determined by titration of the active site with *N-trans*-cinnamoylimidazole (Schonbaum *et al.*, 1961). A purity of about 90% was indicated by this method for the enzyme stock solutions. Dodecyl sulfate concentrations were determined in some instances by extracting a detergent–Methylene Blue complex from aqueous solution (Mukerjee, 1956; Reynolds and Tanford, 1970a) with *o*-dichlorobenzene (Burkhard and Stolzenberg, 1972) and measuring the absorbance in the organic layer at 645 nm. A new calibration curve was prepared from standard detergent solutions for each series of measurements. Quantitative extractions of sodium dodecyl sulfate in the presence of protein were obtained using 20 ml of o-$C_6H_4Cl_2$ and 5.0–5.5 ml of the aqueous layer (dye solution plus sample solution).

All solutions were prepared in pH 5.6 phosphate solution, $\mu = 0.033$. Solutions labeled as pH 4.6 in Figures 1 (some) and 2 (all) contained 0.01 M acetate buffer from the protein stock solution; buffering action of the acetate was sufficient to lower the pH to 4.6. Adjustment of some non-acetate-containing solutions to pH 4.6 with phosphoric acid showed the differences observed in Figures 1–3 to be due to pH and not solvent composition. All subsequent mixtures contained only protein, detergent, and phosphate.

Protein concentrations of 1×10^{-5} to 2×10^{-5} M were used in most cases, except that concentrations as high as 5×10^{-5} M were prepared for titration with *N-trans*-cinnamoylimidazole. Detergent concentrations are expressed as the ratio of the total detergent molarity in the mixture (based on stock solutions prepared by weight) to the molar concentration of enzyme. The resulting mole ratio reflects the overall composition of the mixture, not the number of detergent molecules bound. In mixtures from which chymotrypsin was partially precipitated the percentage remaining in solution was calculated from the absorbance at 280 nm of the supernatant liquid; no corrections for changes in absorbancy were applied.

Except where indicated in Figures 6 and 7, circular dichroism spectra are the equilibrium results obtained at times varying from 1 hr to 1 day after mixing of the solutions. For high ratios of sodium dodecyl or tetradecyl sulfate the conversion of the protein to the denatured state was too rapid to be followed by scanning through the near-uv spectrum. Equilibrium results are the average of three consecutive scans which showed no change as a function of time. The times given in Figures 6 and 7 are the elapsed intervals between mixing the solution and starting a near-uv scan at 320 nm. Elapsed times at any other wavelength can be calculated from the scan rate of 5 nm/min.

Dialysis was performed in Visking cellulose casings which had been boiled in phosphate buffer and in distilled water. Dialysis was at 25° against 4 l. of distilled water, stirred magnetically and changed at 8-hr intervals. Samples were removed from the dialysis bag periodically for measurement of the CD spectrum and for determination of the sodium dodecyl sulfate concentration by extraction with Methylene Blue.

Results

Comparison of Protein–Detergent Interactions at Two pH's. Chymotrypsin–sodium dodecyl sulfate mixtures were prepared at two pH's, 4.6 and 5.6. The protein–detergent complexes were soluble at pH 5.6 for all ratios of sodium dodecyl sulfate to enzyme studied. At pH 4.6, however, an insoluble complex was formed as the detergent concentration increased; for 2×10^{-5} M chymotrypsin the minimum solubility was at $[\text{sodium dodecyl sulfate}]_{total} \cong 7 \times 10^{-4}$, or a molar ratio of about 35, as illustrated in Figure 1. The precipitated complex redissolved when the detergent concentration was raised further, reaching complete solubility at a mole ratio of about 65.

The difference of interactions of detergent with protein at the two pH's which is suggested by the solubility behavior was confirmed by an examination of the CD spectra of several

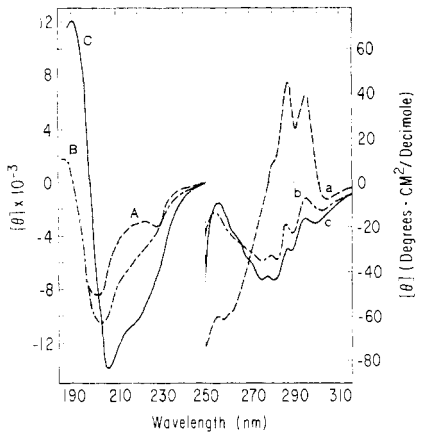

FIGURE 3: Circular dichroism spectra of chymotrypsin in sodium dodecyl sulfate solutions at pH 5.6. Detergent ratios = 24 (A,a); 67 (B,b); 215 (C,c). Protein concentration ranges and intervals between solution preparation and recording of the spectra were the same as in Figure 2.

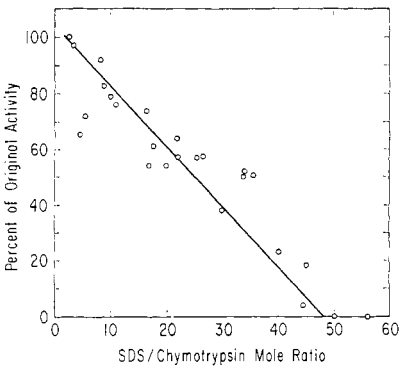

FIGURE 4: Operational normality of α-chymotrypsin in sodium dodecyl sulfate solutions at pH 5.6. Protein concentrations varied from 2×10^{-5} to 5×10^{-5} M. Normalities determined by titration with *N-trans*-cinnamoylimidazole are expressed as percentages of the activity of a solution the same concentration of protein but no detergent.

solutions; such spectra are shown in Figures 2 and 3. The far-uv curves in Figure 2 are representative of three distinct types of chymotrypsin spectra observed for the pH 4.6 solutions. (A) In the presence of no sodium dodecyl sulfate (or amounts insufficient to cause precipitation) the protein's CD is characterized by negative peaks at 229 and 202 nm. (B) In solutions containing the "redissolved" detergent–protein complex (mole ratios $\gtrsim$ 70) the CD spectrum below 250 nm contains a single negative peak at ~213 nm and a positive peak at 191 nm. (C) At higher detergent concentrations a negative shoulder appears at about 218 nm, the negative peak shifts to 207 nm, and the positive peak moves to 190 nm. The relative magnitudes of these peaks are indicated in Figure 2.

The near-uv spectra of the two detergent–protein complexes (b and c in Figure 2) are similar to each other and quite different from the spectrum of the native enzyme (a in Figure 2), which contains positive maxima at 296 and 287 nm and negative peaks at 305 nm and near 260 nm. The positive portion of the native spectrum is obliterated by increasing negative ellipticity near 280 nm as the detergent concentration is increased. A positive ellipticity contribution at 254 nm is present in the pH 4.6 detergent solutions, being more prominent in mixtures closer to the precipitation ratios.

The CD spectrum of chymotrypsin at pH 5.6 in the absence of detergent is virtually identical with that at the lower pH (A and a, Figure 2). However, at pH 5.6 in the presence of sodium dodecyl sulfate, far-uv spectra similar to curve B in Figure 2 are not observed. Instead, as the detergent concentration is increased there is a gradual transition (A, B, and C in Figure 3) from native-like spectra to curves similar to those observed for the high-detergent solutions at pH 4.6. The protein spectra in the near-uv region are qualitatively similar to those of Figure 2; two points are to be noted, however. (1) The addition of dodecyl sulfate to chymotrypsin at moderately low mole ratios produces more positive ellipticities at 296, 287, and 255 nm than are observed in the native protein (compare the a curves in Figures 2 and 3). (2) The $[\theta]_{255}$ becomes less negative as the detergent concentration is increased, but it does not become positive in sign even at high detergent ratios.

The changes in protein conformation indicated by the circular dichroism spectra of the detergent-containing solutions were also reflected by decreases in activity. The normality of chymotrypsin solutions at pH 4.6, as indicated by titration of the active site of the enzyme with *N-trans*-cinnamoylimidazole, decreased rapidly as the concentration of dodecyl sulfate was increased. At a ratio of 20 detergent molecules/1 chymotrypsin molecule in the overall mixture, less than 10% of the original activity remained in solution, even though only about one-fourth of the protein had been precipitated. However, the precipitated protein is at least partially active; precipitate collected at pH 4.6 could be redissolved at pH 6 and titrated, showing an activity between 30 and 40%. (The CD spectrum of such material was still recognizable as chymotrypsin, although altered from that of the native enzyme.) At pH 5.6 the lack of precipitation of the complex permitted titration of all the mixtures as the detergent concentration was increased. Results of such titrations are shown in Figure 4. The decrease in activity is proportional to the detergent excess, with all active sites being disrupted at this pH with a sodium dodecyl sulfate molar excess of as few as 50 detergent molecules/enzyme molecule (0.58 g of sodium dodecyl sulfate/g of protein).

Attempts to Remove the Detergent by Dialysis. The far-uv curves in Figure 2 are quite similar to those reported by Visser and Blout (1971). In particular, the similarity is notable between curve B of Figure 2 (chymotrypsin in the presence of a 74-fold molar excess of sodium dodecyl sulfate) and the spectrum obtained by the previous authors after dialyzing a chymotrypsin–detergent mixture for 24 hr to remove sodium dodecyl

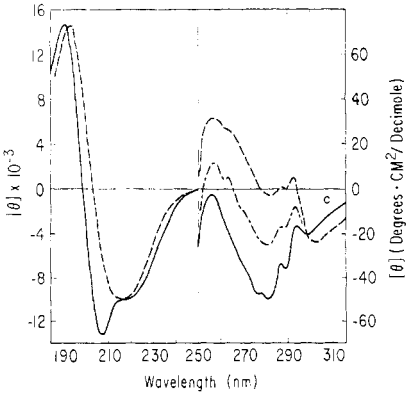

FIGURE 5: Circular dichroism spectra of a chymotrypsin–sodium dodecyl sulfate mixture at various stages of dialysis: (—) before dialysis; (— — —) after 27 hr; (— —) after 74 hr; chymotrypsin concentration $\simeq 2 \times 10^{-5}$ M.

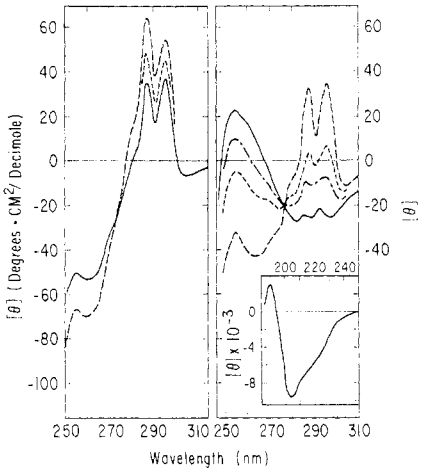

FIGURE 6 (left): Near-uv circular dichroism spectra of 1.1×10^{-5} M chymotrypsin in sodium tetradecyl sulfate, molar ratio = 22; pH 5.7. Spectra were recorded starting at 320 nm at various intervals after mixing of the solution: (— —) 1 min; (- - - -) 36 min; (——) 15 hr.

FIGURE 7 (right): Near-uv circular dichroism spectra of 1.4×10^{-5} M chymotrypsin in sodium decyl sulfate, molar ratio = 1100; pH 5.8. Spectra were recorded starting as 320 nm at various intervals after mixing of the solution: (— —) 1 min; (- - - -) 17 min; (— - —) 34 min; (——) 20 hr. Inset: far-uv spectrum after 21 hr.

sulfate. Similar experiments in this laboratory have shown that the detergent is released from the protein only very slowly, not being fully removed even after 6-days dialysis. Circular dichroism spectra obtained in one such experiment are shown in Figure 5, and detergent analysis results for this and two other trials are given in Table I. The protein's CD spectra in both the near- and far-uv regions after dialysis for 96 hr were identical with the 74-hr results shown in Figure 5; the far-uv spectrum remained unchanged after 141 hr. However, the protein solution became slightly turbid on the 6th day of dialysis; the magnitude of the ellipticity near 255 nm was reduced by about 50% but other features of the near-uv spectrum showed little change from the 74-hr results. The initial pH of the dialysis mixture whose spectra are shown in Figure 5 was 5.4; after 27-hr dialysis the pH was 4.6, at which level it remained.

Circular Dichroic Changes as a Function of Time and of Detergent Chain Length. The more positive near-uv ellipticity

TABLE I: Detergent Remaining in Sodium Dodecyl Sulfate–Chymotrypsin Mixtures during Dialysis.

Dialysis (hr)	[Sodium Dodecyl Sulfate] $\times 10^4$	Detergent/Protein
0	118.7	670
18	15.1	88
27	10.9	60
43	6.5	36
74	4.5	25
96	3.6	20
141	3.0	~16
0	480	2200
24	50.2	294
75	4.2	35
143	1.9	~16
~140	2.6	18

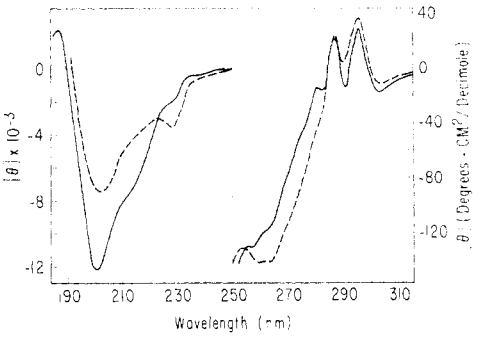

FIGURE 8: Circular dichroism spectra of chymotrypsin (— —) and chymotrypsinogen (——); pH 5.7; protein concentration = 1.4×10^{-5} M.

for detergent–protein complexes (for example, curve a of Figure 3) than for the native enzyme when the mole ratios are low is a time-dependent effect. The peak enhancement at wavelengths greater than 280 nm is even greater initially than when the solutions have reached equilibrium. An opposite trend occurs in the region near 260 nm; $[\theta]$ in this range continues to become less negative until equilibrium is attained. This effect on the chymotrypsin CD spectrum is produced both by sodium dodecyl sulfate and its homolog sodium tetradecyl sulfate when present in small excess; the shorter chained detergents octyl sulfate and decyl sulfate are ineffective at low concentrations in producing any changes. The decyl sulfate and octyl sulfate ions are able to cause conformational transitions of the chymotrypsin, but much higher detergent-to-protein ratios are necessary. Examples of the changes brought about by tetradecyl sulfate and decyl sulfate are shown in Figures 6 and 7, respectively. Initial enhancement of the sort illustrated in Figure 6 was greatest in dodecyl sulfate solutions at mole ratios of ~30; in tetradecyl sulfate solutions the $[\theta]_{287}$ was initially as high as 70 (deg cm²)/dmol for a solution containing [detergent]/[enzyme] = 11 at pH 5.7. (The relative magnitudes of the 296- and 287-nm peaks also were reversed for native chymotrypsin in 1.5 M NaCl, without significant change in either the 260-nm ellipticity or the far-uv region.) Increasing amounts of tetradecyl sulfate caused changes analogous to those associated with dodecyl sulfate solutions in the previous figures; a molar excess of as low as 56 tetradecyl sulfate ions/chymotrypsin at pH 5.9 was sufficient to produce spectra similar to curve C of Figure 3. In contrast, the far-uv spectrum in the insert of Figure 7 indicates that a ratio of 1100 decyl sulfates/chymotrypsin at pH 5.8 caused roughly the same conformational change as did 67 sodium dodecyl sulfate molecules at pH 5.6 (curve B of Figure 3). Positive ellipticities near 255 nm were observed for both decyl and octyl sulfate containing solutions at high detergent concentrations, but the only such observations for tetradecyl sulfate were for mixtures found to contain small amounts of precipitate.

Circular Dichroism of Chymotrypsinogen and Albumin. The near- and far-uv CD spectra of chymotrypsinogen at pH 5.7 are compared with those of chymotrypsin in Figure 8. The chief difference in the near-uv regions is the decreased ellipticity in the 260-nm band for the zymogen and the appearance of a small peak at 281 nm. At higher wavelengths the peak positions for the two proteins are identical (305, 296, 291, and 287 nm). The far-uv spectrum of bovine albumin was recorded for the protein in buffer alone (pH 5.7) and in two different concentrations of dodecyl sulfate, [sodium dodecyl sulfate]$_{monomer}$ = 7×10^{-4} or 3×10^{-3}. The per cent

α helix for the protein under these conditions was approximated from the 208-nm ellipticity using method I of Greenfield and Fasman (1969): without sodium dodecyl sulfate, $[\theta]_{208}$ = 22,000 and % α helix = 62; in 7×10^{-4} M sodium dodecyl sulfate monomers, $[\theta]$ = 20,000 and % helix = 55; in 3×10^{-3} M sodium dodecyl sulfate, $[\theta]$ = 19,000 and % helix = 52.

Discussion

Reynolds and Tanford (1970a) report the formation of complexes of identical stoichiometry when sodium dodecyl sulfate is bound to a wide variety of proteins without disulfide bonds. Two levels of binding were observed, having detergent-to-protein weight ratios of 0.4 and 1.4 g per g. For proteins with intact disulfide bonds the amount of detergent bound is somewhat less, since the unfolding of the molecules is restricted (Fish *et al.*, 1970). The maximum sodium dodecyl sulfate binding ratio for unreduced proteins is between 0.9 and 1.0 g per g (Pitt-Rivers and Impiombato, 1968), but there have been no reports of a complex analogous to the 0.4-g/g binding level for disulfide-containing proteins. The sodium dodecyl sulfate–chymotrypsin interactions illustrated in Figures 1 and 2 may provide an example of such complex formation, however. For a 2×10^{-5} M enzyme concentration, the free detergent concentrations necessary for the formation of the two binding levels (Reynolds and Tanford, 1970a) are provided by total sodium dodecyl sulfate to protein molar ratios between 60 and 75 for 0.4 g/g of bound detergent (35 mol/mol[1]) and by total molar ratios of 125 or greater for 1.0 g of sodium dodecyl sulfate bound per g of chymotrypsin (85 mol/mol). (The 0.4 and 1.0 g per g levels are chosen rather arbitrarily, but the exact values will not matter in the discussion that follows as long as these levels are approximately correct.)

Chymotrypsin forms an insoluble complex with sodium dodecyl sulfate at pH 4.6 without undergoing extensive changes in conformation. At higher detergent concentrations the saturated complex containing the lower level stoichiometry is formed, resulting in the dissolution of the precipitated material (Figure 1) and the extensive reorganization of the protein (curve B of Figure 2). Further increases in sodium dodecyl sulfate concentration lead to formation of the 1.0 g/g of saturated complex, accompanied by a second conformational transition to a form having a CD spectrum such as curve C of Figure 2. Changes in conformation upon formation of the sodium dodecyl sulfate complexes were also observed by Reynolds and Tanford (1970b) for several reduced proteins; they concluded, however, that the overall shapes of the complexes at the two saturation levels are similar and rodlike. In spite of similarities in the rodlike units at the two levels, different structures for the protein within the complexes may be possible. Curves B and C of Figure 2 suggest that such differences exist for chymotrypsin in sodium dodecyl sulfate solutions at pH 4.6; a recent investigation of sodium dodecyl sulfate interactions with bovine serum albumin has indicated different structures and differences in the mechanism of detergent binding at low and high ratios, with a transition in the range of g of sodium dodecyl sulfate/g of bovine serum albumin $\simeq$ 0.4 to 1.0 (Costello and Robertson, 1973). This transition does not significantly alter the far-uv circular dichroism of bovine serum albumin, however, unlike the result for chymotrypsin.

If the 0.4 g/g of sodium dodecyl sulfate–chymotrypsin complex contains the polypeptide chain in a conformation which is characterized by CD of the sort represented by curve B of Figure 2, then the failure to observe such spectra for enzyme–detergent solutions at pH 5.6 is difficult to understand. If the equilibrium constant for unfolding of the native protein to the 0.4-g/g state is strongly pH dependent, then the free-ligand concentration required to cause this transition at pH 5.6 might be so high that the protein goes directly into the transition to the maximum saturation level (Reynolds *et al.*, 1970). However, both saturation levels were observed for the reduced precursor, chymotrypsinogen, at pH 7.2 (Reynolds and Tanford, 1970a,b). It is possible that curve B of Figure 2 does not represent the 0.4 g/g of sodium dodecyl sulfate–saturated chymotrypsin CD spectrum, but is characteristic of some other complex which occurs at pH 4.6 but apparently not at 5.6.

CD Spectra of the Polypeptide Backbone. The spectrum of the detergent-free enzyme (curve A of Figure 2) has features described by Fasman *et al.* (1970) as characteristic of an aperiodic constrained polypeptide chain: a negative band between 200 and 205 nm and a negative shoulder between 215 and 230 nm. The negative band at 229 nm does not arise from a peptide transition but rather from a local chromophore in an environment sensitive to small conformational changes in the active-site region; Morrisett and Broomfield (1971) suggest that the 229-nm ellipticity results from tryptophan-141 perturbations occurring when that residue forms a complex with histidine-40. Such a specific interaction is not reflected in the tryptophan CD in the near-uv region; the tryptophan bands above 285 nm are more clearly resolved in chymotrypsinogen than in chymotrypsin, while the 220-nm band is observed only as a small shoulder in the zymogen (Figure 8; Strickland *et al.*, 1969; Hess *et al.*, 1970).

As chymotrypsin is exposed to increasing amounts of sodium dodecyl sulfate at pH 5.6 the backbone conformation is gradually altered enough to disrupt both the active site (zero activity by cinammoylimidazole titration, Figure 4) and the histidine-40–tryptophan-141 complex (disappearance of the 229-nm CD peak, as in curve B of Figure 3).

Curve C of Figure 3 is characteristic of chymotrypsin in high concentrations of sodium dodecyl sulfate (Visser and Blout, 1971). The band positions in curve C fit those observed for α-helical conformations (Holzwarth and Doty, 1965; Townend *et al.*, 1966; Greenfield and Fasman, 1969). Using method I of Greenfield and Fasman (1969) and the chymotrypsin ellipticity at 207 nm in curve C of Figure 3, the approximate α-helix content calculated for the protein in the detergent-saturated complex is 34%. A comparison of the C curves in Figures 2 and 3 indicates that the protein–detergent complexes at the maximum binding level at pH 4.6 and 5.6 have the same general structure and approximately the same helix content, as would be expected.

Curve B in Figure 2 resembles spectra of polypeptides in the β conformation (Townend *et al.*, 1966; Greenfield and Fasman, 1969), though the band positions (191 and 213 nm) do not exactly match those reported for β polypeptides (195 and 217 nm) or those computed for a β form (194 and 214 nm) based on protein spectra (Chen *et al.*, 1972). This could mean that the chymotrypsin–sodium dodecyl sulfate complex does not contain predominately β-like structure. The symmetry of the 213-nm band makes a significant α-helical fraction, with its strong negative contribution at ~206 nm, unlikely.

[1] Occurrence of the minimum chymotrypsin solubility at pH 4.6 in sodium dodecyl sulfate mixtures with detergent/protein ratios ~35 is probably merely fortuitous. Rough attempts to determine the mole ratio in the precipitated material gave ratios between 15 and 20.

CD Spectra of the Side-Chain Chromophores. The bands above 285 nm in both the native enzyme and its precursor arise from tryptophan (Figure 8; Strickland *et al.*, 1969). The broad band near the 260- and the 255-nm extremum probably have their origins in disulfide transitions (Beychok, 1965; Beychok and Breslow, 1968; Coleman and Blout, 1968; Takagi and Ito, 1972; Barnes *et al.*, 1972). The small amount of fine structure which is observed in some curves in the 260-nm band (curve a of Figure 2) is suggestive of a phenylalanyl contribution to the ellipticity, but the required 7-nm spacing for phenylalanine bands (Horwitz *et al.*, 1969; Strickland *et al.*, 1970) is either not present or not well resolved.

By comparing the spectra of a series of solutions with gradually increasing detergent ratios, or by following changes in the spectrum of one solution as a function of time (such as in Figure 7 for chymotrypsin in sodium decyl sulfate), it is apparent that the extrema at 299, 294, 289, and 287 nm in the spectrum of the saturated sodium dodecyl sulfate–chymotrypsin complex (c curves of Figures 2, 3, and 5) are the tryptophan bands which occur between 305 and 287 nm in curve a of Figure 2. The position of the longest wavelength bands in chymotrypsinogen (and thus the enzyme also) suggests that the optically active tryptophan residues are enclosed within the protein in partially polar regions, not extensively exposed to the solvent (Strickland *et al.*, 1969, 1971). The shift to shorter wavelengths in the presence of sodium dodecyl sulfate may reflect an increased but still incomplete exposure to the aqueous medium for chymotrypsin tryptophans.

The maximum negative ellipticity in the near-uv spectrum of sodium dodecyl sulfate–saturated chymotrypsin (c curves of Figures 2, 3, and 5) occurs in a new band at 281 nm with a shoulder near 275 nm; the structure and position of the peaks suggest that they result from tyrosine residues with hydroxyl groups fully exposed to the solvent (Horwitz *et al.*, 1970). The magnitude of the tyrosyl ellipticity varies with pH for these solutions, becoming more negative from pH 5.6 to 4.6; in the same spectra the 255-nm band attributed to disulfide transitions becomes more positive. The sensitivity of $[\theta]_{255}$ to the presence of detergent is not readily understood, but it seems probable that the conformational changes indicated by the far-uv CD spectra result in constraints on the four disulfides in chymotrypsin, with accompanying alterations to their optical properties (Coleman and Blout, 1968; Linderberg and Michl, 1970).

Dialysis of Chymotrypsin–Sodium Dodecyl Sulfate Complexes. The chymotrypsin far-uv CD spectra obtained at the end of the dialysis period (Figure 5) were in essential agreement with the previous results of Visser and Blout (1971). The near-uv spectra in Figure 5 reveal changes in the environments of the enzyme side chains as detergent is removed. The tryptophan bands (294–286 nm) become more distant and shift to slightly shorter wavelengths, indicating a small increase in the exposure of the indole rings to water (Strickland *et al.*, 1971). The tyrosine ellipticity remains centered at 281 nm as the dialysis proceeds but the magnitude of the peak decreases to near zero, suggesting increasing flexibility in the orientation of the exposed tyrosyl groups as the detergent concentration decreases. During this process $[\theta]_{255}$ becomes increasingly positive and a new negative peak appears at 302 nm (Figure 5). This is not thought to be a shifted tryptophan peak (although it may contain a tryptophan contribution) for two reasons: (1) the shift in position from 299 to 302 nm is in the opposite direction from that observed for the shorter wavelength tryptophan bands; (2) the peak near 300 nm is more negative after the dialysis, while the ellipticity of the bands between 294 and 286 nm becomes more positive. The new negative band probably arises from the chymotrypsin disulfides, as does $[\theta]_{255}$ (Breslow, 1970; Horwitz *et al.*, 1970; Breslow and Weis, 1972). The near-uv CD spectrum of the protein after three or more days of dialysis is thus predominantly a disulfide spectrum, with tryptophan fine structure superimposed on it in the 280- to 295-nm region and with indications of weak phenylalanine fine structure between 260 and 270 nm (Strickland *et al.*, 1970).

Partial removal of sodium dodecyl sulfate from the most saturated complex by dialysis converts the chymotrypsin to a conformation probably containing a significant amount of β structure. Upon further removal of sodium dodecyl sulfate, the backbone remains fixed in the β conformation but the side-chain aromatic groups lose the asymmetric orientations responsible for the near-uv CD spectra of the complexes. There is no evidence of return to the native conformation. Separation of sodium dodecyl sulfate from chymotrypsin by column chromatography also results in a denatured product (Brown *et al.*, 1972).

Activity of Chymotrypsin–Sodium Dodecyl Sulfate Mixtures. A significant time dependency of the interaction of sodium dodecyl sulfate with elastase has been suggested (Kagan *et al.*, 1972) as an explanation for the apparent resistance of this enzyme under certain conditions to inactivation by the detergent. Such a time dependence has also been observed for carboxypeptidases A and B (Nakagawa and Jirgensons, 1973). The observation of virtually unimpaired catalytic activity by chymotrypsin when added to sodium dodecyl sulfate–substrate mixtures at pH 8.0 (Gaudin and Viswanatha, 1972) must result from a similar sluggishness in the detergent–enzyme interaction (activities were determined from results obtained during the first 2 min after mixing; molar excesses of sodium dodecyl sulfate were as high as several hundred thousand). The data presented in Figure 4, however, show that equilibration of much lower ratios of sodium dodecyl sulfate with chymotrypsin at pH 5.6 before titration of the solutions results in complete destruction of the active site. Equilibration (as indicated by a constant near-uv CD spectrum on repeated scans) required 1–2 hr for some solutions in the concentration range represented by Figure 4. The rate of denaturation increases with the concentration of denaturant, however, and mixtures containing [sodium dodecyl sulfate] $\gtrsim 10^{-2}$ (mole ratios of several hundred or higher) gave spectra indicative of complete inactivation as soon as the first CD scan could be started (30 sec or less), in agreement with the rates of denaturation observed by Rossi and Bernhard (1971). The requirement of a total sodium dodecyl sulfate to chymotrypsin ratio as high as 15 by weight ([sodium dodecyl sulfate] $= 2.6 \times 10^{-2}$ at the highest ratio) for total loss of activity after a 10-min incubation time (Gaudin and Viswanatha, 1972) is thus hard to explain.

Specific Detergent–Protein Interactions. Rosenberg *et al.* (1969) have interpreted the extreme broadening of the nuclear magnetic resonance peaks of sodium dodecyl sulfate bound to phycocyanin as evidence of a tightening of the protein's structure when detergent is bound to a few high-affinity sites. The time-dependent enhancement of the tryptophan peaks in the chymotrypsin near-uv CD spectrum (Figure 6) may be a manifestation of such a change in the enzyme's conformation when a few molecules of sodium dodecyl or tetradecyl sulfate are bound. The detergent ions may cause small realignments in the positions of the backbone strands, temporarily greatly increasing the asymmetry of the environments of the buried tryptophans and thus the intensity of their opti-

cal activity. The peaks above 280 nm become less positive as equilibrium is approached while the 260-nm ellipticity becomes more positive (as does the 229-nm peak), suggesting that the high-wavelength changes are not merely a consequence of the diminishing of the 229-nm band. At equilibrium in the presence of small amounts of sodium dodecyl sulfate (as in curve a of Figure 3) or tetradecyl sulfate the 287 nm ellipticity remains equal to or larger than the 296-nm band. Since a similar relative enhancement of the 287-nm peak is produced by 1.5 M NaCl (and since the two peaks have very nearly the same intensity in chymotrypsinogen), it seems probable that the enhanced tryptophan bands are not the result of direct interactions between those residues and the detergent ions, but rather the indirect product of changes such as the postulated tightening of the backbone structure when a few ions are bound. In bovine and human serum albumins the sites of highest affinity for *n*-alkyl ligands are near tryptophan or tyrosine residues (Steinhardt *et al.*, 1972; Polet and Steinhardt, 1968). In chymotrypsin two of the four tyrosine residues are exposed to the solvent (Havsteen and Hess, 1962) as are four of the eight tryptophans (Williams *et al.*, 1965; Brown and Hartley, 1966). While chymotrypsin may not have sites with high affinity for detergents as serum albumin does, it is probable that the sodium dodecyl sulfate and tetradecyl sulfate bind near the exposed aromatic side chains.

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