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Studies of Soybean Trypsin Inhibitor. II. Conformational Properties*

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Ultraviolet difference spectra and optical rotation measurements were carried out on soybean trypsin inhibitor (STI) at various *p*H's, ionic strengths, and temperatures. At room temperature, the conformation is essentially independent of *p*H in the range 2 to 7, but changes in the alkaline range. The protein undergoes a rather sharp thermal transition (detected by difference spectra) which is reversible at *p*H 6.6 and 9.0 at low ionic strength but irreversible at *p*H 1.3. The transition temperature depends on *p*H and ionic strength. Since the optical rotation data yield essentially temperature-independent b_0-values which are not very different from zero, it is concluded that the native protein may contain mostly randomly coiled regions plus a small percentage of regular structure, the latter being a mixture of left- and right-handed helices, and that the sharp transition observed by difference spectra is due to the conversion of the helices of both senses to random coils. The sign of the changes in optical density implies that tyrosyl and tryptophanyl chromophores pass from water into a region containing non-polar groups and/or negatively charged ones, as the extended helical forms are transformed into the more compact random coil forms. Since spectral changes are produced either by heating or by making the solution alkaline at room temperature, the constancy of the electrostatic factor, w, between *p*H 2 and 7, and its increase at alkaline *p*H (Wu and Scheraga, 1962), are consistent with this conclusion. Optical rotation measurements carried out on the STI-trypsin compound suggest that no significant change in the conformation of either protein accompanies association.

Studies of reversible denaturation can provide information about the nature of side-chain interactions and conformation in protein molecules (Scheraga, 1960, 1961, 1962). Crystalline soybean trypsin inhibitor (STI) was selected for such a study, since its denaturation has been reported to be reversible (Kunitz, 1947). In a previous paper (Wu and Scheraga, 1962) results were reported on several physicochemical properties of

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STI. In this paper, the reversible denaturation of STI at various temperatures and *p*H's will be considered.

The denaturation reaction was studied by means of ultraviolet difference spectra and optical rotatory dispersion measurements. The STI-trypsin compound (Kunitz, 1947) was also examined by these techniques to determine whether any significant conformational change occurs in either STI or trypsin when these two proteins combine to form the compound.

Experimental

Materials.—All materials were the same as in the previous paper. Since STI and trypsin have similar molecular weights (21,500 [Wu and Scheraga, 1962] and around 21,000 [Steiner, 1954; Gutfreund, 1954], respectively), and combine with a very large association constant, the STI-trypsin compound was prepared by mixing equal weights of the two components[1] in *p*H 6.8 buffer (0.083 M KCl + 0.042 M potassium phosphate).

Ultraviolet Difference Spectra.—A Cary model 14 Recording Spectrophotometer (Applied Physics Corporation, Monrovia, California) and 1-cm glass-stoppered silica cells were used to measure the ultraviolet difference spectra of STI. Both cells contained protein at the same concentration. Constant temperature water, flowing through a specially constructed block in which the measuring cell was located, kept the temperature of the solution constant in the solution compartment. The temperature of the solution was varied from 10° to 90°. The temperature in the reference compartment was kept at 25°. The difference spectra were recorded from 350 mμ to about 238 mμ, where the slit width became maximum. Small changes in zero values (about ±0.002) at 350 mμ between runs were corrected for in the calculations. The temperature was changed by 2° to 15°; 10 minutes were allowed for thermal equilibrium after the bath had reached the desired temperature. It was found that this time interval was adequate. The temperature of the bath was constant within 0.1° at lower temperatures and within 0.15° at 90°.

For the difference spectrum of the STI-trypsin compound against separated STI and trypsin, a Beckman DU spectrophotometer with a photomultiplier attachment was used. Two 1-cm Beckman cell holders were fastened against each other in series on an aluminum block. The block in turn was fitted to a 10-cm Beckman cell compartment. Four matched 1-cm cells were used. STI and trypsin (in separate cells in series) were measured against two STI-trypsin cells in series. The total concentrations of all proteins and buffer in each light path were exactly the same. The difference was read from 350 mμ to 200 mμ. The optical density value at 350 mμ was taken as zero and other values were corrected accordingly, in order to compare the data with those obtained with the Cary instrument.

The transition temperature was calculated as the temperature at which one half of the total change in optical density had occurred. The reference solution (set at zero optical density) was always a *p*H 6.6 STI solution at room temperature. The change in optical density, ΔD, in the heated solution, was plotted as a function of temperature at any fixed *p*H. The total change of optical density was taken as the difference in ΔD's between 25° and the temperature where the curve of ΔD *vs.* T levels off. The calculated transition temperature was reproducible to ±0.5° and probably accurate to ± 1°.

Optical Rotatory Dispersion.—The Rudolph photoelectric polarimeter, Model 200, equipped with a quartz monochromator and an oscillating polarizer, was used for optical rotatory dispersion measurements. The light source was a zirconium compact arc lamp with a useful range of 310 to 700 mμ. The symmetrical angle was set at 5°.

The solutions to be measured were contained in a water-jacketed 20-cm quartz polarimeter tube of 7-mm bore with fused quartz end-plates made by Optical Cell Co. (Kensington, Md.). Water was circulated through the jacket from a water bath kept at a constant temperature within 0.1° near room temperature and within 0.15° at 75°.

The indices of refraction, n, of the solvents used were determined with the Abbe refractometer at 589 mμ and at room temperature. No correction of n for wave length or temperature was made.

After 10 minutes was allowed for thermal equilibrium, the optical rotation was measured at 10 to 13 of the following wave lengths in mμ: 700, 650, 600, 589, 546, 500, 460, 436, 400, 380, 370, 360, 340, 325, 320, 316, 314, and 312. At the end of each set of measurements at a fixed temperature the optical rotation was measured again at 546 mμ; no change in optical rotation was noticed. The temperature of the water bath was then changed slowly (about 1° per minute or less) until another desired temperature was reached. Ten minutes later the optical rotation was measured at that temperature again as a function of wave length. Solvent blanks were obtained in the same manner.

Since the optical rotations of the blank varied randomly with wave length, the average rotation at each temperature was subtracted from the observed rotation of the protein solution. The specific rotation $[\alpha]$ was then calculated.

Optical rotatory dispersion curves for the proteins were plotted by two methods. The first method was according to Moffitt (1956):

$$[\alpha]_\lambda = \left(\frac{100}{M}\right)\left(\frac{n^2+2}{3}\right)\times \left[\frac{a_0\lambda_0^2}{\lambda^2-\lambda_0^2} + \frac{b_0\lambda_0^4}{(\lambda^2-\lambda_0^2)^2}\right] \quad (1)$$

[1] The stock trypsin solution was dialyzed against 0.001 M HCl to remove $MgSO_4$.

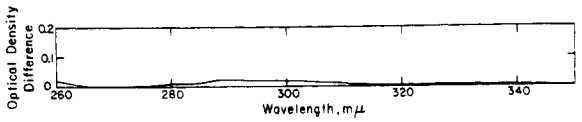

FIG. 1.—Difference spectrum of STI at *p*H 8.98 in 0.60 M KCl + 0.016 M borate at 25.3° *vs.* STI at *p*H 6.53 in 0.30 M KCl + 0.020 M phosphate at 24°. The optical density of the *p*H 6.53 reference solution was set at zero. STI concentration was 1.77 mg/ml in each solution.

where M is the average residue weight and n is the refractive index of the solvent. The value of λ_0 was taken to be 212 mμ in accordance with that found best for poly-γ-benzyl-L-glutamate in a variety of solvents (Moffitt and Yang, 1956). In the wave length range investigated, no deviation from linearity of the Moffitt plot could be detected for λ_0's between 212 and 220 mμ. The parameter b_0 was calculated from $\frac{M}{100}\left(\frac{3}{n^2+2}\right)\Big/\ \lambda_0^4$ times the slope of a plot of $[\alpha](\lambda^2 - \lambda_0^2)$ *vs.* $\frac{1}{\lambda^2 - \lambda_0^2}$.

Since b_0 in equation (1) was found to be small, the data were also plotted according to a modified Drude equation (Yang and Doty, 1957).

$$\lambda^2[\alpha] = \lambda_c^2[\alpha] + k \qquad (2)$$

The parameter λ_c was obtained from the slope of the straight line of $\lambda^2[\alpha]$ *vs.* $[\alpha]$.

Since the concentration of the protein solutions was of the order of 0.16% for STI and 0.32% for the STI-trypsin compound, the degree of precision of $[\alpha]$ was ±2.4° and ±1.2°, respectively, under favorable conditions. Consequently, the expected error in b_0 is about ±15°. Under less favorable conditions the degree of precision of $[\alpha]$ was ±5°.

Activity Analysis and Concentration of Protein.—Activity analyses of STI, trypsin, and STI-trypsin compound were carried out as described in paper I (Wu and Scheraga, 1962), except that a Beckman Model G *p*H meter was used. The STI-trypsin compound (Kunitz, 1947), made from an equal weight of STI and trypsin, was almost free of activity. Analysis showed the compound had 4.5% of the anti-tryptic activity of the STI standard. The concentrations of STI and trypsin were determined spectrophotometrically in a Beckman DU Spectrophotometer or a Cary 14 Recording Spectrophotometer, the factors described in paper I (Wu and Scheraga, 1962) being used. The range of protein concentrations used (mg/ml) was as follows: difference spectra of STI 1.6–2.0; optical rotation of STI and STI-trypsin compound, 1.6–1.7 and 3.2, respectively; difference spectra of STI-trypsin compound *vs.* STI and trypsin in tandem cells, 0.76 in each cell.

pH Measurements.—The *p*H's were measured with a Radiometer TTT1 Titrator, Radiometer *p*H meter 4, or Beckman Model G *p*H meter. Each *p*H meter was calibrated with standard

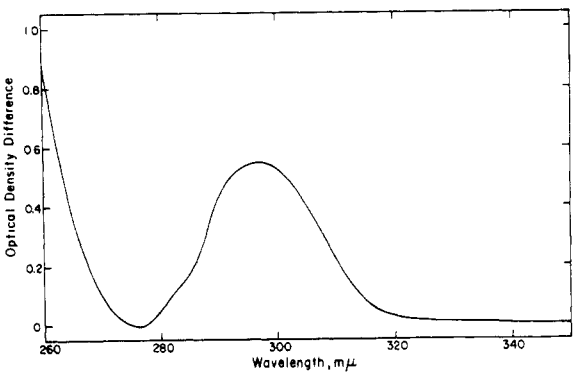

FIG. 2.—Difference spectrum of STI at *p*H 11.87 in 0.084 M KCl + 0.007 M phosphate *vs.* STI at *p*H 6.56 in 0.07 M KCl + 0.02 M phosphate, both at 25°. The optical density of the *p*H 6.56 reference was set at zero. STI concentration was 1.7 mg/ml in each solution.

phthalate, phosphate, and borate buffers prepared as described by Bates (1954).

RESULTS

Difference Spectra of STI.—If a solution of STI at *p*H 8.98 is measured against a reference STI solution at *p*H 6.53 at ~25° there is essentially no observable spectrum in the region 260 mμ to 350 mμ, except for a slight positive deviation of the baseline in the region between 280 mμ and 310 mμ (see Fig. 1). Below 260 mμ there is a small maximum near 240 mμ (not shown in Fig. 1). With a *p*H 6.6 solution used as a reference, the difference spectrum of STI at low *p*H at ~25° appeared similar to that of Figure 1, with only a *slight* augmentation of the peaks (see footnote in Table I). However, at high *p*H (*e.g.*, 11.87) at ~ 25°, a difference spectrum is observed (see Fig. 2). The difference spectrum shown in Figure 2 has not been corrected for tyrosine ionization. With the data of paper I and unpublished results (Wu and Scheraga, 1962) used to correct for tyrosine ionization, the corrected values of ΔD would be 0.194 and 0.105 at 295 mμ and 288 mμ, respectively, instead of those shown in Figure 2.

At higher temperatures (*e.g.*, with the *p*H 8.98 solution at 77.4° and the *p*H 6.53 reference solution at 32.9°), the difference spectrum of Figure 3 is obtained. Large maxima appear at 289 and 297–298 mμ; also, there is one (not shown in Figure 3) at 240 mμ. Several smaller maxima are evident in the region between 260 and 283 mμ. The sign of ΔD, the optical density difference, is positive (the *p*H 6.53 reference solution at 32.9° having been set at zero optical density); this is in contrast to the negative values of ΔD observed with ribonuclease at elevated temperature (Hermans and Scheraga, 1961). It is clear that heating produces changes in the environment of one or more chromophores of STI.

If a *p*H 6.53 reference solution is kept at room

TABLE I

CHANGE IN MOLAR EXTINCTION COEFFICIENT AND TRANSITION TEMPERATURE OF STI AS A FUNCTION OF *p*H

Reference solution was always at *p*H 6.53 and room temperature.[a] The medium contained KCl + HCl at a concentration of 0.07–0.08 M, and buffer at a concentration of 0.01–0.02 M. Data correspond to Figure 5.

	$\Delta\epsilon$ (liters/mole/cm)			T_{tr} (°C)			
*p*H	240 mμ	289 mμ	297–298 mμ	240 mμ	289 mμ	297–298 mμ	Av.
1.28	2910	600	760	39.7	40.1	40.1	40.0
2.29	4330	810	1150	44.3	45.8	44.7	44.9
2.94	5440	1090	1300	49.3	50.5	50.0	49.9
3.10	5970	1000	1300	51.8	52.0	52.0	51.9
6.58	6440	1100	1490	61.0	60.5	60.5	60.7
9.03	7460	1040	1980	58.4	58.3	59.1	58.6
11.87	—	—	4240[b]	none	none	45.6	45.6

[a] If the temperature of the measuring solution was also near 25°, the values of $\Delta\epsilon$ at 298 mμ were *very small*, *viz.*, 600, 300, 400, and 240 at *p*H 1.3, 2.3, 2.9, and 9.0, respectively. [b] Not corrected for tyrosine ionization.

temperature and a *p*H 6.53 measuring solution is heated, the value of ΔD at 298 mμ increases with increasing temperature (Fig. 4). It can be seen that there is a rather sharp transition in ΔD with temperature. The temperature at the midpoint of the total change in ΔD is taken as the transition temperature, T_{tr}, which is 63.8° in this case.

If the *p*H 6.53 reference solution is kept at room temperature, and the *p*H of the heated measuring solution is varied, then T_{tr} may be determined as a function of *p*H. Such a curve is shown in Figure 5. The *p*H 6.53 solution at room temperature was the reference for the heating curves at all *p*H's. At *p*H 9.03 and 11.87, the data may contain a small error due to the temperature dependence of the *p*K of tyrosyl groups, which ionize in this range.

The transition temperatures were determined at 240 and 289 mμ, as well as at 298 mμ. These data are shown in Table I together with the change in molar extinction coefficient, $\Delta\epsilon$, at each wave length where a large maximum occurs in the difference spectrum. A molecular weight of 21,500 (Wu and Scheraga, 1962) was used to calculate $\Delta\epsilon$, which is expressed in liters mole^{-1} cm^{-1}.

When the ionic strengths were increased from 0.1 to 0.3–0.6 at *p*H 6.5 and 9, the transition temperatures were increased by 3–5°.

The reversibility of the heating curves was checked at a number of *p*H's in the following manner. After determination of the value of ΔD at any particular temperature, say 50°, the solution was cooled to room temperature. If the room temperature value of ΔD before heating was attained, then the transition was considered reversible *up to the particular temperature from which the solution was cooled* (50° in this case). Using this criterion, none of the transitions was completely reversible from the temperature at which the transition was complete. However, if the solution was not heated above the transition temperature, then the transitions at *p*H 6.6 and *p*H 9 were reversible, whereas that at *p*H 1.3 was not. These observations were made with STI in 0.08 M KCl + 0.02 M phosphate or borate. No buffer (only 0.08 M KCl + HCl) was used for the *p*H 2.9 and 3.1 solutions; only HCl was used for the *p*H 0.8 and 1.3 solutions. If the KCl concentration was increased, the *p*H 6.6 solution in 0.3 M KCl and the *p*H 9 solution in 0.6 M KCl became partially reversible (after having been

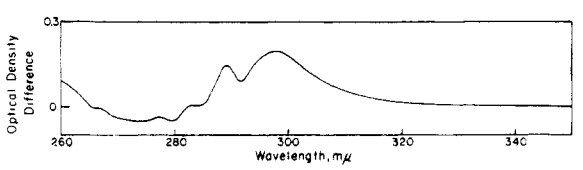

FIG. 3.—Difference spectrum of STI at pH 8.98, 77.4°, in 0.60 M KCl + 0.016 M borate *vs.* STI at *p*H 6.53 (reference set at zero optical density), 32.9°, in 0.30 M KCl + 0.020 M phosphate. STI concentration 1.77 mg/ml in both solutions.

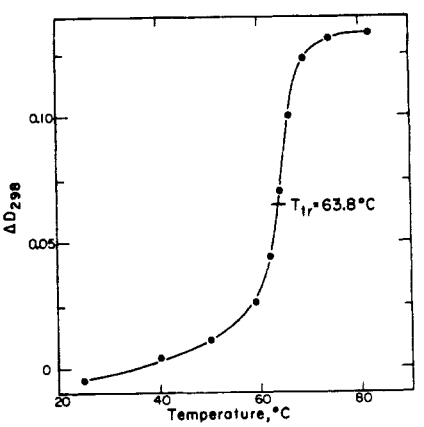

FIG. 4.—Difference spectrum of STI at *p*H 6.53 in 0.30 M KCl + 0.020 M phosphate, STI concentration 1.77 mg/ml. Reference solution was set at zero optical density at room temperature. The change in optical density at 298 mμ, ΔD_{298}, is plotted against the solution temperature. The transition temperature is 63.8°. The ionic strength here is higher than that for the data of Table I; thus T_{tr} is higher here.

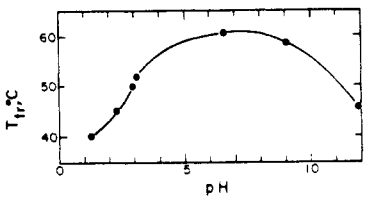

FIG. 5.—Transition temperature of STI, T_{tr} (obtained from ΔD), is plotted against pH. STI concentrations 1.65-1.98 mg/ml. KCl + HCl concentration 0.07-0.08 M, buffer concentration 0.01-0.02 M. Reference was always at pH 6.53 at room temp. No correction was made for tyrosine ionization at high pH because T_{tr} was obtained at constant pH. At pH 9.03 and 11.87, the data may contain a small error due to the temperature dependence of the pK of tyrosyl groups, which ionize in this range.

heated to the transition temperature). The pH 1, 2, 3, and 5 solutions in 0.3 M KCl became turbid when heated to about 50°.

When STI solutions of low pH (0.8, 1.3, and 2.3) were heated beyond the plateau region (equivalent to 80° in Fig. 4), where ΔD leveled off with increasing temperature, a drop in ΔD was observed at 289 and 297-298 mμ. There was no decrease in ΔD at 240 mμ, however. The transition temperature and $\Delta\epsilon$ were calculated as the difference in ΔD between the first plateau and 25°. The value of ΔD at 10° was either the same or slightly less than that at 25°.

Attempts were made to obtain the difference spectrum of the STI-trypsin compound against STI and trypsin at pH 6.8 at 25°, using tandem cells. However, the spectrum changed with time; this was probably due to autolysis of trypsin in the cell not containing STI (M. Laskowski, Jr., private communication).

Optical Rotatory Properties.—The dispersion of the specific rotation, $[\alpha]$, for all solutions studied here was such that linear Drude and Moffitt plots were obtained. Thus, λ_c and b_c were obtained from the slopes of these straight lines. Figure 6 is a Drude plot for STI at 75° (pH 1.22); Figure 7 is a Moffitt plot for the STI-trypsin compound at 25° (pH 6.74). A summary of the optical rotation data is given in Table II.

It can be seen from Table II that there is relatively little variation (within experimental error) in λ_c, b_0, or $[\alpha]_{589}$ for STI and trypsin under the various conditions of pH and temperature. We are not considering the slight increase in the negative values of b_0 upon denaturation to be significant, since the error in each b_0 value is ±15°. The values for trypsin were taken or calculated from the data of Jirgensons (1959, 1961). The STI-trypsin compound had values of λ_c and b_0 similar to those of both STI and trypsin, and a value of $[\alpha]_{589}$ between those of STI and trypsin. The change in optical rotation at 589 mμ upon combination of STI and trypsin, $-87 + 41 \cdot 2 = -64$ compared with -54, was probably within experimental error.

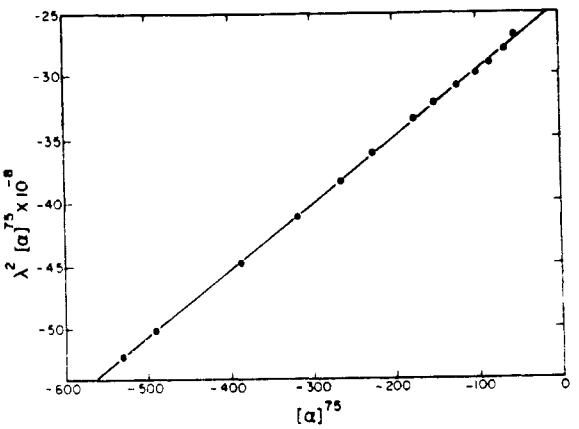

FIG. 6.—A typical Drude plot of the rotatory dispersion of STI at pH 1.22 in 0.069 M HCl at 75°. STI concentration 1.62 mg/ml.

DISCUSSION

At low and neutral pH, the peaks in the ultraviolet difference spectra of proteins below 275 mμ usually arise from perturbations of the phenylalanyl and histidyl chromophores, those from 275-290 mμ from the tyrosyl chromophore, and that above 290 mμ from the tryptophanyl chromophore (see, *e.g.*, Scheraga, 1961). STI has 9 phenylalanine, 2 histidine, 4 tyrosine, and 2 tryptophan groups (Wu and Scheraga, 1962). Although the origin of the peak at 240 mμ is not certain, that near 289 mμ seems to implicate one or more tyrosine residues, and that near 297-298 mμ one or two tryptophan residues. We may assume that changes in the absorption spectrum near 240 mμ reflect, among other things, changes in the peptide backbone (Glazer and Smith, 1960).

Since essentially the same value of T_{tr} is obtained from the dependence of ΔD on temperature at 240, 289, and 298 mμ (Table I), it appears that the observed sharp transitions in STI are due to a cooperative phenomenon which involves simultaneous changes in the conformation of the backbone and in the environment of tyrosine and tryptophan chromophores.

The optical rotation data would, at first sight, imply that STI is randomly coiled under all the

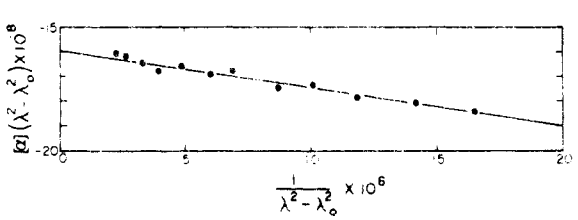

FIG. 7.—A typical Moffitt plot of the rotatory dispersion of STI-trypsin in 0.1 M KCl + 0.027 M phosphate at 25°, pH 6.74. STI-trypsin concentration 3.25 mg/ml.

TABLE II
OPTICAL ROTATORY PROPERTIES OF STI, TRYPSIN, AND THE STI-TRYPSIN COMPOUND

Protein	Solvent	*p*H	Temp. (°C)	λ_c (mμ)	b_0 (deg.)	$-[\alpha]_{589}$ (deg.)
STI	0.07 M KCl + 0.023 M phosphate	6.56	25	221	−50	87
			70	227	−79	78
	0.069 M HCl	1.22	25	222	−70	97
			50	231	−106	80
			75	229	−103	84
Trypsin[a]	0.1 M KCl	3.0	25	227	−55	40
		5.2	25	235	−66	41
STI-trypsin	0.1 M KCl + 0.027 M phosphate	6.74	25	230	−68	54

[a] Jirgensons (1959, 1961).

conditions studied. However, since a rather sharp phase transition is indicated by the difference spectra data, the optical rotation data require an alternative explanation. It is, therefore, suggested that, although STI probably contains some (and perhaps mostly) randomly coiled regions, there may also be helical regions, with helices of *both* screw senses present in the molecule. A mixture of left- and right-handed helices, which transformed to random coils at elevated temperatures, would have the observed optical rotation and difference spectra properties. While we propose this explanation, we add a note of caution, since: (*a*) the interpretation of b_0 in terms of helical content is on a very tenuous basis, and (*b*) β-structures can also have b_0 values near zero. The important point is that there is a cooperative transition in which some organized structure is disrupted upon heating.

The sign of ΔD implies that the tyrosine and tryptophan chromophores pass from an aqueous medium to a non-polar one as the temperature is raised; alternatively, the positive values of ΔD could indicate an increase in the net negative charge distribution in the neighborhood of these chromophores as the temperature is raised[2] (Scheraga, 1961). In either case, the change in environment of the chromophores takes place over a rather narrow temperature range, leading to the postulated phase transition.

Since there is only a very small difference spectrum between low *p*H and *p*H 6 at 25° (see footnote of Table I), and since there is little change in optical rotation at 25° between *p*H 1.2 and 6.6 (see Table II), it would appear that there is no conformational change at 25° induced by a *p*H change at low *p*H. This would be consistent with a constant w between *p*H 2 and 7 (Wu and Scheraga, 1962). The existence of a difference spectrum at *p*H 11, with a postive ΔD, implies a structural change, corresponding to the same kind of change in the environment of the chromophores produced by heating at neutral *p*H; *i.e.*, at 25°, the conformation at high *p*H is different from that at neutral *p*H. This agrees with the fact that a different w had to be used to fit the titration curve in the alkaline region (Wu and Scheraga, 1962). The higher w in the alkaline region[3] implies that the protein is more compact at high *p*H, *if the Linderstrøm-Lang model is applicable.* This is a reasonable conclusion, *i.e.*, the conversion of a mixture of left- and right-handed helices and a randomly coiled region (present at *p*H 7) to a completely randomly coiled molecule (present at alkaline *p*H) could lead to a more compact molecule, perhaps held in the compact conformation in the randomly coiled form by hydrophobic bonds. The ionic strength could affect this transformation by shielding charges in *either* the native or the denatured form; a variety of effects are thus possible, and it is speculation to assign the observations to any one cause. For example, if electrostatic repulsion in the native form contributes somewhat to its instability (due to a non-zero net charge), the transition temperature would be raised by increasing the ionic strength, as observed. The augmentation of the net positive charge at low *p*H and net negative charge at high *p*H would lower T_t at the extremes of the *p*H region, as observed (see Fig. 5). Alternatively, side-chain hydrogen bonding could also be involved in producing the *p*H-dependent behavior illustrated in Figure 5 (Scheraga, 1960); however, in such a case, these hydrogen bonds would have to be *ruptured* when the *p*H is lowered (and we have no spectral evidence for this). The low pK of the carboxyl groups (Wu and Scheraga, 1962) could arise from the postulated local electrostatic effects.

The study of the denaturation of STI suggests that the native protein contains a mixture of random coils and left- and right-handed helices; the helices are transformed to random coils at high temperature. Because of the spectral evidence and the dependence of T_t on ionic strength

[2] Although the formation of a hydrogen bond involving tyrosine would give a positive value of ΔD, it is unlikely that such a hydrogen bond is formed as the temperature is raised.

[3] The tyrosyl groups have a normal pK in this region (Wu and Scheraga, 1962). Apparently the changes in the environment of the tyrosyl groups are sufficient to produce the difference spectrum without affecting its pK.

it appears unlikely that the pH-dependence of T_{11} (Fig. 5) is due to side-chain hydrogen bonding; rather, this behavior is probably the result of electrostatic effects.

If we accept the models proposed for native and denatured STI, the optical rotatory data indicate that a combination of STI and trypsin leads to essentially no change in the conformation of the backbone of either protein. It is not possible to discuss the possible involvement of side-chain tyrosyl or tryptophanyl groups in the association because of the autolysis of trypsin during the measurement of the difference spectrum.

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Some Properties of Urease*

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Samples of urease with reproducible specific activity, 153,000 Sumner units/g, and absorption spectrum, $E^{1\%}_{1cm}$ 7.71 at 272 mμ, have been obtained. Ultracentrifugal analysis indicates a high degree of purity. The molecule (M.W. 473,000) contains about 77 methionyl, 29 cystinyl, and 47 cysteinyl residues. Some of the mercapto groups are very reactive and may be involved in exchange reactions with disulfide groups, causing polymerization. The mercapto groups essential for enzymatic activity are less reactive.

Urease is a very interesting enzyme because of the high efficiency and specificity of its action. It deserves attention also because of its historical importance, as it was the first enzyme to be isolated in crystalline form. Despite this point of special interest, comparatively little progress has been made in the intervening period of time toward elucidation of its chemical nature (Varner, 1960). This paper describes some contributions toward the solution of this problem.

Experimental Procedures

Materials and Apparatus.—Commercially available reagent-grade chemicals were generally used without further purification, but samples were selected which, according to the manufacturer's analyses, had low heavy metal content. Cysteine hydrochloride hydrate, grade B, was obtained from the California Corporation for Biochemical Research, Los Angeles 63; crystalline bovine serum albumin from the Armour Laboratories, Kankakee, Ill.; *p*-chloromercuribenzoate from the Sigma Chemical Company, Inc., St. Louis, Mo.; *N*-ethylmaleimide from the Delta Chemical Works, New York; "Alka-ver" indicator from the Hach Chemical Company, Ames, Iowa. Guanidine hydrochloride, initially of "Aero" (technical) grade from the American Cyanamid Company, Bound Brook, N. J., was purified as described elsewhere (Leslie *et al.*, 1962). The water used was purified by ion exchange and distilled once through an all-glass still, except when otherwise specified.

The jack-bean meal was prepared from beans grown in 1959 by Mr. Ernest Nelson, Route 1, Waldron, Ark. The beans were first ground to pea-sized pieces in a motor-driven stainless-steel

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