Changes in Optical Rotation in the Acid Transformations of Plasma Albumin. Evidence for the Contribution of Tertiary Structure to Rotatory Behavior*

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It was first shown by Jirgensons (1) that there is a pronounced increase in the levorotation of bovine plasma albumin below pH 4. This phenomenon was studied in further detail by Yang and Foster (2,3) who showed that the transition is fast and reversible and is associated with a substantial expansion of the protein molecule. It was further suggested, on the basis of pH value and ionic strength dependences, that expansion is essentially electrostatic in origin and of the character of an α-β transition (helix-coil transition). More recently, it has been shown that this expansion is preceded by an "isomerization" which has been interpreted essentially as a change in tertiary structure with little or no change in secondary structure (4). This transition occurs near pH 4 and is not accompanied by any observable alteration in the optical rotation as measured at the sodium D line.

More recently, Jirgensons (5), on the basis of optical rotation measurements in the ultraviolet (365 mp), has shown in the case of bovine plasma albumin that the large increase in levorotation is preceded by a smaller but definite decrease in levorotation and has suggested that this inverse change corresponds to the isomerization reaction. Surprisingly, human serum albumin did not exhibit this inverse transition to any appreciable extent, a fact which was interpreted as being due to a greater propensity of the human protein for expansion with consequent masking of the smaller effect.

Jirgensons' studies spanned a broad pH range from below 3 to above 11; consequently, the number of data in the pH range of the isomerization was quite limited. In view of this fact it was felt important to investigate in greater detail the rotatory behavior near pH 4. It seemed desirable to compare more carefully the behavior of bovine and human proteins, and to determine the effect of various ions, some of which (thiocyanate, perchlorate) are capable of repressing the expansion almost completely (6). It was hoped by such studies to test more critically Jirgensons' suggestion that the inverse shift in rotation is associated with isomerization. Finally, it was considered desirable to make dispersion measurements at various points on the pH transition curve in an attempt to clarify the extent to which the changes in rotation might be attributable to change in helix content or to other factors. The present communication presents results of a great many measurements made at a single wave length (313 mp) together with a number of dispersion curves. Perhaps the most important conclusion is that the breakdown of the native protein structure must involve at least two cooperative steps before the final molecular expansion. It is further suggested that the observed changes in rotatory behavior cannot be explained on the basis of changes in helix content alone.

EXPERIMENTAL PROCEDURE

Materials—Crystalline bovine plasma albumin (Lot Nos. BX3 and BX4) and crystalline human plasma albumin (Lot No. HX1) were obtained from Pentex, Inc. No evidence for any differences between the two lots of BPA was found. Further, it was not found necessary to defat these preparations before use since in no case did they show evidence of turbidity on standing at low pH values. Isoionic stock solutions of these proteins were prepared with the mixed-bed ion exchange column described by Dintzis (7). The human mercaptalbumin was prepared from Fraction V of human plasma through the mercury dimer utilizing Saroff's modification of the Dintzis method. This material was recrystallized three times, lyophilized, and stored as the mercury dimer in the cold room (−5°C). Before use the dimer was defatted by aging in solution at pH 2.5 to 3.0 for 48 hours in the cold room (1°C). The monomer was then regenerated by passing the solution through the thio glycolate mixed-bed ion exchange column recommended by Dintzis (7). In this case defatting was essential as there was a considerable release of lipid impurity from the protein (and consequent turbidity) at low pH values.

Perchloric acid (G. Frederick Smith Chemical Company) was used without further purification. Thiocyanic acid was prepared by passing reagent grade potassium thiocyanate through a cation exchange column in the hydrogen form. All other reagents were of reagent grade. All water employed was distilled and subsequently deionized by passing through a column demineralizer (Bantam model BD-1) and had a specific conductivity of less than 10−4 mho.

Methods—All optical rotation measurements were carried out at 21°C (±2°C) with a Rudolph model 200 photometric spectrophotometer.

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* The abbreviations used are: BPA, bovine plasma albumin; HSA, human serum albumin; and HMA, human mercaptalbumin.
To allow full spectral intensity in the ultraviolet region, a window polarimeter equipped with an oscillating polarizer. The source employed was an AH-4 mercury arc lamp (General Electric). To allow for full spectral intensity in the ultraviolet region, a window polarimeter equipped with an oscillating polarizer was used. The source employed was an AH-4 mercury arc lamp (General Electric).

In all measurements of optical rotation a 2 dm jacketed polarimeter tube with quartz window was employed. In a few experiments water was circulated through the jacket (25°C ± 0.02°C) but in general this was not considered necessary in view of the constancy of the ambient room temperature. To assure that there was no significant birefringence in the windows, background readings were taken with and without the empty cell in the compartment. There was no detectable difference.

Sedimentation measurements were frequently made on solutions after rotation measurements as a test for homogeneity and absence of aggregation of the protein. Such experiments were conducted in the Spinco model E analytical ultracentrifuge at 20°C. In no case was there any evidence of aggregation beyond the usual 5 to 10% of dimer which is invariably seen in plasma albumin solutions.

The concentration of protein in the isionic stock solution was determined with a Beckman model DU spectrophotometer. The extinction of BPA was assumed to be \( E_{\text{em}}^{1%} = 6.67 \) at 279 mp (8). For both HSA and HMA the extinction at 280 mp was assumed to be 5.30 (9). The uncertainty in determination of the protein concentration (estimated at ±1.5%) is probably almost 10-fold greater than the uncertainty in the measurement of the rotation per se, at least at 313 mp. Since the total change in rotation in the region under consideration was invariably less than 10% it was important to eliminate, in so far as possible, these uncertainties in protein concentration. This was accomplished by preparing an entire series of samples, spanning the pH range of interest from a single isionic stock solution. The stock solution was first clarified by centrifugation at 40,000 r.p.m. (Spinco model L preparative ultracentrifuge) and then equal aliquots were carefully pipetted into 25.0 ml volumetric flasks. With salt and acid solutions of nearly identical normalities the flasks were then filled to the final volume. In this manner the pH value could be varied while the ionic strength was maintained closely constant, and a strict control of the final protein concentration was maintained in a particular experiment. The final protein concentration for rotation readings at 313 mp was in all cases in the range 0.10 to 0.15%. In the case of dispersion measurements it was found that greater accuracy at the longer wave lengths was attained by increasing the protein concentration approximately 2-fold.

All pH measurements were made at room temperature (24°C ± 2°C), with a Beckman model G pH meter standardized against potassium acid phthalate as recommended by the U.S. Bureau of Standards (pH 4.01).

**RESULTS AND DISCUSSION**

Dependence of Rotation of BPA on pH Value and Ionic Environment—The optical rotatory behavior of BPA has been investigated as a function of pH value under a variety of ionic conditions at the single wave length, 313 mp. Figs. 1 and 2 report results in the presence of chloride ion at two ionic strengths, 0.10 and 0.02 M. Fig. 3 gives results in the presence of another relatively weakly bound anion, acetate, at 0.02 M ionic strength. Figs. 4 and 5 show the effect of the very strongly bound anions, thiocyanate and perchlorate (10), again at 0.02 M ionic strength.

In the chloride experiments there is seen to be a single monotonic decrease in levorotation near pH 4. At the lower ionic strength (Fig. 2) the beginning of expansion is evident in the upturn of the rotation at the lowest pH value. At 0.1 M ionic strength this effect is almost swamped out although upturn in rotation would doubtless have been seen if measurements had been extended to lower pH values. In both cases, comparison with the electrophoretic results is facilitated by inclusion of the percentage of N form as found by Aoki and Foster (4). It is apparent that a parallel exists, as pointed out by Jirgensons (5), between the transitions detected by rotation on the one hand and electrophoresis on the other. However, the agreement between the two sets of data is not perfect. In particular, the transition in rotation seems to be distinctly sharper, the pH dependence corresponding formally to an uptake of perhaps 5 or 6 protons rather than approximately 3 as in the case of the N-F reaction deduced from electrophoresis. Further, in the case of 0.02 M chloride (Fig. 2) the N-F transition clearly starts at a
FIG. 3. Dependence of specific rotation at 313 m\(\text{u}\) on pH value, BPA in 0.02 M acetate. Distribution of N and F components as determined by electrophoresis shown for comparison.

FIG. 4. Dependence of specific rotation at 313 m\(\text{u}\) on pH value, BPA in 0.02 M thiocyanate. Distribution of N and F components as determined by electrophoresis shown in upper dashed line, results of perturbation difference spectra by lower broken line.

FIG. 5. Dependence of specific rotation at 313 m\(\text{u}\) on pH value, BPA in 0.02 M perchlorate. Results of perturbation difference spectra shown by broken line.

distinctly higher pH value. This is probably also true in the case of 0.1 M chloride, particularly if cognizance is taken of the fact that the electrophoretic data reported in Fig. 1 were obtained at a temperature near 0\(^\circ\) rather than at room temperature. Whereas the temperature coefficient of the N-F transition is small (4), this curve would be expected to be shifted to a somewhat higher pH value at room temperature. One possible interpretation of these discrepancies is that the boundaries seen in electrophoresis are actually reaction boundaries and cannot be interpreted quantitatively in a straightforward manner (11). However, considerable evidence has been given (4, 12, 13), which need not be reviewed here, that the observed boundaries can, in fact, be treated as corresponding to definite components.

An alternative, and we believe better, interpretation of the minor discrepancies between the pH dependence of optical rotation and of electrophoretic composition can be based on the premise that the over-all transformation of the native protein to the F form consists of a sequence of at least three cooperative steps. This concept was deduced by Foster and Aoki (14) as the only way in which the electrophoretic and titration data could be rationalized. It was, in fact, suggested by them that there should be two additional transitions which would be much steeper than the one observed by electrophoresis. This mechanism has been further discussed in terms of a schematic model of the plasma albumin molecule by one of us (15). That model, designed to explain a large number of phenomena but most notably the important change in solubility properties and detergent-binding behavior in the N-F transition, is based on the concept that the albumin molecule consists of four highly organized folded subunits with three intra-surfaces, two of which are predominantly hydrophobic in character. It was suggested that the N-F transition is essentially an intramolecular dissociation of these subunits with perhaps some small rearrangement of secondary structure within the subunit.

From this point of view, the results presented in Figs. 3, 4, and 5 take on unusual interest. In 0.02 M acetate (Fig. 3) it is seen that the drop in rotation is again very sharp and to the low pH side of the electrophoretic transition. However, this drop is preceded by a small but definite increase in levorotation and the total pH range of the combined transitions in rotation spans exactly that of the isomerization as judged by electrophoresis. Moreover, in the case of the more strongly bound anion, thiocyanate (Fig. 4), the decrease in levorotation gives distinct evidence of taking place in two separate transitions. This double transition is unmistakable in perchlorate which is even more strongly bound than thiocyanate (10). In this case there is seen to be a definite plateau between the two waves, extending from approximately pH 3.0 to 3.5. Furthermore, it is of some interest that the magnitudes of the changes in rotation in the two transitions are equal, within experimental error, in both cases (Figs. 4 and 5). It is suggested that these two transitions result from the successive modification of two halves of the protein molecule, e.g., involving the opening of first one and then the other of the two postulated hydrophobic inner surfaces.

In the case of thiocyanate it is seen (Fig. 4) that the first transition in rotation coincides rather closely with the isomerization observed in electrophoresis, although again the transition is sharper. The second transition is not seen in electrophoresis. On the other hand, the second transition (and not the first) is clearly seen by the solvent perturbation technique of Herskovits and Laskowski (16-18). In this technique the difference spectrum between two protein solutions at the same pH value and ionic strength, one in a simple aqueous environment and the other in presence of a perturbing solvent (e.g. 20\% sucrose), is measured at a wave length of 280 m\(\text{u}\). From the magnitude of the difference spectrum those authors have shown that it is
possible to estimate the extent to which the tyrosyl residues of the protein are accessible to perturbation by the solvent environment. Herskovits and Laskowski have concluded that only approximately 30% of the tyrosyl residues of plasma albumin are subject to such perturbation in the native form but that in the N-F transition there is a further exposure of an additional 20% of the tyrosyls. In 0.02 and 0.25 M chloride this exposure takes place in a single step near pH 4 (16-18) and parallels very closely the single transition in rotation seen in Figs. 1 and 2. However, in thiocyanate and in perchlorate the results (dotted curves in Figs. 4 and 5) give a single wave, again corresponding to the exposure of approximately 20% of the tyrosyls, at a much lower pH value, namely in the region of the second transition in rotation. In terms of the model discussed above this would imply a gross asymmetry in the plasma albumin molecule, i.e. approximately four tyrosyls in one of the hydrophobic inner surfaces and none in the other.

Further evidence exists for the separation of the N-F transition into two separate but related physical transformations by the presence of anions with strong binding affinity. Precipitation of BPA by addition of concentrated KCl or Na₂SO₄ in presence of such weakly bound anions as chloride, acetate, or lactate yields a single monotonic variation with pH value which can be interpreted as resulting from the low solubility of the F form. The change in solubility takes place over the pH range 4.5 to 3.5 (9, 19). However, Cann found that BPA exhibits a more complex solubility behavior in presence of 0.15 M perchlorate (20). Indeed, he showed that in the case of solutions which were aged for 4 days there existed two waves in the plot of the amount of precipitate against pH value. The pH range of the two waves corresponds very closely with the two waves in Fig. 5.

The total change in rotation in going from the isoionic N form to the unexpanded F form appears to differ somewhat in the different ionic environments (ranging from approximately 40° in chloride to about 20° in perchlorate). It is of interest to inquire whether this difference results from modification by the ionic environment of the rotation of the N form, of the F form, or both. To test this point, solutions were prepared at selected pH values covering the entire pH range of interest in the various ionic environments discussed above, employing a common isoionic stock. All solutions at pH 5 or greater were found to yield the same specific rotation to within ±2° (i.e. ±0.4%). It can thus be concluded that the conformation of the N form is remarkably resistant to perturbation by the ionic environment. On the other hand, the F form shows a variation in rotation of nearly 25° between 0.1 M Cl⁻ and 0.02 M ClO₄⁻, a variation of 5%.

Jirgensons (5) has mentioned evidences of difference in the pH dependence of optical rotation near pH 4 between BPA samples, depending on whether or not they had been deionized. The results in Fig. 1 on deionized as compared to deionized material clearly suggest such effects to be minor or absent.

Several experiments were conducted to demonstrate the reversibility of the rotational changes in chloride-containing media. In one experiment the native protein was first converted to the F form by adding acid, then the acid neutralized. It was found that [α]₂₅° was unchanged from the native value. In another experiment an aliquot of native BPA was dialyzed against a nearly identical aliquot of the protein in the F form. After equilibration [α]₂₅° was found to be the same for both solutions within experimental error. In a final experiment, aliquots of BPA at low and at high pH values were mixed directly to give solutions which were at different pH values in the N-F range. The change of optical rotation at 313 mμ was found in all cases to be completely reversible with respect to change in pH value.

Finally, mention should be made of the absence of time effects in these studies. In general, initial readings were made on all solutions within 4 to 10 minutes after mixing. In many cases the solutions were reread after standing in the cold room for 24 to 48 hours. In none of the systems reported here was there found to be any significant time dependence. There has been considerable discussion of the question of the rate of the isomerization reaction in plasma albumin (15, 21) and it was to some extent in the hope that optical rotation might provide a means of studying the kinetics of this process that the present work was undertaken. It would appear that the present results do not throw any further light on this complex problem.

Human Mereaptalbumin and Human Serum Albumin—Results on HMA in 0.10 M chloride are given in Fig. 6. In this case there is seen to be a remarkably good correlation between the transition in optical rotation and the electrophoretic results but again, if anything, the electrophoretic transition lies at slightly higher pH values. In Fig. 7 results are given for HSA in both 0.10 M chloride and 0.02 M thiocyanate. There appears to be no detectable difference between HMA and HSA and the results are clearly in general agreement with those on BPA. Also clearly the human protein gives a double transition in thiocyanate which is very similar to that shown by BPA. The most salient differences between the human and bovine proteins in so far as these experiments are concerned are (a) the evidence of an earlier onset of expansion (the upturn in rotation in chloride below pH 3.4) in agreement with the conclusion of Jirgensons (5);
and (b) the increase in rotation between pH 5.8 to 5.8 and pH 4.4. Both effects are eliminated by thiocyanate.

Dispersion Data—Whereas the chief interest in the present study was in using optical rotation at low wave length as an empirical tool for following the low pH transitions in plasma albumin, it was also of interest to seek some understanding of the molecular basis for the observed changes. In this connection numerous dispersion data have been obtained throughout the low pH range in the presence of both chloride and thiocyanate anions. The recent interest in the relationship between helical folding and optical rotation of polypeptides and proteins is well known and need not be reviewed here. It has been found, in general, that the denaturation of native globular proteins results in an increase in levorotation and this phenomenon finds its counterpart in the similar change which occurs on going from the helical to the coil conformation in some (but not all) polypeptides. From this point of view the decrease in levoration which takes place near pH 4 in plasma albumins might be interpreted as resulting from a small increase in helical folding. However, exceptions to the general rule have been pointed out by Jirgensons (22), and Tanford, De, and Taggart (23) have raised a serious question as to whether changes in helical content are the predominant cause of the rotational changes observed in protein denaturation.

The theoretical considerations of Moffitt (24) have predicted that the helical fold should give rise to complex dispersion according to the general equation

$$\delta = a \frac{\lambda^2}{\lambda^2 - \lambda_0^2} + \frac{b}{\lambda^2}$$

where

$$\delta = \frac{1}{n^2 + 2 \varepsilon}$$

is a reduced mean residue rotation. It has been generally found that synthetic polypeptides in the helical conformation do indeed yield complex dispersion of the type predicted whereas in the random coil conformation the dispersion obeys essentially the simple Drude equation ($b \sim 0$ in Equation 1).

Dispersion data at seven pH values in the presence of chloride are presented in Fig. 8 in the form of the plot recommended by Moffitt and Yang (25). In all cases $\lambda_0$ has been taken as 212 m$\mu$, and correction for the dispersion of the refractive index $n$ has been made. The mean residue weight, $M_0$, was taken as 118. Experimental points are shown in only two cases to avoid undue congestion in the figure. However, it can be stated that in all cases the fit of the data to the straight lines drawn was equally good. All of the dispersion data are summarized in Table I in terms of $a$ and $b$ (obtained from the intercept and slope in the Moffitt-Yang plots).

An interesting result is immediately apparent from the data. The decrease in levoration at 313 m$\mu$ is associated with a
small decrease in the b term in Equation 1, i.e. formally there is a decrease in helix content as judged from dispersion data. This is exactly counter to the conclusion based on magnitude of the rotation at 313 mµ. On the other hand, the increase in rotation at lower pH values is associated with a further decrease in the magnitude of b (data at pH below 3.6 in the 0.02 M chloride series, Table I). A further interesting result is the fact that the dispersion curves in the pH range of the inverse transition tend to cross at a wave length in the region of the sodium D line (550 to 600 mµ). This explains the fact that this transition was not observed in the earlier studies of Yang and Foster (2, 3) which were confined to this wave length. By contrast, in the expansion range the dispersion curves tend to cross at approximately 260 mµ.

In seeking some further understanding of these results it is useful to consider them in terms of an empirical modification of Equation 1 as presented by Doty (28):

\[ m' \alpha = (2a_0^\alpha + fbo_0^\beta) - \frac{x_0^\alpha}{X_\alpha - X_\beta} + \frac{fbo_0^\beta}{X_\alpha - X_\beta} \]  

(2)

Here \( \Sigma a_0^\alpha \) is the total intrinsic residue rotation and \( a_0^\mu \) and \( b_0^\mu \) are the contributions to the a and b terms due to 100% helical folding. The fraction of helical folding is given by f. The value of \( b_0^\mu \) might be expected to be constant from protein to protein and indeed similar to that found for model polypeptides, provided that only a single sense of helix were involved. The individuality of the protein should be expressed in \( \Sigma a_0^\alpha \) and in f, but probably also in \( a_0^\mu \). Doty has suggested values of +650 and -630 for \( a_0^\mu \) and \( b_0^\mu \), respectively, for right-handed helices. However, it seems probable that \( a_0^\mu \) differs from one polypeptide to another and perhaps even from solvent to solvent. It might be expected, therefore, that the appropriate value of this parameter would depend on the specific amino acids participating in the helical fold and perhaps also on their tertiary environment.

In a study of the dispersion behavior of BPA, Imahori (29) has arrived at the following best values:

\[ \Sigma a_0^\alpha = -620 \]
\[ a_0^\mu = +680 \]
\[ b_0^\mu = -600 \]

It is important to note that \( a_0^\mu \) and \( b_0^\mu \) have opposite signs, not only in this case but in all polypeptide systems so far studied. (For left-handed helices both signs would be reversed.) Consequently, it can be seen from Equation 2 that there should exist a wave length at which the contribution of the helical conformation to the rotation would be zero. This wave length is clearly given by

\[ \frac{-a_0^\mu}{b_0^\mu} = \frac{X_\mu}{X_\alpha - X_\mu} \]  

(3)

For plasma albumin, with either Imahori's or Doty's values of \( a_0^\mu \) and \( b_0^\mu \), this wave length is found to be in the range 290 to 295 mµ. The actual point of intersection of the dispersion curves in the expansion region, 290 mµ, corresponds to an \( a_0^\mu \) of over 1000 assuming Doty's best value of -630 for \( b_0^\mu \). The previously mentioned fact that the dispersion curves through the region of isomerization tend to cross in the range 550 to 600 mµ suggests a very different origin for the observed changes in rotation in that case.

From Equation 2, if we let I represent the intercept and S the slope in the Moffitt-Yang plots, we obtain

\[ \frac{I}{S} = \frac{(\Sigma a_0^\alpha + fa_0^\mu)X_\mu}{fbo_0^\beta} = \frac{1}{X_\mu b_0^\mu} + X_\mu \Sigma a_0^\alpha \cdot \frac{1}{S} \]  

(4)

Thus, in a plot such as Fig. 9 the slope should yield a best value of \( \Sigma a_0^\alpha \) and the intercept a best value of \( a_0^\mu/b_0^\mu \). Linearity of such a plot should be a rigorous test of the constancy of the parameters \( a_0^\mu/b_0^\mu \) and \( \Sigma a_0^\alpha \). The actual data in the expansion range are seen to conform to a straight line within experimental error. The best values deduced from this curve are 1300 for \( a_0^\mu \) and -870 for \( \Sigma a_0^\alpha \) assuming \( b_0^\mu = -630 \). The data through the N-F transition range deviate very significantly from this straight line. Clearly, at least one of the supposed constant parameters is not constant. A mathematical analysis of these results based on the assumption that the over-all curve consists of two straight line segments as drawn in Fig. 9 yields the following results:

1. The results are not compatible with a change in \( b_0^\mu \) alone. Furthermore, as previously mentioned, the evidence suggests that this parameter should be reasonably constant.

2. These values clearly differ widely from those deduced by Imahori (29). However, this is not surprising since his value of \( \Sigma a_0^\alpha \) was based on measurements in concentrated aqueous urea. In the first place it seems possible that urea may have a profound effect on \( \Sigma a_0^\alpha \). In the second place our values, which appear to be constant through the acid expansion, may not remain constant on further disorganization of the residual 30% or so of helix which appears to persist in acid solution but which was apparently destroyed in Imahori's urea experiments. It should be emphasized that the deductions we make are not based on any assumed values for \( a_0^\mu \) and \( b_0^\mu \) but only on the assumption of a constant value for the ratio \( a_0^\mu/b_0^\mu \) throughout the pH range under consideration.
have pointed out that rupture of disulfide bonds in native globular proteins would be expected to result in substantial changes in optical rotation. In the present case, the possibility of such contributions is discounted on the grounds that disulfide bonds should be quite stable under the conditions employed, both as to rupture and to exchange. Further, the fact that the alterations observed are in all cases rapid and reversible argues against such effects.

3. Changes in secondary structure other than helix-coil transformations. Such contributions certainly cannot be ruled out. Thus, for example, it has been suggested that the β-keratin structure makes a finite contribution to the a term of the Moffitt equation but that its contribution to b is zero (32). Any changes in such structure would thus contribute to the term $\Sigma_{a_0}^R$ in the analysis employed.

4. Changes in tertiary interaction. Finally there must be considered the possible result of changes in R group interactions resulting from the postulated separation of hydrophobic surfaces in the N-F transition. Such alterations might be imagined as arising from rupture of side-chain hydrogen bonds, changes in vicinal interactions, or simply from a change in the polarizability of the environment of key chromophoric groups (33). It is suggested that it is, in fact, such changes in tertiary environment that are responsible, in the main, for the apparent change in $\Sigma_{a_0}^R$ associated with the N-F transformation. Such a conclusion is in accord with the recent suggestion of Tanford et al. (23). However, it must be emphasized that any such deduction is only tentative and a full interpretation of the results reported herein must await a more complete understanding of both the theory of optical rotation and of the structure of the protein employed.

**SUMMARY**

The course of the reversible breakdown of the native structure of plasma albumin in acid solution has been traced by following the change in rotation at 313 mp as a function of pH value in the presence of various anions. The isomerization which takes place near pH 4 is accompanied by a substantial decrease in levoration as was pointed out earlier by Jirgensons; however, the transition as measured by rotation is a somewhat sharper function of pH value in chloride-containing media than that seen in electrophoretic studies. Furthermore, in presence of acetate and especially in systems containing thiocyanate or perchlorate, the isomerization occurs in at least two steps. In the last two mentioned systems these two steps result in equal increments in the optical rotation (at 313 mp) and might result from successive opening of the two hydrophobic inner surfaces previously postulated to exist in the plasma albumin molecule. Approximately four buried tyrosyl residues appear to be exposed to the solvent environment in the second of these steps, none in the first. Probably neither of these steps is identical with that revealed by electrophoresis; rather, they probably represent subsidiary steps in the transition sequence previously postulated. Only minor differences in rotatory behavior between bovine and human albumins have been observed.

In an attempt to clarify the origin of the change in optical rotation associated with isomerization, results of 19 dispersion measurements are presented. There is an apparent decrease in right-handed helix content (from approximately 48% to approximately 40%) associated with isomerization. A further decrease to approximately 30% accompanies optimal expansion in presence of minimal concentrations of chloride ion. Evidence is presented...
which indicates that most of the observed change in rotation at 313 rnp associated with isomerization is due to structural changes other than the loss of helix and it is suggested that the most important factors are alteration in hydrogen-bonding, in vicinal interactions, and in the polarizability of the environment of chromophoric residues, resulting from changes in tertiary structure. It is pointed out that, on the basis of the Moffitt theory, dispersion curves obtained throughout a helix-coil transition in simple polypeptides should cross at a wave length near or below 300 μ. At this wave length the helix would make no contribution to the rotation. Hence, it may be true for protein transformations generally that rotational changes observed at wave lengths near 300 μ will be found to reflect, in the main, alterations in structural features other than the helix content.

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