# The Effect of Dodecyl Sulfate on the Ultraviolet Spectra of Proteins*

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The effect of the detergent, sodium dodecyl sulfate, on two proteins—bovine serum albumin and bovine pancreatic ribonuclease—has been studied by the technique of ultraviolet difference spectrophotometry. The ultraviolet spectra of the proteins were observed as a function of the dodecyl sulfate concentration at *p*H 6.1. The nature and magnitude of the spectral shifts observed indicate that each protein interacted in two ways with the detergent. At low concentrations of detergent the albumin underwent a small conformational change in which tryptophyl but not tyrosyl residues were involved. At higher concentrations a larger conformational change involving tyrosyl but not tryptophyl residues occurred. At low concentrations sodium dodecyl sulfate precipitated ribonuclease with no observable conformational change. At higher concentrations of detergent the enzyme became soluble with a concomitant conformational change involving one of the tyrosyl residues.

The effects produced by the interaction of sodium dodecyl sulfate and similar detergents with serum albumin have been of interest to many investigators (Putnam and Neurath, 1945; Duggan and Luck, 1948; Karush and Sonenberg, 1949; Yang and Foster, 1953; Pallansch and Briggs, 1954; Markus and Karush, 1957; Aoki, 1958. For a brief review see Foster, 1960). Proteins have a high affinity for such compounds with high surface activity, binding them in significant amounts at very low ambient detergent concentrations. Besides that, the detergents exert a wide variety of effects on proteins, depending on the protein under study and on such conditions as the temperature, the *p*H, and the ionic strength.

*Difference Spectrophotometry.*—Difference spectrophotometry, a technique introduced to protein chemistry by Laskowski *et al.* (1956), has recently been applied by several investigators to problems in which changes in protein conformation are involved (Scheraga, 1957; Sela and Anfinsen, 1957; Bigelow, 1960). Glazer *et al.* (1957) and Williams and Foster (1959) have used the technique to examine the behavior of bovine serum albumin at low *p*H. At *p*H values below 4 the molecule expands (Yang and Foster, 1954) and the ultraviolet spectral peak at 280 mμ shifts to shorter wave lengths. This peak, which is commonly observed in protein spectra, is now well known to be due to the presence of two aromatic chromophores in the protein—the side-chains of tyrosyl and tryptophyl residues. The spectral shifts for serum albumin when the *p*H is lowered, as observed by Glazer *et al.* (1957) and Williams and Foster (1959), and similar blue shifts observed for other proteins under denaturing conditions, are therefore intimately connected with changes in the environment of these two chromophoric amino acids. Just what sorts of changes in the environment are responsible is not yet known, but the disruption of hydrogen bonds (with tyrosyl donors) or hydrophobic bonds involving tyrosyl or tryptophyl residues has been suggested. For discussions of these questions the reader is referred to Williams and Foster (1959), Bigelow and Geschwind (1960), Bigelow (1960), Leach and Scheraga (1960a), and Yanari and Bovey (1960).

In view of the marked conformational changes (to be discussed later) known, from the work of earlier investigators, to accompany the interaction of serum albumin and dodecyl sulfate, it was of particular interest to determine whether such changes manifested themselves in spectrophotometric changes and, if so, to what extent.

Difference spectrophotometry also offered an opportunity to obtain information about the chromophoric groups of serum albumin in its interactions with dodecyl sulfate. Such information might be correlated with data obtained by other techniques to yield specific information about the involvement of tyrosyl and tryptophyl residues in the conformational changes.

Ribonuclease has frequently been examined by the method of difference spectrophotometry, partly because the interpretation of results is somewhat easier than for other proteins. There is no tryptophan in the molecule, so that the observed effects can be related to one amino acid only. Furthermore, three of the six tyrosyl residues are known to be abnormal; they cannot be titrated until a *p*H higher than 12 is reached (Shugar, 1952; Tanford *et al.*, 1955). It appears that when some or all of these tyrosyl residues are normalized (for example, by unfolding part of the molecule with a denaturing agent), the difference spectra which can be observed between the denatured and native forms of ribonuclease can be quantitatively related to the number of abnormal tyrosyl residues which have been normalized (Bigelow, 1960, 1961).

It was, therefore, of interest to see whether dodecyl sulfate would exert any effect on ribonu-

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clease, not only *per se*, but also because it might add to the considerable amount of information we now have about ribonuclease difference spectra.

## Experimental

*Materials.*—Crystalline bovine serum albumin was purchased from Armour and Company. Five-times-recrystallized ribonuclease (protease-free) was purchased from Sigma Chemical Company (Lot No. R21B-68). A sample of this ribonuclease was subjected to chromatography on IRC-50 (Hirs *et al.*, 1953) and found to contain approximately 20% ribonuclease B. Sodium dodecyl sulfate was obtained from E. I. du Pont de Nemours. Stock solutions of protein (approximately 0.7%) were made up in 0.025 M phosphate buffer at *p*H 6.1. Stock solutions of dodecyl sulfate (0.208 M, 0.0208 M, and 0.00208 M) were made up in the same buffer. Dilutions of the stock dodecyl sulfate solutions were made with the buffer.

*Methods.*—A Beckman model DU spectrophotometer was used for the measurements. The instrument was equipped with a photomultiplier and four thermospacers. Water from a bath at 20° was circulated through the thermospacers. Solutions were equilibrated at 20° before mixing in the cuvets. The reference solution in any set of experiments was made up by mixing 1 ml of the stock solution of protein and 1 ml of the buffer in a cuvet. Each experimental solution was made up by mixing 1 ml of the stock protein solution with 1 ml of an appropriate solution of dodecyl sulfate. Thus each cuvet contained the same concentration of protein (0.35%) and buffer, the only difference being the presence of dodecyl sulfate in the experimental solution.

Difference spectra were determined in the region between 270 mμ and 310 mμ except in those cases where the difference spectra did not approach zero at the higher wave length. In these cases the readings were continued out to 500 mμ. Difference spectra were determined for both proteins with concentrations of dodecyl sulfate between $4.16 \times 10^{-5}$ M and 0.104 M.[1]

Concentrations of protein were determined by measuring the ultraviolet absorption of suitably diluted solutions. For bovine serum albumin, the measurements were made at 280 mμ and a molar extinction of 45,500 (Tanford and Roberts, 1952) was used. For ribonuclease, the measurements were made at 277.5 mμ and a molar extinction of 9800 was used (Bigelow, 1960). The molecular weights of the proteins were assumed to be 69,000 and 13,700 respectively.

## Results

*Serum Albumin.*—The difference spectra observed for serum albumin at low concentrations of dodecyl sulfate ($1.04 \times 10^{-3}$ M and lower) are shown in Figure 1. These difference spectra have their minima at 294 mμ. The value of the absorbance difference, $\Delta A$, at this wave length, is shown plotted against the dodecyl sulfate concentration in Figures 2 and 4. The difference spectra observed for serum albumin at higher concentrations of dodecyl sulfate ($1.04 \times 10^{-3}$ M to $2.08 \times 10^{-2}$ M) are

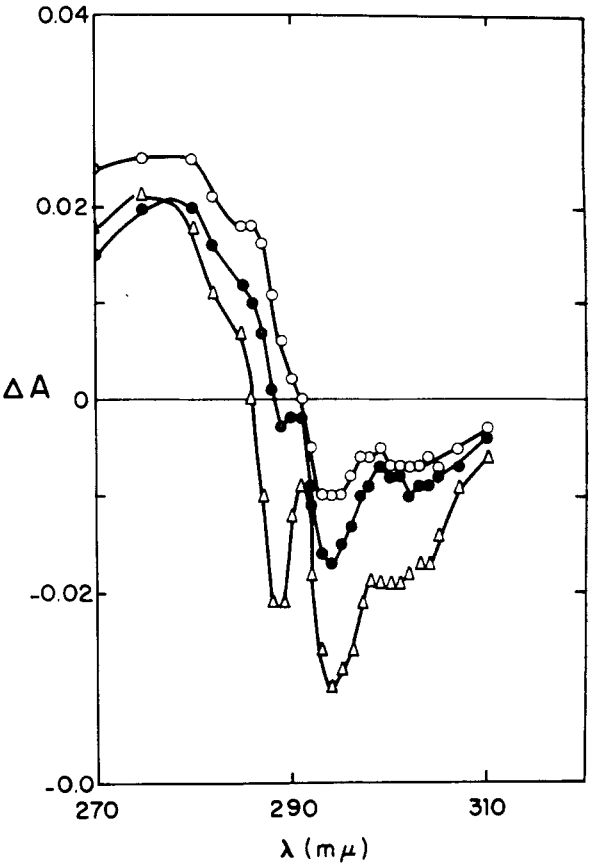


Fig. 1.—Difference spectra for bovine serum albumin in low concentrations of sodium dodecyl sulfate. ○, $2.08 \times 10^{-4}$ M; ●, $3.12 \times 10^{-4}$ M; △, $8.68 \times 10^{-4}$ M.

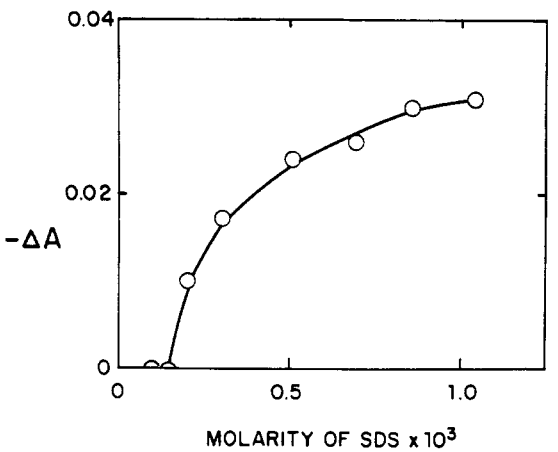


Fig. 2.—Variation of $\Delta A_{294}$ for bovine serum albumin at low concentrations of sodium dodecyl sulfate.

[1] These are the concentrations of *total* dodecyl sulfate, and thus some of the solutions contain detergent above the critical micelle concentration, which is about $6 \times 10^{-3}$ M (Powney and Addison, 1937). Any effects occurring at dodecyl sulfate concentrations much higher than $6 \times 10^{-3}$ M would be caused by dodecyl sulfate micelles.

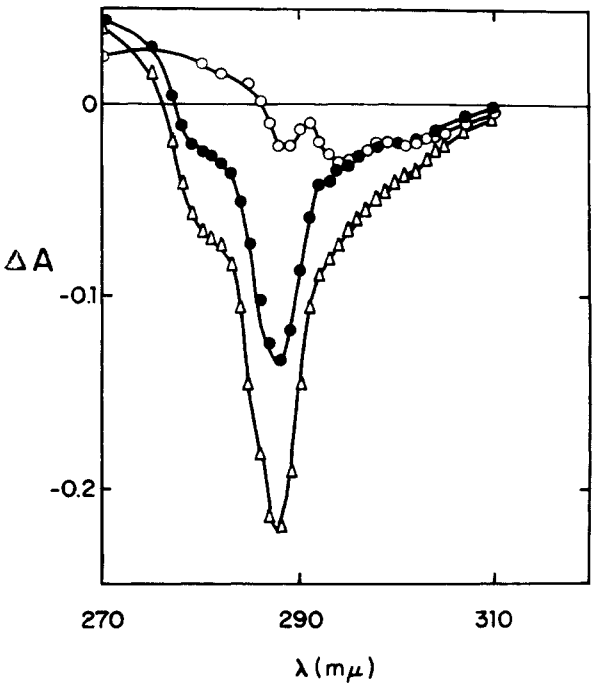


FIG. 3.—Difference spectra for bovine serum albumin at high concentrations of sodium dodecyl sulfate. ○, $1.04 \times 10^{-3}$ M; ●, $4.16 \times 10^{-3}$ M; △, $1.04 \times 10^{-2}$ M.

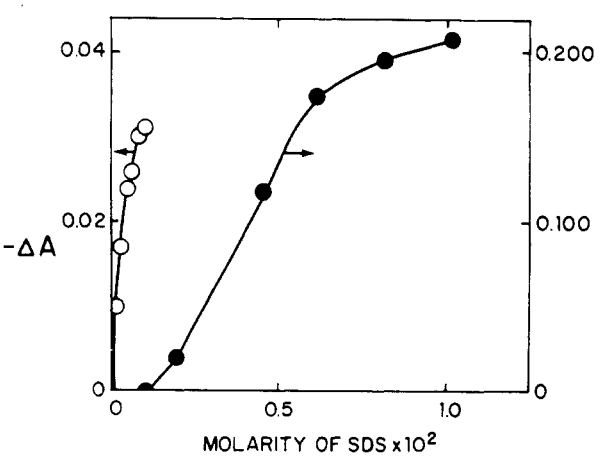


FIG. 4.—Variation of $\Delta A_{294}$ (○) and $\Delta A_{287}$ (●) as functions of the concentration of sodium dodecyl sulfate.

shown in Figure 3. These difference spectra have quite a different shape from those in Figure 1. In Figure 3 the largest difference is at 287 mμ, there being only a shoulder at 294 mμ. There was no further change when the concentration of dodecyl sulfate was raised to $1.04 \times 10^{-1}$ M. The value of $\Delta A$ at the 287 minimum is plotted against the concentration of dodecyl sulfate in Figure 4. The observed value was corrected by subtracting the value observed for a concentration of dodecyl sulfate of $1.04 \times 10^{-3}$ M (that is, the value observed after the first effect was complete). In Figure 5 are shown difference spectra for serum albumin in the higher concentrations of dodecyl sulfate. These have all been corrected by subtracting the $1.04 \times$

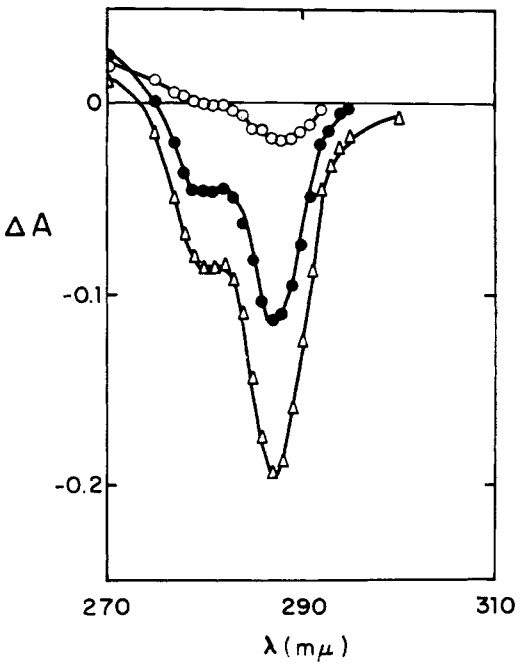


FIG. 5.—Corrected difference spectra for bovine serum albumin at high concentrations of sodium dodecyl sulfate. (See text for corrections.) ○, $2.08 \times 10^{-3}$ M; ●, $4.16 \times 10^{-3}$ M; △, $1.04 \times 10^{-2}$ M.

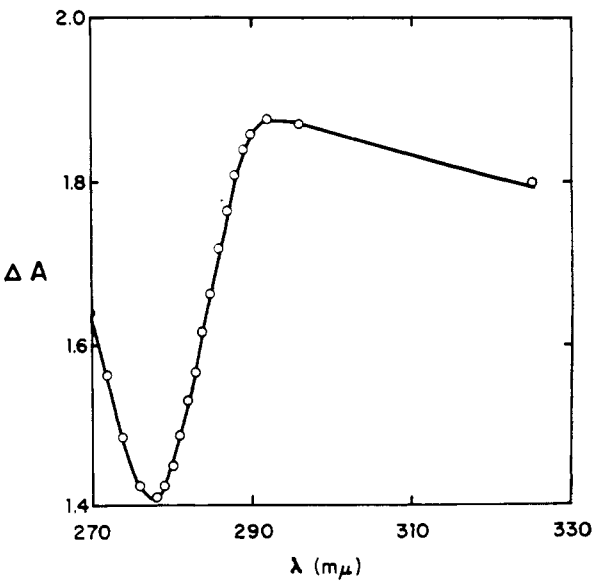


FIG. 6.—Difference spectrum for ribonuclease at concentration of sodium dodecyl sulfate ($2.08 \times 10^{-3}$ M) which precipitated it.

$10^{-3}$ M difference spectrum. They all have the typical shape that is associated with an alteration in the environment of tyrosyl residues. The shoulder observed in the uncorrected data at 294 mμ (Fig. 3) is no longer present, and is thus shown to be significant in the first step only.

*Ribonuclease.*—Dodecyl sulfate also caused two distinguishable phenomena with ribonuclease, though they were different from those observed

with serum albumin. At low concentrations of dodecyl sulfate ($4.16 \times 10^{-3}$ M and below) the solutions containing ribonuclease were opaque because the detergent precipitated the enzyme. (At very low concentrations of dodecyl sulfate [$4.16 \times 10^{-5}$ M and $1.46 \times 10^{-4}$ M] the solutions were transparent on mixing but rapidly became turbid.) Although the solutions were opaque on direct observation, useful ultraviolet measurements could be made on them. For example a "difference spectrum" for ribonuclease in $2.08 \times 10^{-3}$ M dodecyl sulfate is given in Figure 6.

This difference spectrum can be broken down into two contributing effects. One was caused by light scattering by the aggregated molecules (Leach and Scheraga, 1960b) and was observed as the large absorption difference above 290 mμ. (Although it is not shown in Figure 6, $\Delta A$ was still large at 500 mμ, as would be expected for an opaque solution. The values of $\Delta A$ between 300 and 500 mμ gave a linear plot on log-log paper, as would be expected for light scattering. The slopes of such linear log-log plots were invariably smaller than 4, the value expected for molecules considerably smaller than the wave length of the light being used. If the molecular aggregates approach in size the wave length of the light being scattered, the observed slope would be expected to be smaller than 4 [Leach and Scheraga, 1960b].) The second component of the difference spectrum in Figure 6 is the dip at 277 mμ. This is the wave length of maximum absorption for the protein, so it is clear that this dip reflects the trivial fact that some of the protein in the suspension has precipitated out, causing a decrease in the protein concentration in the cuvet which contains the dodecyl sulfate.

At concentrations of dodecyl sulfate above $5.21 \times 10^{-3}$ M (up to $1.04 \times 10^{-1}$ M) ribonuclease was quite soluble and the solutions were quite transparent. Between $5.21 \times 10^{-3}$ M and $8.33 \times 10^{-3}$ M dodecyl sulfate, difference spectra were observed which were caused by changes in the environment of some of the abnormal tyrosyl residues. In this range of concentration of dodecyl sulfate the value of $\Delta\epsilon$ depended on the concentration of dodecyl sulfate, while above this range there was no further change in the spectrum. Difference spectra for ribonuclease in $6.24 \times 10^{-3}$ M and $2.08 \times 10^{-2}$ M dodecyl sulfate are shown in Figure 7.

## Discussion

### *A. Serum Albumin*

Low Dodecyl Sulfate Concentrations.—The data on spectral changes for serum albumin as a function of concentration of dodecyl sulfate (Fig. 1–4) show that two spectrophotometrically observable phenomena occur. At very low concentrations of dodecyl sulfate no change in the light absorption is observed at 294 mμ (Fig. 2), but between $1.5 \times 10^{-4}$ M and $1.04 \times 10^{-3}$ M dodecyl sulfate difference spectra are observed (Fig. 1). These difference spectra arise from blue shifts (shifts to shorter wave lengths) of the protein spectrum and have minima at 287 mμ, 294 mμ, and 302 mμ. The fact that there is a minimum at 294 mμ indicates that tryptophyl residues are implicated (Chervenka, 1959). The minimum at 302 mμ has not, to our knowledge, been observed before with proteins. Whether this arises from the interaction of dodecyl sulfate with some nonprotein component of our sample of serum albumin is not known, but this is one possibility.

This spectral change occurred over a fairly narrow range of concentrations of dodecyl sulfate (roughly between 1.5 and $10 \times 10^{-4}$ M), above which further increases in detergent concentration caused a quite different type of difference spectrum. The difference spectra for this first event never attained very large numerical values of $\Delta A$. At 294 mμ, the largest value of $\Delta A$ was $-0.030$, equivalent to a molar extinction difference of $-540$.

Chervenka (1959), Bigelow and Geschwind (1960), and Donovan, Laskowski, and Scheraga (1961),[2] have presented difference spectra. determined for tryptophan and related compounds under a variety of conditions. Bigelow and Geschwind studied the effect of solvent changes on the absorption, while Donovan *et al.* examined the effect of changes in *p*H, and hence changes in the state of ionization, on the spectra of the compounds. The two groups of workers observed quite different types of difference spectra. Changes in the solvent caused difference spectra with three maxima at wave lengths 274 mμ, 284 mμ, and 291 mμ (Bigelow and Geschwind, 1960). These were very similar to those which had been observed by various workers with proteins (Chervenka, 1959; Blumenfeld and Perlmann, 1959; Bigelow and Geschwind, 1960). On the other hand, the difference spectra reported by Donovan *et al.* (1961) were quite different. They did have a peak at 294 mμ, but they became negative in the vicinity of 290 mμ, and the other peaks which had been observed when the solvent was changed were not observed. They found similar difference spectra on changing the *p*H of solutions of lysozyme and attributed these difference spectra to alterations of charges in the vicinity of indole chromophores. Evidence that the effect was the larger the lower the ionic strength was taken to show that the effect could be transmitted through the medium (Donovan *et al.*, 1961).

The difference spectra we have shown in Figure 1 are very similar to those reported by Donovan and his colleagues (admittedly the minimum at 288 mμ, which may be due to tyrosyl effects, gets more significant at higher concentrations of dodecyl sulfate) and quite different from those reported by Bigelow and Geschwind. We therefore think it probable that the effect of the detergent which was observed was caused by an alteration of the ionic charges in the vicinity of one or both of the indole chromophores. According to Donovan *et al.*

[2] We would like to thank Dr. Scheraga for the opportunity of reading this manuscript before it was published.

(1961), a red shift is observed at 294 mμ with increases in the *p*H of solutions of their model compounds (tryptophan and glycyl tryptophan) or of lysozyme. Therefore, it is possible that a minor conformational change, in which a carboxylate ion or ions are removed from the vicinity of an indole group, could cause the blue shifts we have observed here. The coming together of a charged amino group and an indole chromophore could also cause such an effect. We can be reasonably sure that such a conformational change is minor, because there are only two tryptophyl residues in the protein molecule (Tristram, 1953). A larger conformational change would probably have involved at least some of the tyrosyl residues, of which there are nineteen per molecule (Tristram, 1953).

The observed differences cannot be explained as the result only of the change in the solvent from buffer to dodecyl sulfate in buffer. Solvent effects on residues exposed to the environment are normally insignificant at the low concentrations used here (Bigelow and Geschwind, 1960). Furthermore, dodecyl sulfate is known to cause a slight *red* shift in the spectra of *N*-acetyl esters of tyrosine, tryptophan, and phenylalanine at concentrations above the critical micelle concentration, owing to the inclusion of these compounds in micelles (Yanari and Bovey, 1960). There is therefore no reason to expect dodecyl sulfate to cause a blue shift solvent effect on the absorption of the protein. It therefore seems necessary to conclude that a small conformational change has occurred in the serum albumin molecule, even at these very low concentrations of detergent.

Previous investigators of serum albumin difference spectra (Glazer *et al.*, 1957; Williams and Foster, 1959) have not found any involvement of tryptophyl residues. It therefore appears that the conformational change observed here occurs in a different part of the molecule from the expansion and isomerization which occur at low *p*H (Foster, 1960).

In the present study at *p*H 6.1, no precipitation of serum albumin was noted at low concentrations of detergent. Earlier workers had observed such precipitation of serum albumin and several other proteins only at *p*H values lower than the isoelectric point. The isoelectric point of serum albumin is 4.8 (Putnam and Neurath, 1944).

High Dodecyl Sulfate Concentrations.—Beginning at a dodecyl sulfate concentration of about $1 \times 10^{-3}$ M, the difference spectra observed took on a new character as the detergent concentration was raised to $6 \times 10^{-3}$ M, which is approximately the critical micelle concentration (Powney and Addison, 1937). Beyond $6 \times 10^{-3}$ M (to $1 \times 10^{-1}$ M) no significant further change was noted (Fig. 3–5). The two types of difference spectra occurred at different detergent concentrations, and by subtracting that observed at a concentration of dodecyl sulfate of $1.04 \times 10^{-3}$ M from those observed at higher concentrations, it was possible to determine what type of difference spectrum was observed for the second step alone (Fig. 5). These difference spectra can be regarded as the difference spectra that would be observed if, instead of serum albumin in phosphate buffer as the reference solution, serum albumin in buffer containing $1.04 \times 10^{-3}$ M dodecyl sulfate had been used.

As can be seen from Figure 5, the difference spectra observed in the dodecyl sulfate concentration range from $1 \times 10^{-3}$ M to $2 \times 10^{-2}$ M are typical tyrosyl difference spectra. They have a large minimum at 287 mμ and a smaller one at 280 mμ. The largest value of $\Delta\epsilon_{287}$ observed is −4400, at the top of the second step in Figure 4. This value for the blue shift on denaturation is of the same order of magnitude as that observed by Williams and Foster (1959) in the expansion of the molecule at low *p*H. Williams and Foster observed values ranging from −4000 to −7000, the exact value depending on the supporting electrolyte (and hence, probably, on the ions bound to the protein). This is suggestive, but of course is not proof, that the same parts of the molecule are involved in the conformational changes observed at low *p*H and in concentrations of dodecyl sulfate of $10^{-3}$ M and higher.

It can be noted that if, from the value of $\Delta\epsilon_{287}$ in the difference spectra for the second conformational change of serum albumin, values for $\Delta\epsilon_{294}$ are calculated assuming that only tyrosyl residues are involved, then within the experimental error the calculated and observed values agree with each other. This shows two things: (1) the first conformational change, which has its greatest extinction difference at 294 mμ, is complete when the concentration of dodecyl sulfate is $10^{-3}$ M, as we have assumed in our treatment of the data; and (2) solvent effects of the dodecyl sulfate on exposed tryptophyl residues, which might be expected to cause a red shift in the spectrum at higher concentrations (Yanari and Bovey, 1960), do not play any significant role at the concentrations used here.

Comparison with Other Observations.—The two spectrophotometrically observable changes noted when serum albumin interacts with dodecyl sulfate at concentrations between $10^{-1}$ M and $10^{-4}$ M are consistent with other evidence of conformational changes. Markus and Karush (1957) observed first a decrease in the levorotation of 1% solutions of serum albumin (human, *p*H 7.4) with concentrations of dodecyl sulfate between $5 \times 10^{-4}$ M and $1.6 \times 10^{-2}$ M, with a minimum reached at $10^{-3}$ M, a concentration at which approximately 10–14 moles of detergent are bound by each mole of serum albumin (bovine, *p*H 6.1, Karush and Sonenberg, 1949). Because 0.35% solutions were used in the present study, it is not strictly comparable with that of Markus and Karush. However, the detergent-protein mixing ratios for the two phenomena are very nearly the same. At higher detergent concentrations, Markus and Karush observed the levorotation to increase to values higher than that of the native protein. In the present study with bovine serum albumin the

initial spectrophotometric change at 294 mμ (Fig. 2) was noted at a detergent concentration of $2.1 \times 10^{-4}$ M. If the data for human and bovine serum albumin are compared,[3] it appears that the spectrophotometrically observable change in tryptophyl environment and those changes in secondary and tertiary structure that cause the optical rotation to change occur together. Furthermore, Markus and Karush decided, as we have, that this conformational change is a small one. The second spectrophotometric step in the present study occurred at a detergent concentration of $2 \times 10^{-3}$ M, a concentration above which Markus and Karush observed an increase in the levorotation.

The results of the present study are also consistent with the observations of Pallansch and Briggs (1954), who noted only a single electrophoretic boundary at dodecyl sulfate–serum albumin ratios below 8 or 10. As the detergent-protein ratio increased above this range, there appeared a second boundary of higher mobility. This component had a different mobility from the serum albumin in the absence of detergent. Thus, two distinct events corresponding to molecular conformational changes can be observed as the detergent-protein ratio is increased. That the critical ratio for the first of these is between 8 and 10 is further attested by the observations of Duggan and Luck (1948) that the relative viscosity of serum albumin–dodecyl sulfate solutions only increases progressively after a detergent-protein ratio of 8 to 10 is reached.

In later experiments Aoki (1958) observed that the reaction of horse serum albumin with dodecyl sulfate occurred in three steps at *p*H 6.8. The first step corresponded to statistical binding with the formation of complexes in which the ratio of detergent to albumin was from 10 to 12. In the second step, an albumin-detergent complex, $AD_n$, with $n$ equal to approximately 50, was formed, and in the last step another albumin-detergent complex, $AD_{2n}$, was formed. If our concentrations are converted to mixing ratios such as Aoki used, it is found that the tryptophyl difference spectra we observe at dodecyl sulfate concentrations around $2.1 \times 10^{-4}$ M correspond to the protein-detergent ratios at which Aoki's first step occurred. The second spectrophotometric change first appears at a dodecyl sulfate concentration of $10^{-2}$ M, a range which corresponds to the protein-detergent ratio of Aoki's third step, so we feel that the tyrosyl difference spectra observed are more likely to be accounted for by the conformational changes in the third step, that is, in the conversion of $AD_n$ to $AD_{2n}$.

Leonard and Foster (1961) have recently studied the conformational change at low *p*H by the techniques of optical rotation and ultraviolet spectrophotometry. In the presence of the strongly bound anions thiocyanate or perchlorate, the optical rotation of bovine serum albumin changes in two steps as the *p*H is lowered. Changes in the ultraviolet spectra, measured by the solvent perturbation technique of Herskovits and Laskowski (1960), show that approximately four tyrosyl residues become exposed to the solvent in the transition, and further that all of these are exposed to the solvent in the *p*H range where the second optical rotation step occurs.

It may therefore be possible to draw a parallel between the second and third steps observed by Aoki (1958), only the latter of which is accompanied by ultraviolet changes, and the two steps observed by Leonard and Foster (1961). The albumin molecule may unfold in two steps, which are similar whether the molecule is treated with dodecyl sulfate or with low *p*H.[4] In view of the large value of $\Delta\epsilon_{287}$ (−4400) in our second spectrophotometric step, it may well be that several tyrosyl residues are being normalized, or exposed to the medium, in the conversion of $AD_n$ to $AD_{2n}$. It should be kept in mind of course that we are comparing results obtained with horse serum albumin by Aoki (1958) with our own results on bovine serum albumin.

Although studies of optical rotation suggest that there is a disruption of secondary structure of serum albumin in the presence of dodecyl sulfate, and although there are data about alterations in tertiary structure, there is little information about specific intramolecular interactions. It is tempting to speculate about the role of aromatic residues which are implicated in the conformational changes. The difference spectra obtained in the present study suggest the involvement of the aromatic residues in maintaining the protein structure, perhaps by hydrogen bonding of the phenolic hydroxyl group to unidentified acceptor groups. Alternatively, these difference spectra would be consistent with the disruption of hydrophobic bonds in which tyrosyl groups are involved (Williams and Foster, 1959; Bigelow and Geschwind, 1960; Yanari and Bovey, 1960). Such conclusions have been suggested previously on the basis of ultraviolet difference spectra of serum albumin under the influence of urea or changes in the *p*H or ionic strength (Williams and Foster, 1959). It is doubtful that the effect of the detergent in the concentrations employed in this study is the consequence of its effect on the dielectric constant of the environment. Since the binding of detergent molecules to protein is well established, it appears more likely to be the result of protein conformational changes.

The examination of the ultraviolet spectra of proteins can be a valuable adjunct to other techniques which have been used to study the binding of small molecules. Because bound molecules may themselves be optically active (or may contribute to the optical rotation after being bound) ultraviolet spectra of proteins may be more useful than optical rotation in studying conformational changes

[3] The measurements with human serum albumin were made in 0.02 M phosphate buffer at *p*H 7.4 with 0.15 M NaCl (Markus and Karush, 1957), and our measurements were made in 0.025 M phosphate buffer at *p*H 6.1.

[4] We would like to thank Dr. J. F. Foster for drawing this interesting parallel to our attention, and for allowing us to read his manuscript (Leonard and Foster, 1961) before it was published.

caused by such binding. Although the bound molecules themselves may absorb ultraviolet light in the same spectral region as the protein (and therefore difference spectra may be observed for *them*), it should not be difficult to separate effects on the protein spectrum from effects on the spectra of at least the great majority of types of bound molecules. Finally, a comparison of the difference spectra observed when a single protein binds different molecules may be expected to shed some light on the problem of "configurational adaptability" (Karush, 1950).

*B. Ribonuclease*

Low Dodecyl Sulfate Concentrations.—In concentrations of dodecyl sulfate below $4.16 \times 10^{-3}$ M, ribonuclease is precipitated by the detergent, with no spectrophotometrically observable change to indicate changes in the molecular conformation. The fact that no conformation change is *observable* is proved by the appearance of the "difference spectrum" in Figure 6. As was explained above (see Results) this is the sum of two optical effects—light scattering in the experimental solution caused by the precipitated protein in suspension, and a ribonuclease *spectrum* arising from the fact that the precipitation makes the experimental solution less concentrated than the reference solution. It may be true that there are both precipitated (suspended) and dissolved molecules present in the cuvet, but we believe that the "difference spectrum" shows that neither is spectrophotometrically different from native ribonuclease. If there had been any spectrophotometrically observable conformational change, this would have been manifested by a third component, a *difference spectrum* with a minimum or maximum at 287 mμ. It should be emphasized that although no blue shift is observed on denaturation it does not necessarily follow that no conformational change occurs. It is, nevertheless, true that a molecule of 124 amino acid residues (Hirs *et al.*, 1960), three of which are abnormal tyrosyls (Tanford *et al.*, 1955), cannot undergo a very drastic conformational change without normalization of some of these abnormal residues.

The precipitation of ribonuclease at *p*H 6.1 is similar to earlier observations of the precipitating action of dodecyl sulfate on proteins at *p*H values below their isoelectric points (Putnam and Neurath, 1944). In the case of horse serum albumin, which was the protein most thoroughly studied by Putnam and Neurath (1944), the protein was precipitated by low concentrations of dodecyl sulfate at *p*H values below its isoelectric point. It is interesting to note that Putnam and Neurath decided that the precipitation of horse serum albumin by dodecyl sulfate caused an irreversible conformational change in the protein. They reached this conclusion from experiments on the hydrodynamic properties of the protein after the detergent had been removed by dialysis.

High Dodecyl Sulfate Concentrations.—Figures 7 and 8 show that between dodecyl sulfate concentrations of $5 \times 10^{-3}$ M and $8 \times 10^{-3}$ M (just above the critical micelle concentration) typical tyrosyl difference spectra are observed for ribonuclease. This means that the enzyme molecules are unfolding over this concentration range of the detergent, and that the unfolding occurs in such a way that some of the abnormal tyrosyl residues become exposed to the medium. From $10^{-2}$ M to $10^{-1}$ M dodecyl sulfate there is no further change in the difference spectrum.

Two conclusions are possible from Figure 8. First, it is seen that, if the points in the range of dodecyl sulfate concentrations where the conformational change occurs are extrapolated back, they reach the axis near the detergent concentration below which the protein is insoluble. This verifies that no blue shift on denaturation occurred in the

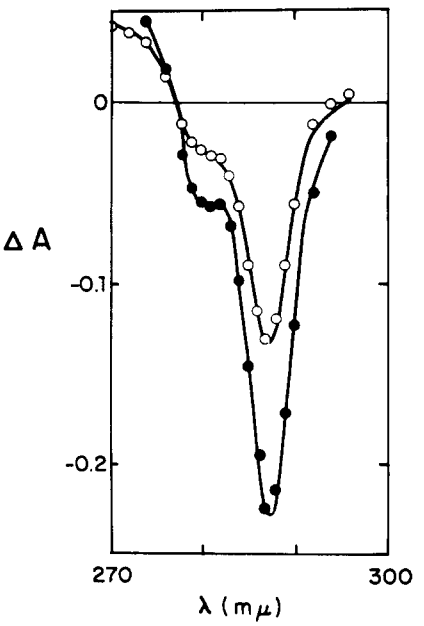


Fig. 7.—Difference spectra for ribonuclease at high concentrations of sodium dodecyl sulfate. ○, $6.24 \times 10^{-3}$ M; ●, $2.08 \times 10^{-2}$ M.

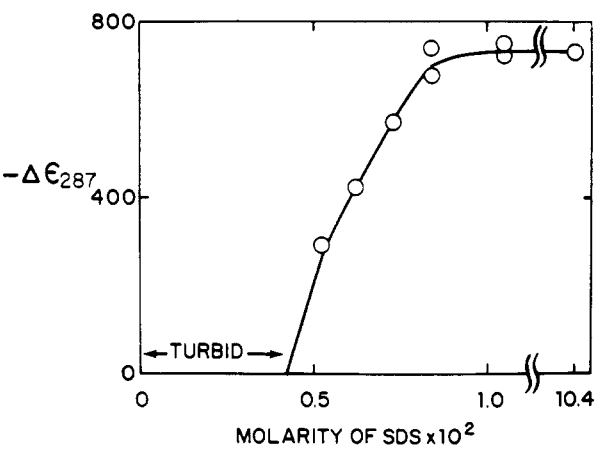


Fig. 8.—Variation of $\Delta\epsilon_{287}$ for ribonuclease as a function of the concentration of sodium dodecyl sulfate.

precipitation. Second, the value of the denaturation blue shift for the conformational change is $\Delta\epsilon_{287} = -740$. Difference spectra for a wide variety of ribonuclease derivatives and denatured forms have recently been examined, and they lead to the conclusion that the values of the blue shift on denaturation can be determined for the normalization of the individual abnormal residues (Bigelow, 1960, 1961). The abnormal residues, whose positions in the peptide chain so far remain unknown, have been called A, B, and C. A and C are each associated with blue shifts on denaturation of $\Delta\epsilon_{287} = -1000$; B is associated with a blue shift on denaturation of $\Delta\epsilon_{287} = -700$. It would therefore appear that dodecyl sulfate causes ribonuclease to undergo a conformational change in which only residue B is normalized.

Comparison with Other Observations.—It is interesting to consider our results in the light of some recently reported experiments of Stark *et al.* (1961) on the inactivation of ribonuclease by carboxymethylation with iodoacetate. They have shown that in native ribonuclease none of the four methionyl residues will react with iodoacetate at *p*H 5.5, although methionyl residues in a random coil will react. They also found that unexpected reactivity, presumably due to some feature of the molecular folding, is conferred on one of the four histidyl residues. They found that in 0.2 M dodecyl sulfate the unusual inertness of the methionyl residues was maintained but the unusual reactivity of the histidyl residue was destroyed. They suggested that an increase in the net negative charge of the molecule caused by the binding of negatively charged detergent molecules might make the approach of iodoacetate ions to the critical histidyl impossible.

Our results show that there is another possible explanation for the observations of Stark *et al.* (1961) on the course of the carboxymethylation in dodecyl sulfate solutions. We think it is likely that the conformational change we have observed is responsible for the alteration of the reactivity of the abnormal histidyl residue. It is clear from the results of Stark *et al.* (none of the abnormal methionyl residues is affected) and from our results (only one of the three abnormal tyrosyl residues is affected) that the conformational change is a small one.

If the histidyl residue which is abnormal in native ribonuclease is the one at position 119 (Hirs *et al.*, 1960) of the peptide chain, as has been concluded by Barnard and Stein (1959), it is possible to speculate on the causes of the normalization of abnormal tyrosyl B. Several possibilities which require only the derangement of the carboxyl-terminal portion of the molecule exist. For example, if abnormal tyrosyl B were the residue in position 115 of the peptide chain (Hirs *et al.*, 1960), and if it were abnormal because of interaction with other parts of the molecule, dodecyl sulfate would only have to unfold the carboxyl-terminal part of the chain as far as residue 115, and such a process could account for the normalization of both tyrosyl 115 and histidyl 119. Other possibilities of course exist, and further experiments will be needed before a decision can be made between them.

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