TEMPERATURE DIFFERENCE SPECTRA

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The optical activity of a protein solution is very sensitive to changes in the protein configuration. Thus, the large increase in levorotation that commonly occurs when a protein solution is heated has been interpreted as the result of a protein unfolding process. A detailed study of the effects of temperature and urea on the unfolding of ribonuclease and lysozyme has just been completed in this laboratory. (Foss and Schellman 1959, Foss, submitted for publication.) While the optical activity of a protein is a sensitive measure of configurational changes it gives no direct information about what amino acid residues are participating in the unfolding. This paper is a preliminary report on some spectral measurements which clearly implicate some of the side chain chromophores in the heat induced unfolding process.

EXPERIMENTAL

In order to measure the changes in spectra that occur when ribonuclease and lysozyme unfold the following simple procedure was used. A protein solution was placed in the sample chamber of a Cary 11 spectrophotometer and the solvent in the reference chamber. Both were in 1 cm cuvettes. The protein solution was heated to a temperature high enough to cause it to unfold--as determined from the rotation-temperature data obtained earlier. Difference spectra were run in the same way except that identical protein solutions

were placed in both cuvettes.

It was found that the sample temperature can very easily be controlled as follows. Remove the sliding shelf in the sample chamber and the base from the Cary 1 cm cuvette holder. Remount the metal pillars of the cuvette holder on a narrow brass strip and clamp them between Beckman "Thermospacers". When the Thermospacers rest on a 1 cm support (with inlet and exit tubes pointed upwards) the cuvette will be the same distance from the base of the chamber as originally. Lateral adjustments should be made to minimize interference with the light beam. Make a new sample chamber cover with short copper tubes going through it for water circulation and for electrical leads for a thermistor if desired.

In the experiments to be reported the heated samples were taped shut to reduce evaporation and the sample temperatures were not measured directly with a thermistor probe. It was learned earlier that even at 70° the sample temperature would come within one degree of the bath temperature in less than 10 minutes after the water circulation was started. For this reason the spectra of all the heated proteins were measured 10 minutes after circulation began.

The ribonuclease (Lot 381-059) and lysozyme (Lot D-638040) were both purchased from Armour and Co. The ribonuclease was made up in .1 M potassium chloride at a concentration of 1.02 mg/ml. The lysozyme was made up in a solution .1 M in potassium chloride and 8 M in urea. Its concentration was .38 mg/ml. No buffers were used.

RESULTS

Figure 1 shows the ordinary spectra and the difference spectra for the ribonuclease and lysozyme samples. It can be seen that the light absorbance between 270 and 300 mμ decreases for both proteins when they are unfolded by heating. There is a slight increase in

the absorbance of the ribonuclease below 270 mμ and of the lysozyme above 290 mμ.

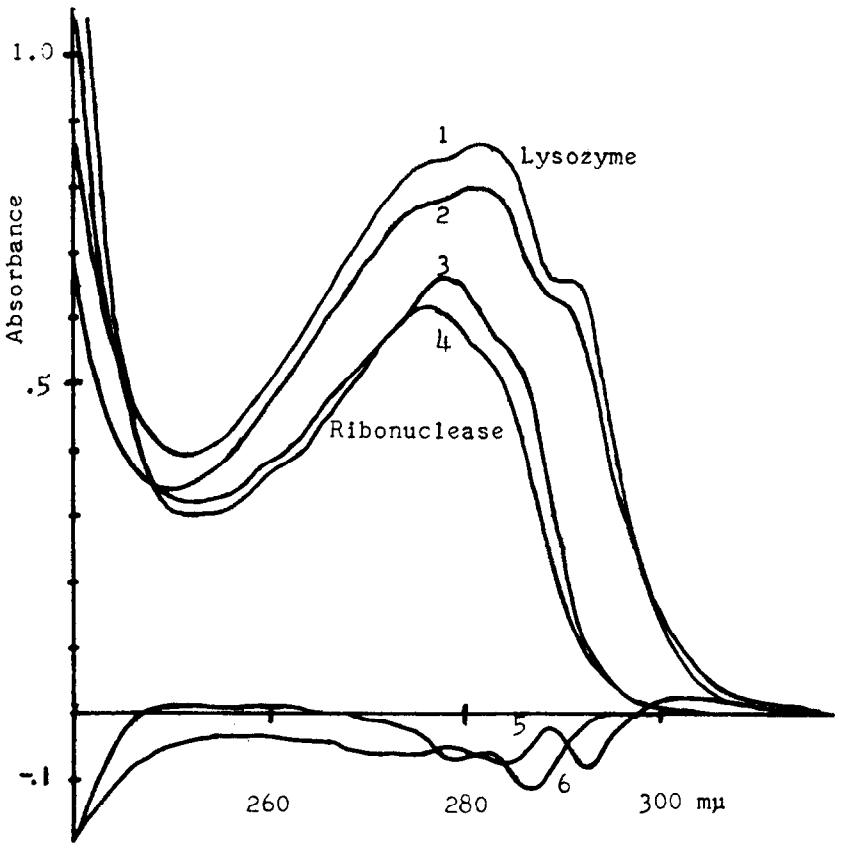

Fig. 1. Spectra and temperature difference spectra of lysozyme and ribonuclease. 1) Lysozyme in 8 M urea (and .1 M potassium chloride) at room temperature. 2) The same but lysozyme at 55°. 3) Ribonuclease in .1 M potassium chloride at room temperature vs. .1 M potassium chloride at room temperature. 4) The same but ribonuclease at 70° 5) Temperature difference spectrum of different aliquots of the above lysozyme solution. Reference solution at room temperature, sample at 55° 6) Temperature difference spectrum of different aliquots of the above ribonuclease solution. Reference solution at room temperature, sample at 70°.

When the ribonuclease undergoes this heat induced configurational transition its spectral change is very similar to that reported by Sela and Anfinsen who unfolded the protein with 8 M urea at room temperature. (Sela and Anfinsen 1957) The difference spectrum between the heated and unheated ribonuclease has two prominent peaks

at 287 and 279 mμ with peak heights (ΔA) of $\Delta A_{287} = -.166\ A_{278}$ and $\Delta A_{279} = -.106\ A_{278}$. Again this is very similar to the results obtained by Sela and Anfinsen.

Lysozyme is more difficult to unfold and for this reason the first experiments were performed in 8 M urea. The results are qualitatively similar in that there is a general decrease in absorbance on unfolding but the difference spectrum has peaks at 292 and 284 mμ with $\Delta A_{292} = -.094\ A_{282}$ and $\Delta A_{284} = -.082\ A_{282}$.

In all of these experiments the spectral changes were completely reversed when the protein solutions were returned to room temperatures. The time of heating was never more than thirty minutes.

DISCUSSION

The technique described above adds another method to the large number now available for studying proteins. To distinguish spectra obtained in this way from the usual "pH difference spectra" they will be referred to as "temperature difference spectra". Since the protein is observed while it is actually at a different temperature than the reference it is possible to study reversible changes.

Ribonuclease contains no tryptophan and consequently the observed changes must result from changes in the environment of the tyrosine residues. Similar changes in the pH difference spectrum of ribonuclease have been interpreted as the result of changes in tyrosyl-carboxylate hydrogen bonding (Scheraga 1957). But recent work vitiates this interpretation (Wetlaufer et al 1959).

The temperature difference spectrum for lysozyme is quite different from that of ribonuclease. It also differs from the pH difference spectrum for lysozyme reported by Donovan (Donovan 1959) A 5 Å shift in the tryptophan absorbance spectrum would lead to results similar to those observed (Chervenka 1959). Because of the large tryptophan-tyrosine ratio in lysozyme changes in the

tryosine spectrum would probably be masked.

This study shows very clearly that there are changes in the environment of the side chains of both ribonuclease and lysozyme when the molecules unfold. This is a rather conservative conclusion which would have been anticipated. But more interesting results should come from a careful study of the temperature dependence of these changes and a comparison with the optical activity-temperature data. In this way it may be possible to learn if the unfolding of the protein backbone is preceded or followed by changes in the side chains. The required experiments will be performed in the near future in this laboratory and will be described later.

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