SPECTRAL CHANGES ACCOMPANYING THE THERMAL DENATURATION OF COLLAGEN

G. C. WOOD

School of Pharmacy, Royal College of Science and Technology, Glasgow.

Received August 12, 1963

The denaturation of some globular proteins is accompanied by a marked increase in extinction coefficient in the range 190 - 200 mμ (Goldfarb, 1951; Rosenheck and Doty, 1961) and by a decrease in extinction coefficient in the range 220 - 240 mμ (Glazer and Smith, 1960, 1961). As a result of spectral studies on synthetic α-helical polypeptides and the changes of spectra which accompany the α-helix——random coil transition (Imahori and Tanaka, 1959; Tinoco et al, 1961; Rosenheck and Doty, 1961; see Wetlaufer, 1962 for a review) it seems certain that the change in the lower range reflects the effect of main-chain conformational changes on absorption of the peptide group. Glazer and Smith (1960, 1961) suggested that the spectral changes between 220 mμ and 240 mμ can also be attributed to the effect of main-chain conformational changes on peptide group absorption but it seems likely that other phenomena also contribute to the changes in this range.

During a study of the thermal denaturation of collagen solutions observations were made of spectral changes, particularly in the more accessible 220 - 240 mμ range. Solutions of acid soluble calf skin collagen and neutral salt soluble rat skin collagen in 0.1 M NaCl adjusted to pH 4.2 with 0.005 M acetate buffer were prepared as described by Wood and Keech (1960) and Wood (1962). Spectra were determined either in a Unicam SP 500 spectrophotometer with a constant temperature cell holder (stray light $< 0.1\%$ at 220 mμ, $< 0.3\%$ at 210 mμ) or in a Hilger Uvispek spectrophotometer with nitrogen-flushing (stray light $<$ 0.5% at 195 mμ, $<$ 2% at 190 mμ). Below 200 mμ, the above solvent was replaced by dilute HCl, pH 4.

The difference spectrum (Figure 1) of a solution of heat denatured acid soluble collagen relative to the unheated solution shows a prominent peak at 223 mμ whose magnitude is of the same order as that observed in globular proteins (Glazer and Smith 1960, 1961) but which is of opposite sign. An effect of the same magnitude was observed with neutral salt soluble collagen. A plot of the extinction of a collagen solution against temperature (Figure 2) shows a sharp discontinuity between 35°C and 40°C which clearly associates the change in the spectrum with denaturation as detected by viscometry, optical rotation etc. This discontinuity is superimposed on a gradual change with temperature, presumably due to temperature broadening of the spectrum.

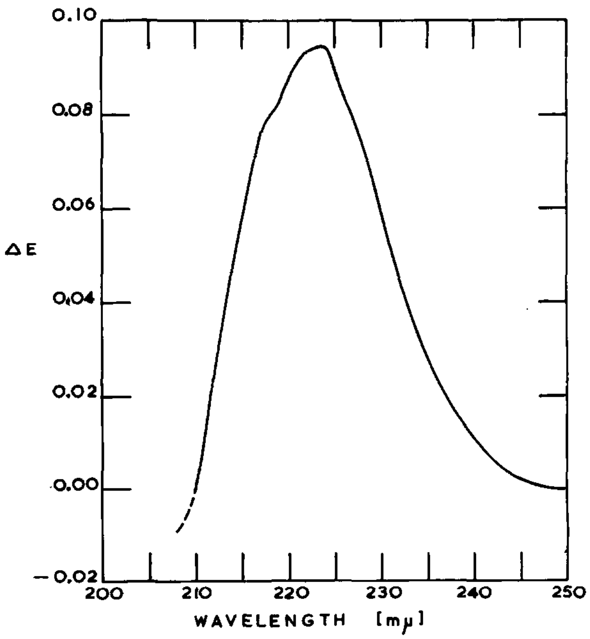

Figure 1. Difference spectrum at 25°C of a solution (0.014g./100 ml.) of calf skin acid soluble collagen in 0.1 M NaCl pH 4.2 heated to 45°C for 15 min. and immediately cooled to 25°C. Reference cell — unheated collagen solution.

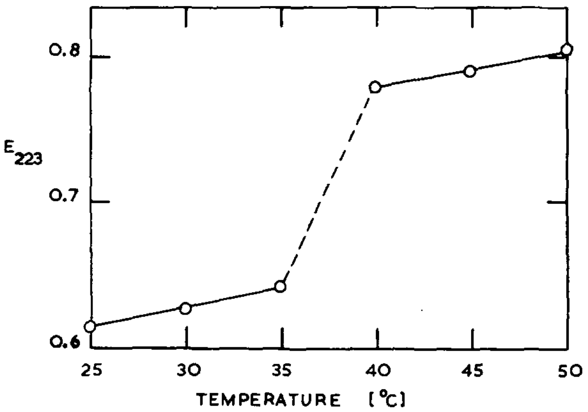

Figure 2. Extinction (223 mμ) of a solution of calf skin acid soluble collagen at different temperatures. Reference cell 0.1 M NaCl, pH 4.2. Solution maintained at each temperature for 15 min. before extinction recorded.

Determination of the absorption spectra of native and denatured collagen in the range 190 - 230 mμ in the second instrument, while confirming the change at 223 mμ showed (Figure 3) the absence of pronounced permanent changes at lower wavelengths although there is a small decrease of extinction between 198 mμ and 207 mμ. The nature of these spectral

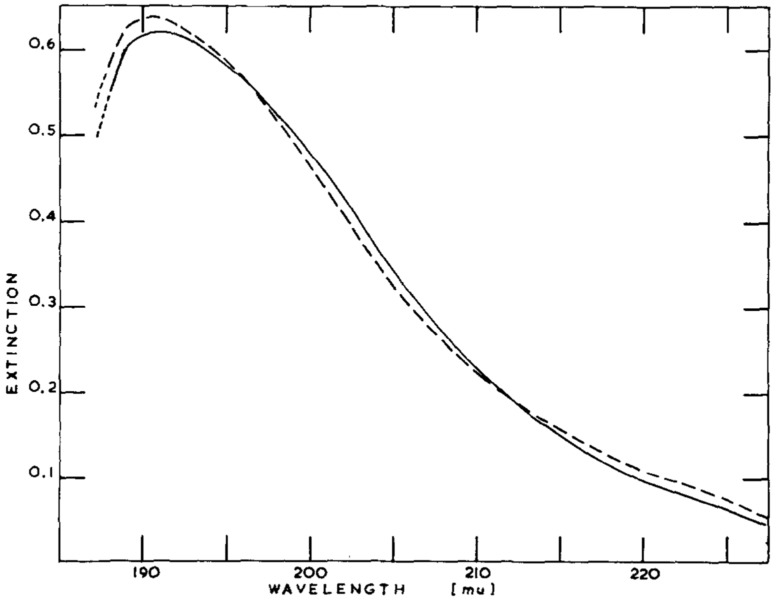

Figure 3. Absorption spectra of a solution of calf skin acid soluble collagen at room temperature (full curve) and of a sample of the same solution which had been heated to 60°C for 15 min. and immediately cooled to room temperature (broken line). (Conc. 0.0012g./100 ml.).

changes is as yet unknown but one may speculate that they are a result of conformational changes in the polypeptide chains. It may be significant that the low wavelength change occurs in the region of the imide absorption band of poly - L - proline (Fasman and Blout, 1963) and that Blout et al (1963) have recently observed a pronounced negative Cotton effect in the optical rotatory dispersion of collagen, with a minimum at 207 mμ and an inflexion at about 195 mμ, whose magnitude is greatly reduced on denaturation.

Study of spectral changes may prove to be a useful additional means of investigating the denaturation of collagen. Figure 4 shows the change of $E_{223\ m\mu}$ and viscosity during denaturation of acid soluble collagen at 37°C.

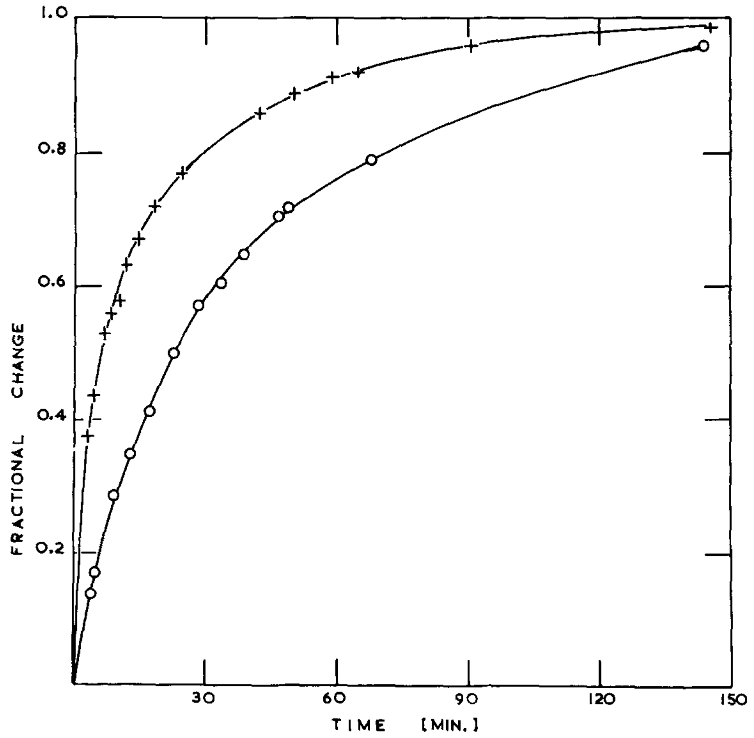

Figure 4. Fractional change in viscosity, $(\eta_0 - \eta_t)/(\eta_0 - \eta_\infty)$, (O) and extinction, $[(E_{223})_t - (E_{223})_0]/[(E_{223})_\infty - (E_{223})_0]$, (+) during denaturation of a solution (0.034g./100 ml.) of calf skin acid soluble collagen at 37°C. η_0, $(E_{223})_0$; η_t, $(E_{223})_t$; η_∞, $(E_{223})_\infty$ are the viscosity and extinction at 223 mμ, respectively, at zero time, time t and 4 hours.

Clearly the spectroscopic change is considerably slower ($t_{0.5} \simeq 1500$ sec.) than the viscosity change ($t_{0.5} \simeq 400$ sec.) indicating that the asymmetry of the collagen molecules (or their aggregates) is reduced before substantial changes occur in polypeptide chain conformation. Several workers (Doty and Nishihara, 1958; Hannig and Engel, 1961; Wood, unpublished results) have found that optical rotation, which is also closely related to chain conformation, changes during thermal denaturation at about the same rate as viscosity. The recent results of Engel (1962) suggest, however, that change of optical rotation lags behind the fall of viscosity to about the same extent as the change of $E_{223\ m\mu}$. Further work is required to clarify this.

The technical assistance of Mr. J. Pickard is gratefully acknowledged.

References

Blout, E. R., Carver, J. P. and Gross, J. (1963). J. Amer. Chem. Soc., 85, 644.

Doty, P. and Nishihara, T. (1958). In "Recent Advances in Gelatin and Glue Research" (G. Stainsby, ed.) p 92. Pergamon Press, London.

Engel, J. (1962). Arch. Biochem. Biophys., 97, 150.

Fasman, G. D. and Blout, E. R. (1963). Biopolymers, 1, 3.

Glazer, A. N. and Smith, E. L. (1960). J. Biol. Chem., 235, PC43.

Glazer, A. N. and Smith, E. L. (1961). J. Biol. Chem., 236, 2942.

Goldfarb, A. R. (1951). Science, 114, 177.

Hannig, K. and Engel, J. (1961). Leder, 9, 213.

Imahori, K. and Tanaka, J. (1959). J. Mol. Biol., 1, 359.

Rosenheck, K. and Doty, P. (1961). Proc. Nat. Acad. Sci. U.S., 47, 1775.

Tinoco, I., Halpern A. and Simpson, W. T. (1962). In "Polyamino acids, Polypeptides and Proteins" (M. A. Stahmann, ed.) p 147. University of Wisconsin Press, Madison, Wisconsin.

Wetlaufer, D. B. (1962). Adv. Protein Chem., 17, 304.

Wood, G. C. and Keech, M. K. (1960). Biochem. J., 75, 588.

Wood, G. C. (1962). Biochem. J., 84, 429.