Slow Exothermic Interactions in Aqueous Solutions of Gelatin

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Dilute aqueous solutions of gelatin have been studied at 25 °C by calorimetry and by circular dichroism. The solutions were preheated to 60 °C and rapidly cooled to 25 °C before the measurements were made. Thus the initial state of the solutions is non-equilibrium, and the slow, strongly exothermic relaxation was followed over periods of hours. Heat effects observed are tentatively ascribed partly to associations of gelatin molecules, and partly to the formation of helical structures of gelatin molecules accompanied by structural changes of water.

Gelatin consists of linear polypeptide chains, obtained by acid or basic treatment of collagen.[1,2] Aqueous solutions of gelatin (concentration >1 %) form semi-solid gels upon being cooled. Cross-links in gelatin gels are non-covalent bonds, and the gels melt reversibly upon being heated. [3-6] It is, however, a general observation that the formation of gel structures after cooling of a gelatin sol to room temperature is a slow process (taking hours or even days), and slow processes in gelatin–water systems heavily burden investigations and characterization of these systems. A gelatin gel is rarely in an equilibrium state: physical properties of a gel depend on the history of the sample considered.

The aim of the present paper is to contribute to the elucidation of the slow interactions in aqueous gelatin solutions. Dilute solutions (0.5–2.5 %) were studied at 25 °C over a number of hours. Immediately before an experiment, the gelatin solution was heated to 60 °C for a few minutes, and then rapidly cooled to room temperature. The aim of this cycle of heating and cooling was to obtain a solution of sub-cooled, randomly coiled polypeptide chains. Heat effects and circular dichroism were measured as the solution relaxed to equilibrium.

Experimental

Gelatin P-104 (210 Bloom) was a gift from Nordisk Gelatine, Copenhagen, and used without modification. The weight average molecular weight, $M_w = 9.9 \times 10^4$, was measured at Department of Chemistry, Risø National Laboratory, by a combination of gel chromatography and light scattering.[7]

Gelatin solutions were prepared by swelling the protein with glass-distilled water at room temperature for one hour, before hot water was added. Concentrations are based on weighing.

Calorimetric measurements were made at the Thermochemistry Laboratory, Chemical Center, University of Lund, Sweden. The calorimeter used was an LKB Thermal

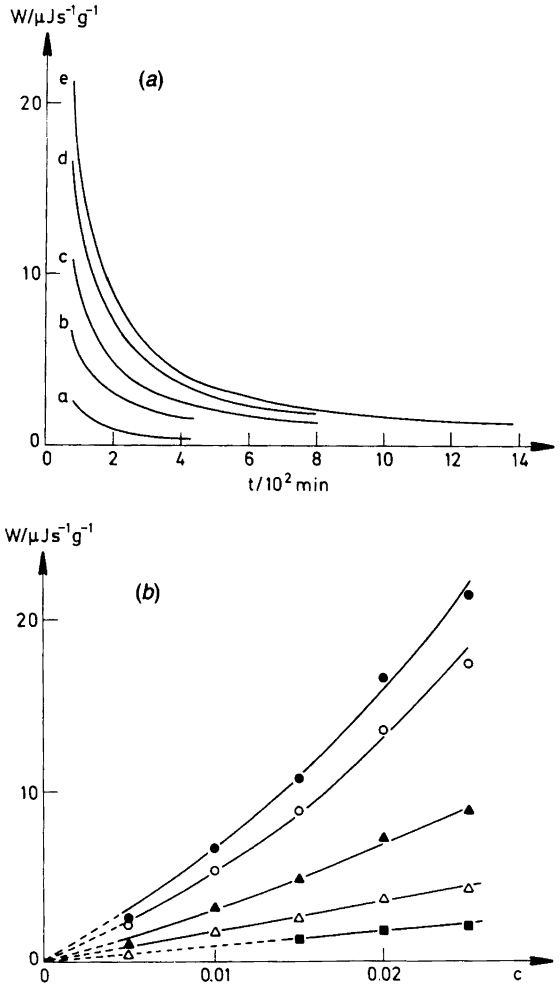

Fig. 1. Heat effects, $W = -dH/dt$, in aqueous solutions, at 25 °C, measured per gram of solution. *c* is the weight fraction of gelatin. As mentioned in the text, the solutions were preheated to 60 °C and cooled to 25 °C immediately before being transferred to the calorimeter. (*a*) *W* as a function of time at five concentrations; a, 0.50 %, b, 1.0 %; c, 1,5 %; d, 2.0 %; e, 2.5 %. (*b*) *W* as a function of concentration at 80 (•), 100 (○), 200 (▲), 400 (△) and 800 min (■).

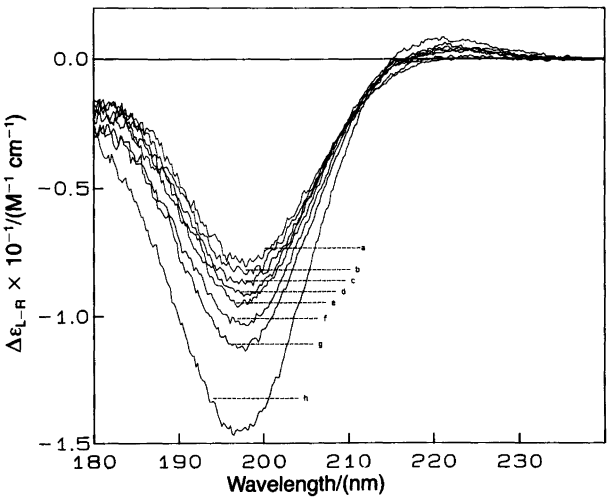

Fig. 2. Circular dichroism of a gelatin solution at 25 °C. The weight fraction of gelatin is 6.1×10^{-3}. The solution was preheated to 60 °C, and then cooled rapidly to 25 °C. The time (min) spent at 25 °C was: a, 13; b, 80; c, 2.5×10^{2}; d, 1.2×10^{3}; e, 2.5×10^{3}; f, $6{,}0\times10^{3}$; g, 1.6×10^{4}; h, 2.6×10^{4}.

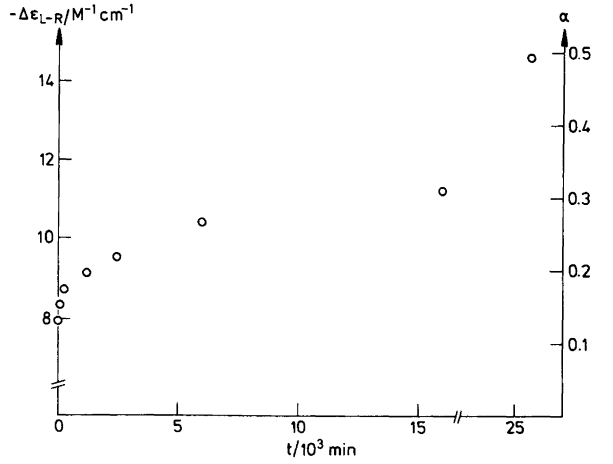

Fig. 3. Circular dichroism at 198 nm (left-hand scale), and helical content (right hand scale) of the gelatin solution studied in Fig. 2. The helical content was calculated according to eqn. (1).

Activity Monitor (TAM).[8] The sample size was 5 g. Owing to the relatively large time constants of the calorimeter the voltage–time lags behind the input power–time until steady-state conditions are reached.[8] Only measurements obtained after more than 80 min are considered in the present paper.

Circular dichroism was measured at the NOVO Research Institute, Copenhagen, using a thermostatted Jobin-Yvon-Dichrograph Mark-V. The optical pathlength was 10 μm.

Results and discussion

Fig. 1 shows heat effects measured in gelatin solution at 25 °C. As mentioned in the introduction, the solutions were heated to 60 °C, and quenched at 25 °C immediately before being transferred to the calorimeter. The process studied is thus the relaxation of a solution of initially randomly coiled gelatin chains.

The heat production $W = -dH/dt$ per gram gelatin solution is shown in Fig. 1(*a*) as a function of time for five protein concentrations, and in Fig. 1(*b*) as a function of concentrations at five time intervals. The relaxation is a slow, exothermic process. The data does not permit any detailed kinetic analysis of the complicated pattern of simultaneous reactions occurring in the solutions,[9–11] but characteristic features do appear.

The rate of heat production decreases significantly with time over the entire time range studied [$dW/dt<0$, Fig.1(*a*)], and increases with increased gelatin concentration [$dW/dc>0$, Fig. 1(*b*)]. The upward curvature of the curves in Fig. 1(*b*) for t = 80 and 100 min ($d^2W/dc^2>0$) indicates that interactions between gelatin molecules contribute significantly to the intensive heat production in the early stages of the relaxation process. The approach to a linear relationship between heat production and gelatin concentration [dW/dc = const. for $t>400$ min, Fig. 1(*b*)] suggests that (pseudo) first-order reactions become predominant at later stages. This behaviour is in accordance with earlier observations that first-order reactions are rate-determining for gelation processes.[4–6,9,12]

In an attempt to specify more closely the slow, apparently first-order interactions we have measured the circular dichroism (CD) of a solution of gelatin concentration 0.62 %. Fig. 2 shows CD spectra in the wavelength range 180–240 nm. Spectral changes, observed at 198 nm, indicate an increased number of helical structures in the solutions with time.[13] Fig. 3 shows Δε at 198 nm as a function of time.

The helical content in gelatin indicated on the right hand scale of Fig. 3 is tentatively estimated on the basis of available values of Δε (198 nm) for collagen (Δε = −24 $M^{-1}\,cm^{-1}$) and randomly coiled gelatin (Δε = −5.5 $M^{-1}\,cm^{-1}$).[13] The fraction of helical structures of the polypeptide chains, α, is calculated as shown in eqn. (1).

$$\alpha = (\Delta\varepsilon/M^{-1}\,cm^{-1} + 5.5)/(-24 + 5.5) \qquad (1)$$

The slow, but steadily increasing amount of helical structure in the gelatin solution is demonstrated in Fig. 3. From the rate of helix formation, $d\alpha/dt$, (Fig. 3) and the heat production in the solution, W [Fig. 1(*b*), for c = 0.0061], the enthalpy change $\Delta H°$ following the helix formation is estimated according to eqn. (2) where $\Delta H°$ is the enthalpy

$$-\Delta H°\,(d\alpha/dt) = W(t)/0.0061 \qquad (2)$$

change per gram of gelatin.

Calculated values of $-\Delta H°$ do vary with time, but within the range 200–300 J g^{-1}, i.e. they are much larger than e.g. the heat of denaturation of globular proteins (typically

20 J g^{-1}),[14] or collagen (70 J g^{-1}).[15] The experimental data support the view that extensive structural changes of water accompany the formation of helical structures in gelatin solution, or that water molecules constitute integral parts of the helical structures.[16]

The behaviour of gelatin, compared with that of globular proteins, may be associated with the high content of the amino acids proline and hydroxyproline. The content in gelatin of these amino acids amounts to 15–25 %,[17] and this causes an excess of CO-groups over NH-groups in the polypeptide chain. Extensive formation of polypeptide structures such as α-helices or pleated sheets is therefore prevented, and thus the basis of globular protein conformations is excluded. The excess of CO-groups are left with water molecules as hydrogen-bond partners, and thermodynamic properties indicate that interactions with water are essential for gelatin and collagen structures.[15,16,18–21]

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References

1. Harrington, W. F. and von Hippel, P. H. *Adv. Protein Chem. 16* (1961) 1.
2. Veis, A. In: Ramachandran, G. N., Ed., *Treatise on Collagen,* Academic Press, London 1967, Vol. 1, Chap. 8.
3. Flory, P. J. and Weaver, E. S. *J. Am. Chem. Soc. 82* (1960) 4518.
4. Harrington, W. F. and Rao, N. V. *Biochemistry 9* (1970) 3714.
5. Eagland, D., Pilling, G. and Wheeler, R. G. *Faraday Discuss. Chem. Soc. 57* (1974) 181.
6. Finer, E. G., Franks, F., Phillips, M. C. and Suggett, A. *Biopolymers 14* (1975) 1995.
7. Almdal, K. *Thesis,* University of Copenhagen, Copenhagen (1989).
8. Suurkuusk, J. and Wadsö, I. *Chem. Scr. 20* (1982) 155.
9. Hauscka, P. V. and Harrington, W. F. *Biochemistry 9* (1970) 3754.
10. Godard, P., Biebuyck, J. J., Daumerie, M., Naveau, H. and Mercier, J. P. *J. Polym. Sci. 16* (1978) 1817.
11. Bochard, W., Bremer, W. and Keese, A. *Colloid Polym. Sci. 258* (1980) 516.
12. Piez, K. A. and Sherman, M. R. *Biochemistry 9* (1970) 4134.
13. Jenness, D. D., Sprecher, C. and Johnson, W. C., Jr. *Biopolymers 15,* (1976) 513.
14. Privalov, P. L. *Adv. Protein Chem. 33* (1979) 167.
15. Privalov, P. L. *Adv. Protein Chem. 35* (1982) 1.
16. Maquet, J., Théveneau, H., Djabourov, M., Leblond, J. and Papon, P. *Polymer 27* (1986) 1103.
17. Eastoe, J. E. In: Ramachandran, G. N., Ed., *Treatise on Collagen,* Academic Press, London 1967, Vol. 1, Chap. 1.
18. Berendsen, H. C. In: Franks, F., Ed., *Water, a Comprehensive Treatise,* Plenum Press, New York 1975, Vol. 5, Chap. 6.
19. James, D. W. and Rintoul, L. *Aust. J. Chem. 35* (1982) 1157.
20. Tenhu, H. and Sundholm, F. *Eur. Polym. J.* (1986) 629.
21. Tenhu, H., Rimpinen, O. and Sundholm, F. In: Kramer, O., Ed., *Biological and Synthetic Polymer Networks,* Elsevier Applied Science, London 1988, p. 87.

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