The Enthalpy of Interaction between Collagen Fibres and Denaturing Solvents

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It is well known that both urea and acid cause collagen fibres to swell and at high concentrations to denature (Rigby, 1961). The mechanisms by which these reactions occur are not well understood. Paz Andrade et al. (1975) have used microcalorimetry to study the reaction between urea and some globular proteins, and have interpreted the results in terms of the binding of urea to the peptide units.

The present work was undertaken to extend these observations to a non-globular protein, collagen, and to compare the results with those obtained for the interaction of collagen with dilute acid (HCl).

The collagen used was a bovine achilles-tendon preparation obtained from Calbiochem (Los Angeles, CA, U.S.A.). An LKB Sorption microcalorimeter (Kusano et al., 1971), which directly measures the heat of interaction between solids and liquids, was used to determine the enthalpy ($\Delta H$) of swelling of a known weight of collagen (5–10 mg) with about 0.4 cm$^3$ of the appropriate solution at 37°C.

Fig. 1 shows the enthalpy of interaction of urea with collagen as a function of the concentration of urea. The reaction of insoluble collagen fibres with water is exothermic and the mean enthalpy for this swelling reaction is $-3730 \pm 240$ J/mol ($-868 \pm 58$ cal/mol) (where a mol is one amino acid residue of mean mol.wt. 91). The effect becomes more exothermic with increasing urea concentrations, reaching a maximum at about 4–5 mol/dm$^3$. Above this concentration, $\Delta H$ decreases (absolute value) to a minimum at 6–7 mol/dm$^3$, and then increases again. We believe these results support the following mechanism for the interaction between collagen and urea. The increase in the exothermal effect observed is due, as postulated by Robinson & Jencks (1965), to the binding of the amine group of urea to the accessible peptide bonds via hydrogen bonds. The initial

![Graph showing the enthalpy of interaction of urea with collagen as a function of the concentration of urea.](https://example.com/image)

**Fig. 1.** Enthalpy of interaction of urea with collagen at 37°C as a function of the concentration of urea

Details are given in the text.

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increase in urea concentration (0–5 mol/dm³) probably involves breaking of intermolecular hydrogen and hydrophobic bonds and binding of the urea molecules to the exposed peptide groups. With further increase in urea concentration the triple helix of collagen collapses endothermically (Finch & Ledward, 1972), so that the resultant heat of interaction becomes less exothermic. At 7 mol/dm³ of urea the triple-helical structure is completely disrupted, exposing further peptide groups that interact with more urea causing an increase in the exothermal effect.

HCl was diluted to give solutions of various pH values. Fig. 2 shows the enthalpy of swelling of collagen in these solutions. As the pH is lowered from 5 to 2 there is a decrease in exothermal effect, which we believe relates to breaking of some intermolecular hydrogen bonds. At pH 2.0 there is a sharp decrease in the exothermal effect due to the endothermal collapse of the triple helix.

It seems that, in denaturing solvents, the collagen fibre loses its integrity in two stages. In the first stage, at low denaturant concentrations, the fibre swells with rupture of some of the more accessible interhelical bonds, whereas the less accessible intrahelical bonds break only at higher reagent concentration. This two-stage hypothesis for denaturation is supported by experiments, described by Rigby (1961), that measured contraction and stress in collagen fibres during denaturation.