A study of the hydration and thermodynamics of warm-water and cold-water fish collagens

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The hydrated volumes, V_h , of collagens extracted from various fish species were calculated by using the Simha–Einstein equation, and it was found that the hydration of warm-water fish collagen is greater than that of cold-water fish collagen (halibut). Although the intrinsic viscosities of warm-water fish (bigeye-tuna, carp and catfish) collagens are almost the same, the hydrated volume of bigeye-tuna collagen is approx. 1.5 and 3 times those of carp and catfish collagens respectively. The extent of hydration at 20 °C is in the following order: bigeye tuna > carp > catfish > halibut. The various thermodynamic activation parameters (ΔG^* , ΔH^* and ΔS^*) were calculated and it was found that they are useful for determining the exact denaturation temperature. It was calculated that the denaturation temperatures of halibut, bigeye-tuna, carp and catfish collagens are 17, 31, 32 and 26–30 °C respectively. The variations of hydration, intrinsic viscosity, denaturation temperature and the thermodynamic parameters with the variation of concentration of catfish collagen were also thoroughly examined. The change of thermodynamic parameters from coiled-coil to random-coil conformation upon denaturation of collagen were calculated from the amount of proline and hydroxyproline residues and compared with viscometric results.

INTRODUCTION

Collagen has unique chemical, physical and biological properties that provide a rewarding study to workers not only in a broad medical field, but also in fibre science and technology. The reason for studying collagen in the solution state is that traditionally biologists have been concerned with the chemistry and function of cells at a molecular level, since important physiological reactions usually occur in solution, and particular attention has been paid to temperature.

Gustavson (1956) observed that the shrinkage temperature, T_s , appeared to be directly related to the hydroxyproline content of the collagen fibres. Subsequently, other workers have shown that both T_s and T_d (thermal denaturation temperature) are linear functions of the total pyrrolidine content (Burge & Hynes, 1959; Piez & Gross, 1960). It has been proposed that steric restrictions to rotations around the polypeptide backbone that arise from the stereochemistry of the pyrrolidine ring act to stabilize the triple-helical structure by reducing the total configurational entropy change in the helix-coil thermal transition (Harrington & Sela, 1958). If it is assumed that the denaturation of collagen in both the macroscopic and the molecular state is a first-order phase transition of the 'melting' type with the free energy of unfolding $\Delta G_u = 0$, then the following relationship is predicted (Flory & Garrett, 1958):

$$T_d = \frac{\Delta H_r}{\Delta S_r} \quad (1)$$

where ΔH_r and ΔS_r are the enthalpy and entropy per residue. Although the evidence of the dependency of the enthalpy and temperature of the thermal transition on

the pyrrolidine content as shown by calorimetric determination (McClain & Wiley, 1971, 1972) should provide a direct method for determining the validity of the theories concerning stabilization of the collagen structure ($\Delta G = 0$ in the denatured state), the thermodynamic picture in this regard is in an unsatisfactory state.

In the present investigation we have measured the viscosity of various concentrations of catfish collagen solutions at various temperatures, and from these we have computed various thermodynamic parameters as well as the extent of hydration at temperatures below and above the denaturation temperature of this collagen. A comparative study of catfish collagen with other warm-water (bigeye-tuna and carp) and cold-water (halibut) fish collagens was also made.

MATERIALS AND METHODS

Extraction and purification of catfish collagen

Marine catfish were collected from Marina Beach, Madras, India. The fish were dissected and the air bladders removed. These were cleaned free of blood vessels and other adhering tissues and washed thoroughly with cold distilled water. The clean tissue was cut into small pieces, and the collagen was extracted according to the method of Piez *et al.* (1963), with 0.5 M-acetic acid at 4 °C for a total period of 48 h. The highly viscous solution was centrifuged at 10000 *g* for 30 min, and the clarified supernatant was dialysed against 0.02 M- Na_2HPO_4 . The precipitate obtained was centrifuged again and redissolved in 0.5 M-acetic acid. This procedure was repeated twice, followed by exhaustive

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Fig. 1. Plot of η/η_0 versus c using Einstein equation (2) for catfish collagen at various temperatures

The solvent medium was 0.1 M-acetic acid, pH 2.8. Temperature: ■ (curve 1), 20 °C; ▽ (curve 2), 25 °C; ▲ (curve 3), 30 °C; ○ (curve 4), 35 °C; ● (curve 5), 40 °C; × (curve 6), 45 °C.

dialysis against 0.05 M-acetic acid, and the collagen solution was then freeze-dried. The collagen samples from the other fish species were prepared according to the method of Kubota & Kimura (1975).

Viscosity measurements

The viscosity of various concentrations of collagen was measured by using an Ostwald viscometer at various temperatures with 0.1 M-acetic acid (pH 2.8). The flow times of both acetic acid (pH 2.8) and water were taken separately at various temperatures in order to enable

determination of the absolute viscosity values of collagen solutions. The viscometer had a flow time for water at 25 °C of 240 s and was calibrated by using 20% (w/v) sucrose solution in water. The temperature of measurement was accurate within ± 0.1 °C. Uncertainties in temperature, density measurement and the flow detection imparted a maximum error of $\pm 0.7\%$ to the measured viscosity.

RESULTS AND DISCUSSION

For spherical-shaped particles, the hydrated specific volume, V_h , of collagen solutions can be calculated by using the following Einstein (1906, 1911) equation:

$$\eta/\eta_0 = \eta_{rel.} = 1 + aV_h \cdot c \quad (2)$$

where $\eta_{rel.}$ is the relative viscosity of the solution, η and η_0 are the viscosities of the solution and the solvent respectively, and c is the concentration of the collagen solution. For spherical-shaped particles $a = 2.5$ and for non-spherical particles $a > 2.5$. The plot of η/η_0 versus concentration of collagen solutions is shown in Fig. 1. The V_h values for elongated rod-shaped particles of collagen were computed after obtaining ν values from the following Simha-Einstein (1940) equation (3) and are given in Tables 1 and 2:

$$[\eta] = \nu \left(\bar{v}_{collagen} + \frac{\delta}{\rho_{H_2O}} \right) \quad (3)$$

where $\bar{v}_{collagen}$ is the partial specific volume of collagen, δ is the g of water bound per g of collagen, ρ_{H_2O} is the density of water and ν is the asymmetry-of-shape factor. $\nu = 2.5$ for spherical shape of the particles and $\nu > 2.5$ for non-spherical shape. The values of δ and $\bar{v}_{collagen}$ are taken from Mandal *et al.* (1986).

Eqn. (2) is generally valid for electrolyte solutions. Although the solutions of collagen employed are not electrolyte solutions, eqn. (2) is valid up to 0.04 g/100 ml

Table 1. Hydrated specific volume, V_h , and intrinsic viscosity, $[\eta]$, of collagens extracted from various fish species

The pH of the solution was 2.8.

Temp. (t) (°C)	[η] (dl/g)				V_h (ml/g)			
	Bigeye-tuna collagen	Carp collagen	Catfish collagen	Halibut collagen	Bigeye-tuna collagen	Carp collagen	Catfish collagen	Halibut collagen
5	15.0	14.5	14.8	10.0	2.70	1.70	1.00	1.80
10	14.2	13.8	14.0	9.0	2.80	1.78	1.03	1.10
15	13.5	13.2	13.4	8.1	2.95	1.85	1.05	0.80
20	12.7	12.5	12.7	6.5	3.05	2.01	1.06	0.94

Temp. (t) (°C)	ν				$\bar{v}_{collagen}$ (ml/g)	δ (g/g)
	Bigeye-tuna collagen	Carp collagen	Catfish collagen	Halibut collagen		
5	1136.4	1098.5	1121.2	757.5	0.75	0.57
10	1118.0	1086.5	1102.2	708.6	0.73	0.54
15	1088.3	1064.1	1080.3	653.0	0.72	0.52
20	1048.8	1032.3	1049.6	379.7	0.71	0.50

Table 2. Hydrated specific volume, V_h , intrinsic viscosity, $[\eta]$, and some physicochemical parameters of catfish collagen at various temperatures

The pH of the solution was 2.8.

Temp. (t) (°C)	V_h (ml/g)	$[\eta]$ (dl/g)	ν	$\bar{v}_{\text{collagen}}$ (ml/g)	δ (g/g)
20	1.06	12.70	1049.6	0.71	0.50
25	1.81	5.50	469.5	0.70	0.47
30	2.30	1.60	141.3	0.68	0.45
35	1.69	1.30	118.3	0.67	0.43
40	1.67	0.80	74.5	0.65	0.42

for catfish collagen (at 20 and 25 °C). Above this concentration the equation is not applicable, as is shown in Fig. 1 by the broken curves. Eqn. (2) is also valid up to 0.035 g/100 ml for bigeye-tuna and carp (i.e. warm-water fish) collagens and 0.045 g/100 ml for halibut (i.e. cold-water fish) collagen (Mandal *et al.*, 1985).

The intrinsic viscosity, $[\eta]$, was determined by using the following equation:

$$\eta_{sp}/c = [\eta] + [\eta]^2 K \cdot c \quad (4)$$

Here, $\eta_{sp} = (\eta/\eta_0 - 1)$ is the specific viscosity of the solution, and K is the Huggins constant. The plot of η_{sp}/c versus c is shown in Fig. 2 for catfish collagen at various temperatures, and the $[\eta]$ values obtained from that plot are given in Table 2.

Hydrated volume

Table 1 shows that the hydrated volume, V_h , as well as intrinsic viscosity, $[\eta]$, of warm-water fish collagen (bigeye tuna, carp and catfish) are greater than those of cold-water fish collagen (halibut at 20 °C). It is generally accepted (Mandal *et al.*, 1980) that the hydration decreases with the increase in temperature in the case of non-ionic surfactants and some proteins. The same phenomenon (A. B. Mandal, P. K. Sehgal, F. Chandrasekaran & M. Chvapil, unpublished work) has also been observed with collagens of shark skin, bovine skin and desamido and type III (human placenta) collagens. In the case of rat tail tendon collagen (A. B. Mandal, F. Chandrasekaran, P. K. Sehgal & M. Chvapil, unpublished work) in a citrate buffer environment (pH 3.7) the hydration volume increases with increase in temperature up to 26 °C (from 5 °C to 26 °C), beyond which it decreases. However, it is not always easy to compare results for proteins in different buffers, even at the same pH. According to Chiancone & Gilbert (1965), acetic acid buffers produce their own characteristic effects because of the penetration of protein coils by undissociated acetic acid, which increases the overall volume.

Although the intrinsic viscosities of warm-water fish (bigeye-tuna, carp and catfish) collagens are almost the same, the hydrated volume of bigeye-tuna collagen is approx. 1.5 and 3 times those of carp and catfish collagens respectively. It was calculated that the hydration at 20 °C is in the following order: bigeye tuna > carp > catfish > halibut. The order of hydration can be changed when the temperature is below 15 °C.

The helix-coil phase transition during thermal de-

Fig. 2. Plot of η_{sp}/c for catfish collagen at various temperatures in order to determine the intrinsic viscosity

The solvent medium was 0.1 M-acetic acid, pH 2.8. Temperature: ○ (curve 1), 20 °C; ● (curve 2), 25 °C; △ (curve 3), 30 °C; ▼ (curve 4), 35 °C; □ (curve 5), 40 °C.

naturation in 0.1 M-acetic acid (pH 2.8) was monitored by specific-viscosity measurements. The plots of $(\eta_{sp}/c)_t / (\eta_{sp}/c)_{t=20\text{ °C}}$ versus temperature at various concentrations of catfish collagen are shown in Fig. 3. The denaturation temperature T_d of collagen was obtained as the temperature at which the η_{sp}/c value was half-completed. The T_d values for catfish collagen at con-

Fig. 3. Plot of $(\eta_{sp}/c)_t / (\eta_{sp}/c)_{t=20\text{ °C}}$ versus temperature for catfish collagen at various concentrations in order to determine the denaturation temperature

The solvent medium was 0.1 M-acetic acid, pH 2.8. Concn. of collagen: □, 0.03 g/100 ml; △, 0.04 g/100 ml; ○, 0.06 g/100 ml.

centrations of 0.03, 0.04 and 0.06 g/100 ml are 28, 27 and 26 °C respectively.

Nikoli & Benedek (1976) found that heat denaturation of lysozyme increases the hydrodynamic radius from 1.85 to 2.15 nm, and not to the 4.9 nm calculated for a random coil. Our results for catfish collagen suggest that hydrodynamic volume (rather than hydrodynamic radius) increases with increase in temperature only when the temperature is below the denaturation temperature of collagen. Similarly the hydrodynamic volume for rat tail tendon collagen (A. B. Mandal, F. Chandrasekaran, P. K. Sehgal & M. Chvapil, unpublished work) increases with increase of temperature below its denaturation temperature.

Thermodynamic parameters

Enthalpy. The enthalpy (or heat content) of activation, ΔH^* , was calculated by using the following Eyring (1980) equation:

$$\log \eta = \log \left(\frac{hN}{V} - \frac{\Delta S^*}{2.303R} \right) + \left(\frac{\Delta H^*}{2.303R} \cdot \frac{1}{T} \right) \quad (5)$$

where η is the absolute viscosity of collagen solutions, h is Planck's constant, N is Avogadro's number, V is the molar volume of the solvent medium, R is the universal gas constant and T is the temperature on the absolute scale.

The plot of $\log \eta$ versus reciprocal temperature on the absolute scale for obtaining transition temperature T_d is shown in Fig. 4. The ΔH^* values of collagen before and after denaturation were calculated from that slope and are depicted in Tables 3 and 4. We have calculated the Gibbs free energy of activation, ΔG^* , by using the following eqn. (6):

$$\Delta G^* = RT \cdot \ln \left(\frac{\eta V}{hN} \right) \quad (6)$$

After insertion of ΔG^* and ΔH^* in the following Gibbs equation:

$$\Delta G^* = \Delta H^* - T \cdot \Delta S^* \quad (7)$$

the entropy of activation, ΔS^* , was calculated at the corresponding temperatures. The ΔG^* and ΔS^* values

Fig. 4. Plot of $\log \eta$ versus $1/T$ using Eyring equation (5) for catfish collagen at various concentrations of collagen

The solvent medium was 0.1 M-acetic acid, pH 2.8. Conc. of collagen: $\triangle$ (scale VI), 0.01 g/100 ml; $\otimes$ (scale V), 0.02 g/100 ml; $\bullet$ (scale IV), 0.03 g/100 ml; $\blacktriangledown$ (scale III), 0.04 g/100 ml; $\circ$ (scale II), 0.05 g/100 ml; $\times$ (scale I), 0.06 g/100 ml.

are also given in Table 3 for various fish collagens. It is possible to calculate the value of ΔS^* from the intercept of the plot of $\log \eta$ versus $1/T$ by utilizing eqn. (5); but the intercept is not sharp enough, so we have avoided such calculations. Details of calculations of thermodynamic parameters from viscosity measurements are provided in our previous publications (Mandal *et al.*, 1980, 1985).

In Table 3 it can be seen that over the entire range of temperature of investigations the Gibbs free energy of activation, ΔG^* , decreased with the increase in temperature and is irrespective of the source of collagen. The observation of decrease of ΔG^* with increase in temperature is consistent with other collagens examined. There is no appreciable change of ΔG^* above the denaturation temperature of collagen, but at the temperature of denaturation and a little below there is a dramatic decrease in ΔG^* .

A similar change occurs in ΔS^* , the denatured state above the denaturation temperature possessing high ΔS^* . The denaturation temperature for various concentrations of collagen is shown in Table 3 with asterisk (*) marks. The values of ΔG^* , ΔS^* and ΔH^* for water at 25 °C are 9.15 kJ·mol⁻¹, 17.05 J·degree⁻¹·mol⁻¹ and 15.01 kJ·mol⁻¹ respectively. The increase of all these thermodynamic activation parameters for collagen in the present study indicate an ordering of the structure of water in these collagens.

In Table 3 it is also seen that all the thermodynamic parameters (ΔG^* , ΔH^* and ΔS^*) increase with increase in concentration of catfish collagen in acidic solutions. Similar increases were obtained with the other fish collagens examined. Although ΔG^* increases with the increase in concentration of both the desamido and type III (human placenta) collagens, there was a decrease of ΔH^* and ΔS^* .

The enthalpy of collagen transition strongly depends on the hydroxyproline content. The dependence between the enthalpy value, calculated per 1000 residues, and the hydroxyproline content, η_{1000}^{Hyp} , can be expressed in the first approximation by the following linear empirical equation (Privalov, 1982):

$$\Delta_{\text{CC}}^{\text{RC}} H_{1000} (25^\circ \text{C}) = (2400 + 35.8 \eta_{1000}^{\text{Hyp}}) \text{ kJ} \cdot \text{mol}^{-1} \quad (8)$$

where $\Delta_{\text{CC}}^{\text{RC}} H$ is the change in enthalpy of collagen from coiled-coil to random-coil conformation.

The first term of this equation corresponds to the transition enthalpy of a collagen-like structure that does not contain hydroxyproline, for example (Pro-Pro-Gly)_n polymer.

Entropy. The entropy of collagen transition should depend on at least three variables: the content of the amino acids, the hydroxyproline and the proline residues. Earlier it was assumed by Harrington (1964) that the entropy contribution of residues containing pyrrolidine rings at collagen 'melting' is zero, while the average contribution of all other residues is 17 J·degree⁻¹·mol⁻¹. However, it is now evident that the entropy contribution of hydroxyproline is far from being zero. By using the values for the proline and amino acid residues, it is possible to estimate the entropy contribution of hydroxyproline from the known entropies of collagen 'melting'. This estimate gives the value of 103 J·degree⁻¹·mol⁻¹. Thus the entropy of collagen

Table 3. Various thermodynamic activation parameters of collagens extracted from warm-water and cold-water fish species at different temperatures

The pH of the solution was 2.8. The solvent medium was 0.1 M-acetic acid.

Temp. (<i>t</i>) (°C)	ΔG^* (kJ·mol ⁻¹)	ΔH^* (kJ·mol ⁻¹)	ΔS^* (J·degree ⁻¹ ·mol ⁻¹)
Halibut collagen (0.03 g/100 ml)			
5	11.25	27.35	57.91
10	10.96		57.92
15	10.41		58.82
17*	9.90		60.17
20	9.73	31.91	75.70
25	9.51		75.17
30	9.37		74.39
Bigeye-tuna collagen (0.03 g/100 ml)			
5	12.79	19.15	22.88
10	12.67		22.90
15	12.56		22.88
20	12.47		22.80
25	12.28		23.05
30	11.60		24.92
31*	10.70	25.53	27.80
32	10.01		50.89
35	9.82		51.00
40	9.58		51.00
Carp collagen (0.03 g/100 ml)			
5	12.55	21.88	33.56
10	12.38		33.58
15	12.20		33.61
20	12.02		33.65
25 ¹	11.85		33.66
30	11.65		33.76
31	11.53	27.48	34.04
32*	10.87		54.46
35	9.91		57.05
40	9.57		57.22
Catfish collagen (0.03 g/100 ml)			
5	10.58	19.15	30.82
10	10.42		30.84
15	10.26		30.86
20	10.10		30.89
25	9.69		31.74
30*	9.20		32.84
35	9.06	25.53	53.47
40	8.92		53.07
45	8.85		52.45
50	8.75		51.95
55	8.69		51.34
Catfish collagen (0.05 g/100 ml)			
5	11.03	23.93	46.40
10	10.79		46.43
15	10.55		46.45
20	10.31		46.48
25	9.94		46.95
30*	9.39		47.99
35	9.15	28.72	63.54
40	9.02		62.94
45	8.95		62.17
50	8.85		61.52
55	8.78		60.80

transition at 25 °C can be written by the following empirical expression:

$$\Delta_{\text{CC}}^{\text{RC}} S_{1000} (25^\circ \text{C}) = [11 \times (1000 - \eta_{1000}^{\text{Pro+Hyp}}) + 5 \times \eta_{1000}^{\text{Pro}} + 103 \eta_{1000}^{\text{Hyp}}] \text{ J} \cdot \text{degree}^{-1} \cdot \text{mol}^{-1} \quad (9)$$

Details for estimating $\Delta_{CC}^{RC}H$ and $\Delta_{CC}^{RC}S$ values are given in the review by Privalov (1982). The calculated $\Delta_{CC}^{RC}H$ and $\Delta_{CC}^{RC}S$ values from the amount of proline and hydroxyproline residues as well as viscometric results are given in Table 4.

Unfolding of the triple helix

The plot of $\log \eta$ versus $1/T$ (Eyring's equation 5) is similar to the Van't Hoff plot of the equilibrium constant of micro-unfolding ($R \cdot \ln K$ versus $1/T$). As can be seen from the value of $\log \eta$, the concentration of the unfolded form in the pre-denaturational temperature range is very low, but it increases with an increase of temperature. On reaching the denaturational temperature range, the functional dependence of the plot of $\log \eta$ versus $1/T$ breaks abruptly. Hence one can conclude that there are two qualitatively different processes of collagen structure unfolding, the non-denaturational and the denaturational. Since the slope of a curve on the Eyring's plot corresponds to the enthalpy of a process, it follows from Fig. 4 that the non-denaturational and the denaturational unfoldings of collagen structure are characterized by qualitatively different enthalpies.

The enthalpy of the non-denaturational process (ΔH_b in our case) is small and is practically the same (about $25 \text{ kJ} \cdot \text{mol}^{-1}$) for all the collagens reported by Privalov (1982). However, we found for our fish collagens that the ΔH_b values obtained ranged from $19 \text{ kJ} \cdot \text{mol}^{-1}$ to $30 \text{ kJ} \cdot \text{mol}^{-1}$, depending on the concentration of collagen. Comparing it with the average calorimetric enthalpy of collagen melting ($5.5 \text{ kJ} \cdot \text{mol}^{-1}$), one can conclude that the non-denaturational unfolding of the collagen structure involves about four to six residues, i.e. one or two triplets, whereas the denaturational process involves a larger number of triplets, which unfold simultaneously.

An additional significant feature is the difference

between the values of $\log \eta$ for various collagens if we compare them at the same temperature. Since $RT \cdot \ln(\eta V/hN)$ is the Gibbs energy of the considered process, it follows that the changes in Gibbs energies from coiled-coil to random-coil conformation, which can be denoted as $\Delta_{CC}^{RC}G$, are different for collagens from various species. The values of $\Delta_{CC}^{RC}G$ obtained are presented in the last column of Table 4. Consideration of Table 4 leads us to the following conclusion: the enthalpy and the entropy of collagen transition from the coiled-coil to the random-coil state are specific characteristics of the given collagen molecule and vary greatly from species to species. Both of them correlate with the stability of collagen and both increase with the increase of stability expressed either as temperature of transition or the Gibbs energy value. This latter fact is in conflict with the relations expected from the existing models of collagen structure. According to the results obtained by Kubota & Kimura (1975), the value of $\Delta_{CC}^{RC}G$ for bigeye-tuna, carp and halibut collagens is negative, whereas the value of $\Delta_{CC}^{RC}G$ from our results for bigeye-tuna, carp and catfish collagens is positive and only negative in the case of halibut collagen. Our results are supported by the report by Privalov (1982). The reason for the discrepancy between the results obtained by Kubota & Kimura (1975) and our study in the case of warm-water fish collagens is probably due to the variation of hydroxyproline content. It is unlikely to be due to the presence of impurities, which do not alter the absolute value of the thermodynamic parameters (Rigby, 1967; Privalov & Tiktopulo, 1970; McClain *et al.*, 1971).

The denaturation enthalpy values determined by McClain & Wiley (1972) using a differential scanning calorimeter were in good agreement with the value of $4.8 \text{ kJ} \cdot \text{mol}^{-1}$ reported by Flory & Spurr (1961), based on the relationship between tensile strength and the equilibrium 'melting' temperature of rat tail tendon collagen,

Table 4. Chemical and thermodynamic characteristics of the helical part of collagens

For catfish collagen $\eta_{1000}^{\text{Pro}} = 107.5$, $\eta_{1000}^{\text{Hyp}} = 90$ and $\eta_{1000}^{\text{Pro+Hyp}} = 197.5$. For bigeye-tuna collagen $\eta_{1000}^{\text{Pro}} = 107.0$, $\eta_{1000}^{\text{Hyp}} = 84.8$ and $\eta_{1000}^{\text{Pro+Hyp}} = 191.8$. For carp collagen $\eta_{1000}^{\text{Pro}} = 116$, $\eta_{1000}^{\text{Hyp}} = 80$ and $\eta_{1000}^{\text{Pro+Hyp}} = 196$. For halibut collagen $\eta_{1000}^{\text{Pro}} = 114$, $\eta_{1000}^{\text{Hyp}} = 69.6$ and $\eta_{1000}^{\text{Pro+Hyp}} = 183.6$. The pH of the solution was 2.8.

Thermodynamic characteristics at 25 °C						
	T_d (°C)	ΔH_b (kJ·mol ⁻¹)	ΔH_a (kJ·mol ⁻¹)	$\Delta_{CC}^{RC}H_{\text{res.}}$ (kJ·mol ⁻¹)	$\Delta_{CC}^{RC}S_{\text{res.}}$ (J·degree ⁻¹ ·mol ⁻¹)	$\Delta_{CC}^{RC}G_{\text{res.}}$ (J·mol ⁻¹)
Catfish collagen (0.01 g/100 ml)	30*	15.32	21.88	6.52	21.75	—
Catfish collagen (0.03 g/100 ml)	30*	19.15	25.53	6.38 (5.62†)	21.70 (18.63†)	— (+ 68.77†)
Bigeye-tuna collagen (0.03 g/100 ml)	31*	19.15	25.53	6.38 (5.44†)	27.95 (18.15†)	— (+ 40.0†)
Carp collagen (0.03 g/100 ml)	32*	21.88	27.48	5.60 (5.27†)	23.88 (17.60†)	— (+ 30.0†)
Halibut collagen (0.03 g/100 ml)	17*	27.35	31.91	4.56 (4.89†)	17.79 (16.72†)	— (- 92.56†)
Catfish collagen (0.05 g/100 ml)	28*	23.93	28.72	4.80	16.50	—
Catfish collagen (0.06 g/100 ml)	26*	30.64	36.04	5.40	19.00	—

* T_d values obtained from the transition temperature of the plot of $\log \eta$ versus $1/T$.

† Values of $\Delta_{CC}^{RC}H_{\text{res.}}$, $\Delta_{CC}^{RC}S_{\text{res.}}$ and $\Delta_{CC}^{RC}G_{\text{res.}}$ calculated from the amount of proline and hydroxyproline residue.

and the value of $4.6 \text{ kJ} \cdot \text{mol}^{-1}$ determined by Kuntzel & Doehner (1939) from their calorimetric studies on bovine hide powder. After applying statistical mechanical theories, Harrington & Rao (1967) derived values of $\Delta H = 4.66 \text{ kJ} \cdot \text{mol}^{-1}$ for rat skin collagen and $4.74 \text{ kJ} \cdot \text{mol}^{-1}$ for cod skin collagen.

Data from our present investigation indicated that the denaturation enthalpy varies with concentration as well as the imino acid content of the collagen (Table 3). Utilizing direct calorimetric measurements, Privalov & Tiktopulo (1970), however, reported an increase in both ΔH and ΔS with increasing imino acid content. Our results are comparable with the findings reported by Privalov & Tiktopulo (1970). At the same concentration of collagen (0.03 g/dl) the denaturation enthalpy for catfish, bigeye-tuna, carp and halibut collagens are 6.38, 6.38, 5.60 and $4.56 \text{ kJ} \cdot \text{mol}^{-1}$ respectively (see Table 4).

The energy per triple helix of collagen in the activated state as calculated by eqn. (6) gives values from 9.9 to $12.8 \text{ kJ} \cdot \text{mol}^{-1}$ for our fish collagens (Table 3) and the data from Privalov & Tiktopulo (1970) revealed values of 18.4, 14.8, 10.8 and $9.6 \text{ kJ} \cdot \text{mol}^{-1}$ for rat, pike, cod and whiting skin collagens respectively.

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