Assembly of Amphiphilic Compounds and Rigid Polymers. 1. Interaction of Sodium Dodecyl Sulfate with Collagen

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ABSTRACT: To elucidate the role of fixed charge and chain rigidity on the assembly of amphiphilic compounds over a polymer substrate, we studied the interaction between sodium dodecyl sulfate (SDS) and crystalline or soluble collagen. Shrinkage temperatures and the equilibrium degree of swelling were measured for cross-linked tendons under isoelectric (pH = 6.0) conditions or in acid (pH = 2.5) solutions. Viscosities and solubilities were measured for soluble collagen to construct pseudo phase diagrams delimiting the field of stability of the helical, random coiled, and crystalline forms. Under isoelectric conditions, increasing concentrations of SDS (up to ≃0.1 M) cause a depression of transformation temperatures which is much larger than that observed with salts, with aliphatic alcohols, or with sodium methyl sulfate. This is attributed to the prevailing hydrophobic interaction of the surfactant with the random coiled form of collagen, as evidenced by adsorption of the surfactant onto the higly swollen, denatured tendon. The cooperativity of the binding process at pH = 6.0 confirms that peculiar micelle-like assemblies are formed by SDS within the disordered random coiled network. Under nonisoelectric conditions the transition temperature is not much affected by conformation. SDS brings about a dramatic collapse of the lateral dimension of tendons at pH = 2.5, as well as precipitation of soluble collagen, even at very low concentrations, revealing a cooperative phenomenon leading to an increased interhelix interaction. The extent of adsorption of the surfactant over the protein, considerably larger at this pH, emphasizes the strength of electrostatic interaction enhanced by the hydrophobic tail of the surfactant. However, poor cooperativity is shown by the binding data at pH = 2.5, suggesting that ordered assembly of cylindrical micelle-like aggregates is prevented by the low charge density of collagen. Tendons at low pH might be described as highly swollen oriented liquid crystalline networks and may undergo a phase transition to the crystalline state as a result of the strong binding between fixed charges and dodecyl sulfate ions.

Introduction

Due to their ability to strongly bind to both polar and hydrophobic residues, and to promote interchain association, amphiphilic molecules naturally play a major role in the assembly of composite structures. An essential role of the chain conformation of the polymeric ligand is anticipated. In a previous paper[1] we investigated the interaction of the anionic surfactant sodium dodecyl sulfate (SDS) with gelatin in fluid solution and in the gel state. Fluorescence of pyrene data suggested the occurrence of rather rigid microdomains in pure gelatin (without SDS) even below the critical gel concentration. The same technique also suggested the occurrence of less rigid micelle-like microdomains in the presence of SDS. Surfactant selective electrodes evidenced a strong binding of SDS to gels, while gel strength and solution viscosity went through a maximum on increasing surfactant concentration.

The interpretation of the above results was complicated by the ill-defined structure of the gelatin gel—a disorganized assembly of microcrystalline and amorphous regions[2]—and by the concurrent effects of SDS in destabilizing the triple helical aggregates[3-5] and in promoting micelle formation in anionic polyelectrolyte solutions.[6] A proper thermodynamic description of the system requires an independent characterization of these two effects, as well as an assessment of the thermodynamic phase (crystalline, amorphous, or liquid crystalline) which favors their occurrence.

In this work, we attempt a systematic investigation of the role of SDS in promoting conformational transitions and micelle formation for collagen in its various thermodynamic phases: crystalline tendons, soluble tropocollagen helices, amorphous cross-linked networks or soluble coils (see schematization in Figure 1). Pseudo phase diagrams delimiting the field of stability of each form as a function of temperature, pH, and SDS concentration are presented along with associated variations of the equilibrium degree of swelling of tendons. Here we follow the approach used earlier by Ciferri and co-workers.[7-11] These data are complemented by evaluation of the surfactant adsorption isotherms under the working conditions.

Theoretical guidelines for the micellization of amphiphilic compounds on coiling polyelectrolyte networks were recently put forward by Khokhlov and co-workers.[12] The collagen/SDS system is of particular interest since it allows an assessment of the effect of conformational rigidity and low charge density on the organization of the surfactant over a polymer substrate containing both hydrophilic and hydrophobic sites. It has been suggested that single coiling molecules can form loops enclosing microdomains of surfactant molecules.[13-15] However, one cannot expect this mechanism to occur with rigid helices. In the latter case one expects several rigid chains to surround hydrophobic clusters of amphiphilic molecules, modeling in principle the self-assembly of several biological structures.[16]

Experimental Section

Materials. Sodium dedecyl sulfate, SDS (Fluka, puriss), sodium methyl sulfate, sodium sulfate, and sodium chloride (Merck, p.a.) were used as received. Rat tail tendons were

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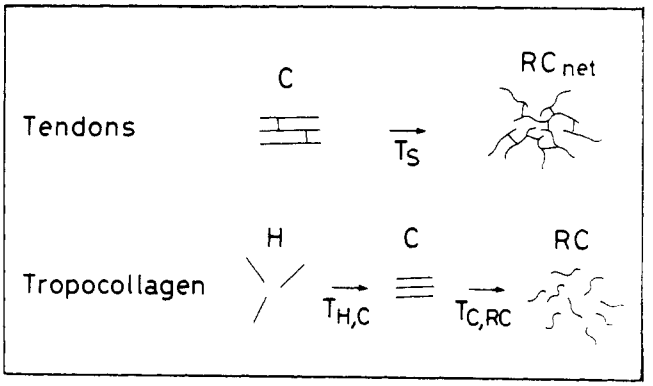

Figure 1. Transformation temperatures involving the insoluble crystal (C), the soluble tropocollagen (H), the insoluble random coiled network (RC_{net}), and the soluble random coiled (RC) forms of collagen.

obtained from 4 month old animals from the Instituto de Nutricion y Tecnologia de los Alimentos, University of Chile. Tendons, selected for uniformity and cross section (diameter ≃ 0.2 mm) were carefully washed with distilled water and stored in water at 4 °C for periods not exceding 10 days prior to use. Cross-linking was performed by equilibrating the tendons in a 0.1% *p*-benzoquinone solution for 1 h at ≃20 °C.[10] The cross-linking step prevented dissolution of tendons at low pH or above the shrinkage temperature, T_s. The latter was 58 and 68 °C for un-cross-linked and cross-linked tendons, respectively. The variability of T_s for individual tendons from the same or different animals was within ±2 deg. Prior to shrinkage or swelling measurements, tendons were equilibrated in a test tube with a large excess of the SDS–water (or salt–water) solutions after adjusting the pH with NaOH or HCl to the desired value (6.0 or 2.5). In order to prepare tropocollagen (i.e. the native triple helix unit of collagen) solutions, un-cross-linked tendons were treated with 0.5 M aqueous NaH_2PO_4 as described by Dimitru and Garrett.[17] Solubilization occurred in 1 day at 4 °C, and the excess salt was eliminated by extensive washing. The phosphorus content in the final solution was below 30 ppm,[7] protein content ≃0.2% (w/v, determined by dry weight), and pH = 4–5. Solutions were stored at 4 °C for less than 10 days with no evidence of protein degradation. Solutions of SDS or salt were added to the protein solution below 10 °C with proper concentrations as to obtain ternary solutions with polymer concentration C_p between 0.016 and 0.06% and SDS concentration (C_{SDS}) in the range 0.1–100 mM. Measurements were carried out under near isoelectric conditions (pH = 4) and when collagen is a weakly charged polyelectrolyte (pH = 2.5).[2]

Transformation Temperatures. In the case of tendons, the variation of length L with temperature was followed with a calibrated scale while the tendon swollen in excess solution was kept in a temperature-controlled (±0.1 deg) bath. The rate of increase of temperature was 3 deg/60 min in the region close to the shrinking temperature. The initial length (L_o) was determined at 20 °C in the corresponding solutions. The shrinkage temperature T_s (Figure 1) was determined from L/L_o *vs* T curves. In the case of tropocollagen, freshly prepared solutions of given pH and SDS (or salt) concentration were kept for about 10 h in a cold room under moderate stirring.[7] These solutions were then transferred to an Otswald viscometer placed in a termostated bath, increasing the temperature at 3 deg/60 min. If the solution remained homogeneous, the flow time (t) was measured. A sharp drop of the specific viscosity n_{sp} [$n_{sp} = (t/t_o) - 1$] was observed upon increasing the temperature and ascribed to the helix → coil transition. The helix to coil transformation temperature $T_{H,C}$ (Figure 1) was determined from n_{sp} *vs* T curves. If a precipitate was visually detected on increasing the temperature, the corresponding temperature was taken as the helix to crystalline transformation temperature $T_{H,C}$ (Figure 1).[7] The fibrous appearance of the precipitates was compatible with its crystalline character. Moreover, precipitates obtained under similar conditions exhibited the 700 Å spacing typical of native collagen,[18] although the crystalline assembly might not always be the same as that prevailing in native tendons.[19] When the precipitate had

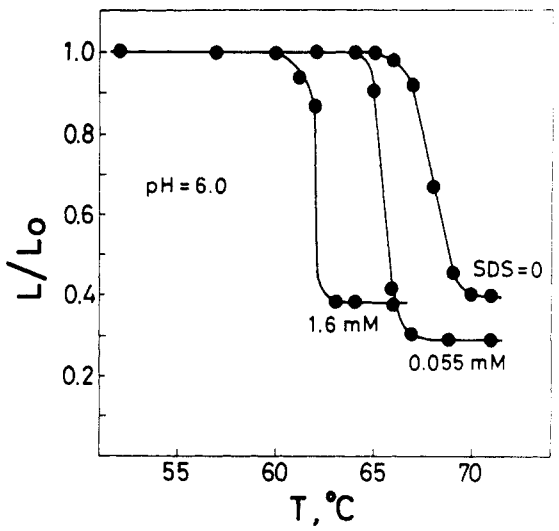

Figure 2. Typical length–temperature curves for cross-linked tendons at the indicated pH and free SDS concentration.

formed, a further increase of temperature led to its redissolution accompanied by the low viscosity typical of denatured gelatin. The visually assessed dissolution temperature was taken as the crystalline to random coil transformation temperature $T_{C,RC}$ (Figure 1). Some arbitrariness in the visual determination of $T_{C,RC}$ was unavoidable, and only the final point of the C → RC transformation could be reliably determined with a reproducibility of ±3 deg. Transformation temperatures determined by these procedures were not significantly affected by a further decrease in the heating rate. For both tendons and tropocollagen, values reported are averages of duplicate or triplicate measurements. The reversibility of all transformation was not investigated in detail but appeared incomplete and similar to that already reported and discussed in the literature.[7,10,11]

Swelling. Mean diameters and sometimes lengths were determined with a microscope with a precision within ±20 μm using short pieces of tendons while in swelling equilibrium with the corresponding solution. The degree of swelling, Φ is defined as the ratio of the volume of the tendon swollen in SDS or salt solution to the volume of the dry, crystalline tendon. Φ was calculated from the corresponding products of cross-sectional areas (assumed circular) and lengths.

Binding Equilibria. The extent of SDS binding to collagen was measured in untreated tendons and in tendons brought to a random coil condition by previous heating at 70 °C. The measurements were carried out at room temperature, taking advantage of the irreversibility of denaturation,[2,10] and at pHs 2.5 and 6.0. The tendons were equilibrated during 20 min with an SDS solution of a given analytical concentration, and the free surfactant concentration was evaluated in aliquots of the supernatant solution by forming a complex between the SDS and added methylene blue, followed by its extraction with chloroform.[20] The absorbance of the blue complex was measured at 570 nm with a UV-160 Shimadzu spectrophotometer.

Results

Shrinkage and Swelling. Length-temperature curves (Figure 2) allow the determination of the shrinkage temperature T_s from the midpoint corresponding to the limiting slopes of the linear portions at low and high temperatures. Significant deviations from an ideal melting transition occur.[21,22] The transformation is not sharp, and as reported elsewhere,[10] only a partial recovery of the initial length is obtained on cooling. Moreover, birefringence studies revealed the persistence of a structural organization even at temperatures much larger than T_s. It was suggested that near the cross-linkages the disorganization of intra- and intermolecular order is prevented.[10]

Swelling data (Figure 3) illustrate volume changes before, after, and during melting. Key features of the

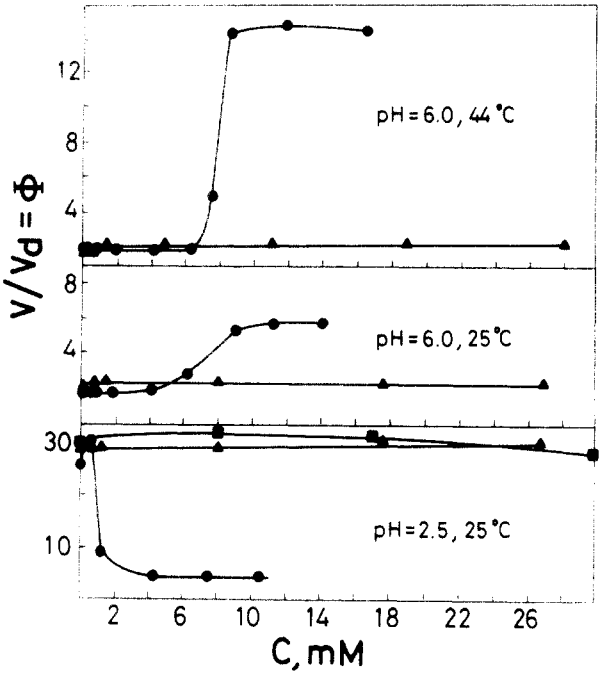

Figure 3. Degree of swelling for cross-linked tendons *vs* SDS (●), sodium methyl sulfate (▲), or sodium chloride (■) concentration at the indicated pH and temperatures. The indicated SDS concentration corresponds to the analytical surfactant concentration that, due to the small mass of tendon considered, also represents the free surfactant concentration.

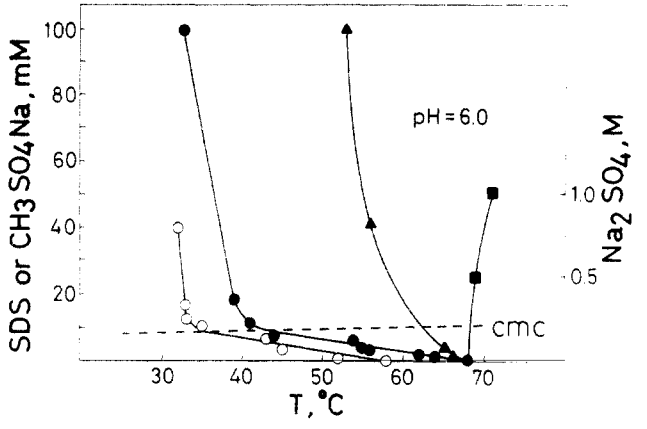

Figure 4. Variation of shrinkage temperature of collagen tendons with SDS, sodium methyl sulfate, or sodium sulfate concentration at pH = 6.0: (●) cross-linked tendons in SDS; (○) un-cross-linked tendons in SDS; (▲) cross-linked tendons in sodium methyl sulfate; (■) cross-linked tendons in sodium sulfate. In this pseudo phase diagram, the field of stability of the crystalline and of the random coiled forms are respectively to the left and to the right of each transformation curve. The broken line gives the CMC of the binary SDS/water system.[40]

results are (i) the large (almost 7-fold) increase in Φ resulting from the melting transition (pH = 6.0, T = 44 °C, C_{SDS} 6–7 mM, compare also with the phase diagram in Figure 4), (ii) the large (almost 13-fold) increase of Φ in pure water when pH is decreased from 6.0 to 2.5, and (iii) a strong deswelling due to SDS at pH = 2.5 and C_{SDS} < ≃2 mM.

It should be noted that the large increase of Φ observed in pure water at pH = 2.5 is achieved by only a 10% reduction of length accompanied by nearly a 13-fold increase of cross-sectional area. This is in marked contrast with the comparable increase of Φ which accompanies shrinkage at pH = 6.0. The unshrunken tendon in water at pH = 2.5 is completely transparent, as generally observed for molten tendons. However, X-ray diffraction studies[10] revealed that the meridional 2.86 Å reflection typical of the residue repeat, as well as the equatorial reflection at 14.3 Å due to spacing between adjacent triple helices,[23,24] is still visible at the large Φ values occurring at pH = 2.5. It is noteworthy that the latter spacing, which is known to be sensitive to hydration (being 10.7 Å for dry tendons[10]) essentially coincided with the value observed in water at pH = 6.0, the large difference in lateral swelling notwithstanding.

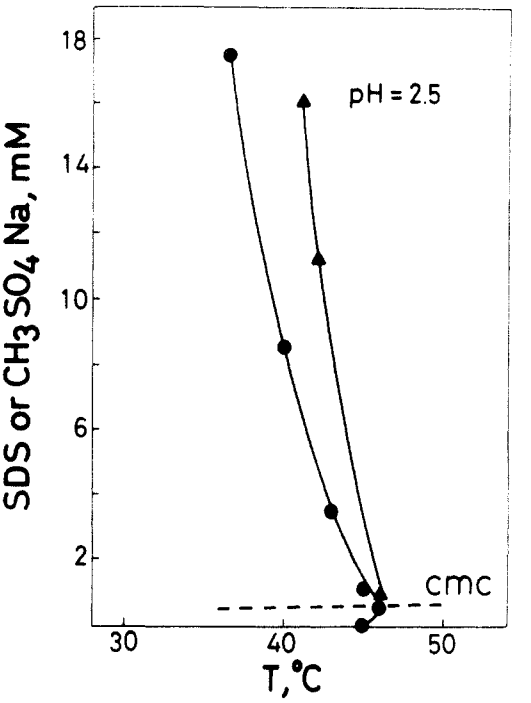

Figure 5. Variation of shrinkage temperature for cross-linked tendons at pH = 2.5 with SDS (●) or sodium methyl sulfate (▲) concentration. The broken line gives the CMC of the binary SDS/water system.[40]

Pseudo phase diagrams, determined by plotting shrinkage temperatures (obtained from data as in Figure 2) *vs* SDS or salt concentration are illustrated in Figures 4 and 5. Under isoelectric conditions (Figure 4) T_s is continuously depressed on increasing C_{SDS} for both un-cross-linked and cross-linked tendons. The latter exhibit a larger T_s which primarily reflects a decrease of water content[11] resulting from cross-linking. The depression due to sodium methyl sulfate is smaller than that due to SDS. Both SDS and sodium methyl sulfate enlarge the field of stability of the RC network. Sodium sulfate instead exhibits a prevailing salting-out effect, as expected from the ranking of Na^+ and sulfate ions in the Hofmeinster series.[25]

At pH = 2.5 (Figure 5) T_s in pure water is depressed to 45 °C, down ≃23 deg from the corresponding value at pH = 6.0 for cross-linked tendons. The shape of the T_s *vs* C_{SDS} curve is somewhat different than under isoelectric conditions. In fact, upon increasing the surfactant concentration, T_s is at first slightly increased (C_{SDS} < 2 mM) and then depressed. At C_{SDS} = 8 mM, T_s is 40 °C, down only 5 deg from the corresponding value in pure water. From Figure 3 the degree of swelling at C_{SDS} = 8 mM appears much smaller than in pure water at pH = 2.5.

Soluble Collagen. Viscosity *vs* temperature curves, illustrated in Figure 6, characterize the transformation from the soluble triple helix to the soluble random coils. $T_{H,RC}$ was obtained from the midpoint corresponding to the linear portions at low and high temperature. Increasing the surfactant concentration drastically reduces the field of stability of the helical form, and already at C_{SDS} ca. 10^{-5}

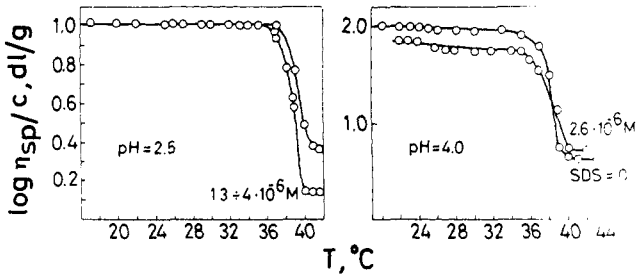

Figure 6. Typical reduced specific viscosity *vs* temperature curves for tropocollagen solutions at the indicated pH and SDS concentration. The unlabeled curve at pH = 2.5 corresponds to zero SDS concentration. Polymer concentration = 0.06%.

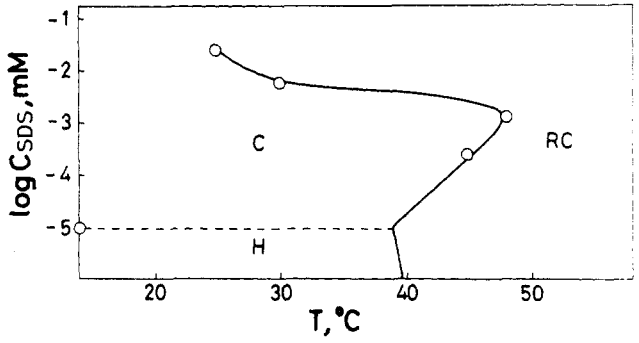

Figure 7. Pseudo phase diagram for soluble collagen. Transformation temperatures $T_{H,C}$ and $T_{C,RC}$ *vs* SDS concentration. Polymer concentration = 0.06%.

M a precipitate appears (pH = 2.5, T = 20 °C). Even in pure water the collagen solution is stable only in the pH range 2.5–4.5. At pH = 6.0 the helical form aggregates. In fact, the kinetics of crystallization of collagen in water and in surfactant solutions at pH = 7 has been extensively studied.[26] The formation of a precipitate observed here at low surfactant concentrations was accompanied by noticeable time effects and a well-known difficulty in redissolving the precipitate,[7,27] particularly when long periods of standing were allowed.

A pseudo phase diagram including the three transformation temperatures (H → C; H → RC; C → RC) *vs* C_{SDS} is given in Figure 7. It is seen that the H form is practically overshadowed by the prevailing C and RC forms. The transformation temperatures for the C → RC process are likely overestimated due to the difficulty in detecting the initial point of the transformation (see Experimental Section). Nevertheless, it appears that the C → RC line has a positive slope at low C_{SDS}, followed by a slope reversal at larger surfactant concentrations. Even the T_s *vs* C_{SDS} line for tendons at pH 2.5 (Figure 6) shows a similar trend.

Binding Isotherms. The amount of SDS adsorbed per gram of tendons was evaluated from the difference between the surfactant analytical concentration and that measured in the supernatant solution. The values obtained are plotted, as a function of the free surfactant concentration, in Figure 8. The amount adsorbed at pH = 6.0 is significantly smaller than that adsorbed at pH = 2.5. Moreover, at the lower pH binding is essentially unaffected by conformation, in marked contrast to the behavior under isoelectric conditions when adsorption is larger for the denatured (70 °C) than for the native (20 °C) form.

Discussion

Residual time effects, incomplete reversibility, and lack of sharpness conflict with the identification of the transition temperatures and the diagrams in Figures 4, 5, and

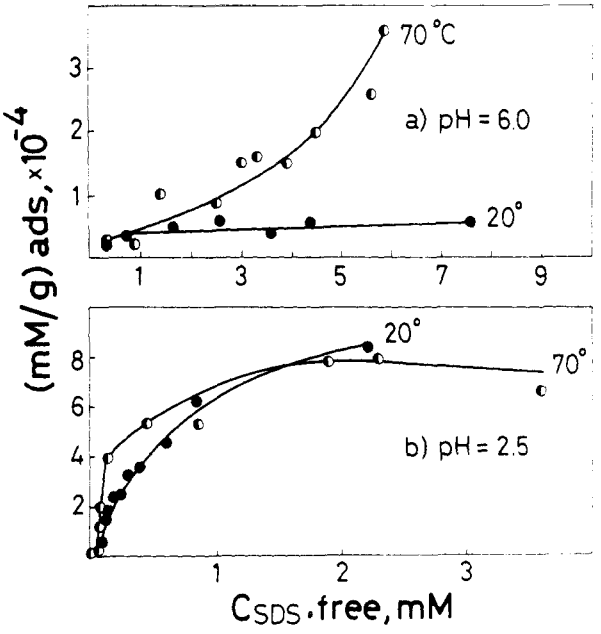

Figure 8. Binding adsorption isotherms. mM/g *vs* free SDS at pH = 2.5 and 6.0 for tendons in the native (●) and denatured (◐) forms.

7 to true thermodynamic equilibria. Nevertheless, the usefulness and the validity of an underlying phase transition model associated with the shrinkage of tendons has been established.[31,32] In particular, the H → C transformation has been shown to be an "inverse" solubility process[7,27-29] occurring on increasing temperature and implying that the overall enthalpy of the H → C transformation is a negative quantity.[28-30]

The surfactant molecule considered here exhibits electrostatic and hydrophobic interactions. Its role on the stability of collagen may therefore be conveniently analyzed in the context of the role of salts and of aliphatic alcohols previously investigated,[7-11] which we find useful to briefly review. Under isoelectric conditions, typical salting-in agents such as KSCN or $CaCl_2$ cause a continuous depression of T_s, $T_{C,RC}$, and $T_{H,RC}$ up to, at least, 1 M concentration.[7,9-11] For salting-out agents such as NaCl or CsCl, an initial depression at low (<0.1 M) salt concentration is invariably followed by an increase of transition temperatures at larger concentrations. Under nonisoelectric conditions, the effect of salts is similar to that observed at pH = 6 only for salt concentrations, C_s > ≃0.6 M.[7,11] From 0–0.6 M added salt, transition temperatures are at first depressed (an effect poorly understood) and then reincreased, irrespective of salt type, due to screening of the polyelectrolyte charge. The trend of isothermal swelling *vs* C_s under isoelectric conditions parallels the trend of transition temperature, going through a maximum, both below and above T_s,[11] and exhibiting a sudden jump during the transition. Alphatic alcohols[8] depress T_s and $T_{H,RC}$ with an effectiveness increasing with the size of the aliphatic tail in the series:

$$\text{methanol} < \text{ethanol} < \text{propanol} \qquad (1)$$

For all the alcohols considered, the degree of swelling decreases with concentration but does not exhibit the maximum noted with salts.

The above results were interpreted[28-30,33] in terms of a strong binding of salts or of alcohols to specific sites along the chain and of a "diluent effect" including a host of less specific interactions (for instance effects of "water structure") involving the bound polymer and the whole diluent.

Binding accounts primarily for the strong depression of transition temperatures, while the diluent effect accounts primarily for swelling. The respective contributions are represented by the first and second right-hand terms of the equation[29,30]

$$\Delta H_f(1/T_m - 1/T_m^\circ) = A \ln(1 + KC_s) + B(v_1 - \chi v_1^2) \quad (2)$$

where ΔH_f is the melting enthalpy, T_m is the melting temperature of the undiluted protein, K is the binding constant, χ is the diluent interaction parameter,[9] and v_1 is the volume fraction of diluent. The above theory requires that binding will lower the melting temperature *provided it occurs preferentially* to the final (RC) form. Peptide bonds are more exposed to the solvent during the C → RC or H → RC transformations. In fact, binding of salting-in agents to the peptide bond *at* the $T_{C,RC}$ temperature of collagen was experimentally demonstrated.[34] A selective enrichment of alcohols was also demonstrated[8] by working with collagen films. Although binding of –OH groups to the peptide bond does occur,[35] the observation that binding increases according to series (1) reveals a direct interaction between the hydrophobic tail of the alcohol with apolar groups of collagen which becomes exposed during melting.

Returning to the present results, we observe that under isoelectric conditions the depression of T_s and $T_{C,RC}$ due to sodium methyl sulfate is comparable to that of salting-in agents and alcohols, while a greater depression is exhibited by SDS. For instance, the depression of T_s (Figure 4) from pure water to 0.1 M SDS or sodium methyl sulfate is respectively ≃35 and ≃15 deg. This may be compared to the corresponding depressions of ≃5 and ≃10 deg observed in 0.1 M KSCN[11] or *n*-propanol,[8] respectively. A prevailing role of the diluent effect in depressing T_s is ruled out by an evaluation of the second right-hand term in eq 2[9,11,30] on the basis of the general tendency toward deswelling due to SDS or sodium methyl sulfate.

In analogy to the case of other ligands, the depression observed here is in line with the binding data in Figure 8 (top) showing a more efficient binding of SDS to the denatured than to the native form of collagen. Expressing the binding constant as[34,36]

$$K = \frac{v/n}{(1 - v/n)C_{SDS}} \quad (3)$$

where v is the average number of SDS bound per mole of protein having a total number n of binding sites, it should be possible to calculate K and n from plots of v/C_{SDS} *vs* v. At variance with the case observed with salts such as KSCN, the present data at pH 6.0 and T = 70 °C did not yield satisfactory straight lines. This effect could be attributed to more than one class of sites or to the cooperativity evident from the upward curvature in Figure 8 (top) (we note that for KSCN K was on the order of 1–10 M^{-1} and n was about 15% of the potential binding sites).

Concerning the nature of the binding sites, since sulfate anions do not bind to peptide bonds,[25] the polar head of the surfactant interacts mostly with ε-amino residues. However, the polymer is under isoelectric conditions and, moreover, the exposure of ε-amino sites is not greatly affected by conformation. Hence the component favoring denaturation is the direct interaction of apolar tails of SDS with some of the apolar residues of collagen which become exposed during melting. It is shown that in type I collagen, except for the telopeptide ends composed of 15–25 amino acids at the NH_2 or COOH terminals of the 2α1 and α2 chains, glycine occurs in every third amino acid in the sequence. Due to the 3_1 symmetry of each of the three constituent helices, glycine forms an apolar edge which stabilizes the triple helical aggregate by hydrophobic interaction. Sites where SDS may bind even to native collagen also occur. For instance, conformational disordering at the telopeptide sequences due to binding of alkane sulfonate detergents was noticed at concentrations which did not produce any modification in the main triple helical structure.[26,37] A model for self-assembly of collagen in vitro[38] suggests that a thermally induced conformational stiffening at particular sequences is needed before nucleation begins.

The swelling of tendons under isoelectric conditions does not show the continuous decrease of Φ with concentration exhibited by alcohols. In addition to the large change of Φ which accompanies the transition at 44 °C, the smaller increase of Φ at 25 °C in the case of SDS is similar to that observed with salts and may be associated with the electrostatic interactions of sulfate groups. As noted elsewhere,[10,11] it is surprising that tendons undergo significant variations of swelling while in the crystalline state at pH = 6 (it is normal instead to expect such changes during melting or in the amorphous state[9]). Regions of reduced organization (telopeptides or special sequences) appear to be able to interact with the diluent in the amorphous-like manner.[11]

Turning to nonisoelectric conditions, the depression of T_s resulting from the decrease of pH in pure water is attributed to destabilization of the helix due to the net positive charge (about 5% residues are lysine or 5-hydroxylysine) and the associated Donnan effect revealed by the large swelling. The positive slopes at low C_s in Figures 6 and 7 reflect a stabilization of the helix when charged ε-amino residues are screened by dodecyl sulfate ions. The observation that SDS causes only a small depression of T_s may be attributed to binding occurring primarily at charged ε-amino sites, the exposure of which is conformation independent. This also explains why no significant difference is observed by the binding data at 20 and 70 °C. Moreover, consistent with the "inverted" nature of the H → C transformation, more hydrophobic binding sites are likely exposed in the case of soluble helices, or swollen tendons at low pH and temperature, than in the case of crystalline tendons at pH = 6.0. These sites are available to tails of bound and free SDS molecules. The hydrophobic binding pattern prevailing at pH = 6.0 appears therefore altered when the tendon is highly swollen and electrostatic binding occurs.

The observation that the extent of binding is larger at pH = 2.5 than at pH = 6.0 (particularly at C_{SDS} < 0.5 mM) reflects the larger strength of electrostatic interaction. Analysis of the data in Figure 8 (bottom) in terms of eq 3 yields satisfactory straight lines and values of $K \approx 1500$ M^{-1} and of n corresponding to one site per 10 residues (about twice the number of ε-amino groups, based on reported values for the amino acid composition of collagen).

The strong deswelling operated by SDS at pH = 2.5 has characteristics of cooperative phenomena and cannot be simply described as the usual screening of electrostatic charges and the reduced Donnan effect since no such large deswelling is observed with sodium methyl sulfate or sodium chloride. The hydrophobic tail of dodecyl sulfate ions may magnify the usual screening effect by a reduction of the local dielectric constant favoring strong binding with the fixed charges on the polymer. Ion pair formation in mixed organic–water solvents was recently invoked by Khokhlov and Kramarenko[39] to describe the phenomenon

of gel collapse, which is accompanied by the coil to globule transition of flexible network chains.

Another contribution of SDS toward interhelix stabilization would be the direct association, or interdigitation, of the hydrophobic tails of the bound surfactant.[16] Collapse of polyelectrolyte networks due to absorption of surfactants was also reported by Tyabina et al.[12] They suggested the formation of network surfactant aggregates which could attain micellar or lamellar form at a CMC (critical micelle concentration) much lower than in the case of binary surfactant–water solutions. A strong interaction between triple helices promoted by SDS, with concomitant expulsion of water and decrease in Φ, is also in agreement with the precipitation of tropocollagen upon addition of minute amounts of SDS both at pH = 6.0 and pH = 2.5. We have superimposed on the pseudo phase diagrams in Figures 4 and 5 the expected temperature variation of the CMS for the binary SDS/water system as obtained from the literature.[40] The association of the surfactant to the protein evidenced in Figure 8 at concentrations considerably lower than the CMC, particularly at pH = 2.5, appears to be in line with the proposed interpretation.

To further explore the details of the aggregates, we consider the cooperativity exhibited by the binding data in Figure 8. Langmuir plots of these data yield satisfactory straight lines and can be analyzed in terms of the fractional binding saturation β:[41]

$$\beta = 1 + (K\omega C_{SDS} - 1)/[(1 - K\omega C_{SDS})^2 + 4KC_{SDS}]^{1/2}/2 \quad (4)$$

where ω is the cooperativity parameter and K is the intrinsic equilibrium constant. Kω represents the equilibrium constant for binding to a site adjacent to an occupied one. For the concentration C'_{SDS} at the midpoint $\beta = 0.5$:

$$C'_{SDS} = (K\omega)^{-1} \qquad d\beta/d \ln C'_{SDS} = \omega^{1/2}/4$$

Plots of β *vs* the log of the free SDS concentration could be fitted with values of $K \simeq 1800\ M^{-1}$ and $\omega \simeq 1$ for the isotherms at pH = 2.5, revealing strong binding, but no cooperativity. The data at pH = 6.0 could be handled only in an approximative way since no saturation is reached. Nevertheless high cooperativity is evident from the data at $T = 70$ °C (Figure 8 (top)) and a value of ω on the order of 20 should be expected.

The present binding data, and our earlier fluorescence data, thus reveal that micelle-like aggregates are formed only when the protein is in the isoelectric state and in the coiling form. Since SDS and denatured isoelectric collagen are bound via hydrophobic interaction, the resulting "micelle" must posses a rather unusual organization, with parts of the polymer chain being able to form loops, and partake in the hydrophobic core.

The lack of cooperativity shown at pH = 2.5 is in contrast to the observations that binding of surfactants to flexible polyelectrolytes, and even to poly(L-lysine) and DNA, is usually cooperative.[42-45] We believe that the main factor is the low charge density of the rigid triple helix, resulting in binding sites being too far apart for hydrophobic interaction between bound surfactant to cooperatively develop. Wei and Hudson[46] have recently investigated binding of SDS to cationic chitosan. The cooperativity parameter was found to decrease when the degree of diacetylation (reflecting charge density) decreased.

Cooperative hydrophobic interaction between electrostatically bound ligand is generally regarded[44-46] as a reinforcement of the Coulombic interaction between the charged groups. Shirahama et al.[45] have shown that the binding of dodecylpyridinium cations to DNA exhibits two cooperative steps. They suggested electrostatic binding resulting in the formation of a first layer of hydrophobic tails, followed by interdigitation of excess surfactant into a kind of a bilayer, with cationic groups of the ligands exposed to water helping to keep DNA in solution. It was later suggested[16] that this interdigitation mechanism could lead to soluble clusters involving several DNA molecules. It is plausible that a second step interdigitation mode is also occurring in the present system during the deswelling–precipitation process, actually contributing to the cooperative character of the transition. Note in fact, from Figure 3, that the largest reduction of volume at pH = 2.5 and $T = 25$ °C occurs at C_{SDS} near 1 mM, when binding of SDS (Figure 8 (bottom)) is already near saturation. It appears that further search for regular cylindrical aggregates should consider polyelectrolytes which not only are intrinsically rigid and highly charged but also are able to form soluble aggregates.

A final comment concerns the dimensional changes of the tendon in pure water upon transfer from pH = 6.0 to pH = 2.5. A 13-fold increase of sectional area, accompanied by only a 10% reduction in lengths, and the permanence of the 14.3 Å reflection are difficult to explain. This tendon could be described as an oriented network with a prevailing liquid crystalline order (stabilized by electrostatic repulsion with a minor conformational disorder in the triple helix) and a few regions where the native crystalline structure is preserved. The latter could be stabilized by particular sequences or by the cross-linkages. In fact, the uncommon persistence of some local order was noted for the present[10] as well as for other networks[47] cross-linked in the oriented state well above the shrinkage temperature. The shrinkage of tendons in pure water at pH = 2.5 should then be regarded as a transition from a prevailing liquid crystalline network to a prevailing amorphous network. Similarly, the cooperative phenomenon involving the SDS-induced collapse of tendons at pH = 2.5, $T = 20$ °C, and $C_{SDS} < 1$ mM should reflect the liquid crystal → crystal transition. Occurrence of liquid crystallinity in polymer gels having either chemical[48] or physical[49] cross-linkages was, in fact, observed. The verification of the above model will be undertaken at a later time.

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