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The Effect of Anionic Detergents on Collagens of Mammals and Teleostei

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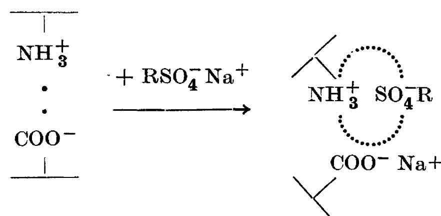
By treating globular proteins with solutions of anionic detergents, as sodium dodecyl sulphate (= SDS) or dodecylbenzene sulphonate (= DBS), the proteins are denatured [1] and acquire fiber forming properties [2]. This effect of anionic agents is of great theoretical interest and also of some technical importance. Recent reviews of Putnam [3] on the theory of the reaction and of Lundgren [2] on the problem of fiber formation give excellent expositions of this field and complete literature references.

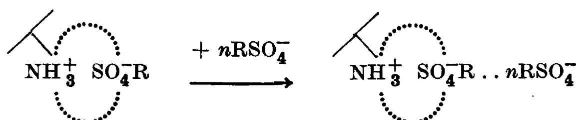
In the denaturation and unfolding of the chains of globular proteins by means of anionic detergents, the primary reaction is apparently the interaction of the anions with the cationic protein groups [4]. This attachment leads to a weakening or partial breaking of the saltlinks. In micellar systems containing a large excess of detergent over the equivalent of basic protein groups, a secondary type of reaction probably is also involved, considered by some investigators [5, 7] to be directed towards the peptide groups, *i. e.* the imide nitrogen which consequently should result in rupture of hydrogen bonds. Other investigators, notably Lundgren [6], believe that forces of the type which bind detergent ions into micelles are responsible for the secondary attachment of the amount of detergent in excess of the equivalent of ionic protein groups.

The first reaction (electrovalent) of the anionic detergent may according to Lundgren [6] be schematically illustrated as follows:



The rather long chains of the organic anion tend to wedge apart the chains; thereby impairing the strength of the saltlike crosslinks of the polypeptide chains.

The second type of reaction (non-electrostatic) may be represented by the schematic structure given by Lundgren [6]:



The unfolded protein chains with laterally attached anions of the detergent will thus build up other layers of negative charge (micelles) which tend to repel groups of similar charge on adjacent protein chains and hence, to labilize the protein structure (rupture of hydrogen bonds). It has been indicated analytically that the primary reaction is directed towards the basic protein groups; one molecule of detergent being fixed by each of the basic groups of the protein. Some investigators, *e. g.*, Pankhurst and Smith [7] consider that the maximum binding occurs when each nitrogen atom of the protein (gelatin) has combined with one molecule of dodecyl sulphate (2.88 g SDS is then fixed by 1 g gelatin). However, it has been amply demonstrated by Lundgren [6] that by extraction of the protein-detergent compound with 60 % aqueous acetone in the absence of salt, the ionically bound detergent not being removed, the composition of the final product will approximate the limiting proportion of one detergent anion for each equivalent of the basic groups of the protein. It is interesting to note that in the presence of salt the compound is completely split up in its constituents, probably due to ionic exchange [6]. According to evidences at hand, two factors are requisite for interaction: 1) the ionic group of the detergent. 2) its specific affinity which is related to the size and structure of the detergent, resulting in stabilization by the mutual affinity of the hydrocarbon portions of adjacently bound detergent ions [3].

The effect of detergents of the SDS and DBS types on collagen and related fibrous proteins has not been studied. It has been noted in a single experiment that the shrinkage temperature of collagen is considerably decreased by treatment in solutions of 1 *M* strength of SDS; the hydrothermal test being carried out in the SDS-solution [8]. Keratin resists the action of detergents; cleavage of the -S-S-bridge being required for any effect [9]. Of great interest is the finding of Steinhardt and Fugitt that pre-treatment of keratin with SDS facilitates its deamidation in acidic medium [5]. Silk is not reactive towards these reagents; a finding to be expected from our knowledge of its structure and low content of ionic groups.

In the denaturation of globular proteins, the chains are unfolded and brought nearer to the fibrous state, whereas the denaturation of collagen and other fiber proteins takes the reverse course towards the thermodynamically more probable, coiled configuration. The fibrous state is hence artificial and bound up with the presence of stabilizing crosslinks. It is to be expected that investigations of the effect of anionic detergents and related compounds on collagen will provide indication of the types of bonds which stabilize this protein and supply additional information on the molecular organization of fibrous proteins. Further, information may be forthcoming on the still unsettled problem of the nature of the combination of detergents with proteins, and the important question whether the disruption of the protein structure initiated by its combination with the detergent is completely reversible.

EXPERIMENTAL

Besides chemical pure preparation of SDS and DBS, a number of technical products ("fatty alcohol sulphonates") have been studied. The latter types consisted entirely of SDS or mixtures of homologues, containing SDS as the main component together with sulphates of C_8-C_{10}-compounds as well as those of $C_{14}-C_{16}$ chains. The technical products were employed: 1) as received and 2) after the removal of inorganic salts by extraction of the original products with ethanol. On the whole, the various preparations gave the same effect on collagen, compared on the basis of effective detergent; the alkylbenzene compounds being in some instances slightly more effective. The data given in the present paper pertain to the chemical pure SDS-compound. It is to be noted that the SDS-solutions used had pH values higher than those corresponding to the isoelectric point of the bovine collagen preparations. Hence, the anion affinity should be expected to be the driving force in systems on the alkaline side of the isoelectric point of the protein.

As collagen substrates, isoelectric calf skin pelt (limed and subsequently delimed), American standard hide powder and skins of cod (*Gadus morrhua*), only mechanically cleansed, were employed. Standard hide powder and pelt in the deaminated state were also used (method of Thomas and Foster [10]). The hide powder was denatured by 2 min. immersion in water of 70° C. The analytical methods were the standard procedures generally employed except for the determination of sulphur which was carried out according to Grote-Krekeler [11]. Further, in the determination of the shrinkage temperature (*Ts*) the freely suspended strips were introduced into water of a temperature 1—2° C below the indicated *Ts*. After 1 min. the bath was heated ("the shocking effect"). The pertinent analytical data of the collagen preparations employed are given in Table 1.

The isoelectric points of the pelt and hide powder were 5.5 and 5.2 respectively. Cod skin showed pH 7.0—7.5. The deaminated collagens were isoelectric at pH 4.0.

In comparative experiments solutions of sodium polymetaphosphate, with an average molecular weight of 7 600, determined by means of the end group method of Samuelson [12] were employed. The solutions used were adjusted to pH 2.0 (hydrochloric acid). The average molecular weight was after 4 h 6 800 and after 24 h 3 600. Since in the present instance more than 90 per cent of the total fixation of polymetaphosphate by colla-

Table 1. Composition of collagen preparations.

No.	Type	% N	% Ash	% S	HCl-binding capacity (in meq. HCl per g collagen)	*Ts* in °C
1	Pelt	18.0	0.1	(.6	0.92	67
2	Hide Powder	17.9	0.1	0.4	0.88	—
3	Deaminated pelt	17.6	0.3	0.6	0.64	66
4	Deaminated hide powder	17.5	0.2	0.4	0.60	—
5	Skin of cod	18.3	0.4	1.0	0.94	43
6	Heat-denatured hide powder	17.9	0.1	0.4	0.88	—

gen occurs in the first two h, the molecular weight of the fixed metaphosphate anion is probably not far from the average value of the sodium polymetaphosphate (7 600).

Further, β-naphthalene sulphonic acid of analytical grade was used in 0.2 *M* solution. The special technique of determining the degree of swelling of the collagen preparations will be described in the experimental part.

RESULTS AND DISCUSSION

a. The effect of SDS on various types of collagen

The effect of the concentration of solutions of SDS on their dissolution of various preparations and types of collagens of skin is shown in Fig. 1. In these series, 2.0 g collagen was treated for 96 h in 50 ml of the SDS-solutions at final pH 6.4—6.8 (covered with toluene), containing increasing amounts of SDS. In the concentrations of SDS employed (> 0.1 %), micelle formation is prominent. See ref. 3.

Evidently, mammalian collagen in the form of skin, both native and alkali-pretreated (limed) is not solubilized by SDS, even in the most concentrated solutions. A small quantity of the heat-denatured skin is brought into solution. Hide powder which is finely ground hide, thoroughly limed and then delimed, is solubilized to a marked extent by the concentrated solutions and by denaturation this tendency is greatly enhanced. Most interesting is the finding that native cod skin collagen is rather completely brought into solution by moderately concentrated solutions of SDS. This is not surprising in view of the established fact [12] of the low degree of intermolecular stabilization of collagen of *teleostei* compared to the mammalian type, as shown by its low degree of hydrothermal stability (shrinkage temperature), its great susceptibility to proteinases, acids and alkalis, its greater reactivity for coordination-active tanning agents [13] and the destructive effect of certain sulpho acids on its macro

structure [14]. The different behaviour of fish skin collagen compared to bovine skin collagen does not appear to be a problem of different amino acid composition. The recently reported, complete analysis of fish skin collagen [15] shows practically the same contents of the polar and non-polar amino acids forming the main part of the collagens; the only difference being the greater percentages of the rarer amino acids (serine, threonine and methionine) in the collagen of *teleostei*. The possibility of different sequence of residues and the presence of polypeptide units of different composition must be left open as an explanation. The accumulated evidences appear to be best explained on the basis of molecular organization; macro-structural details as revealed histologically probably also being involved.

The following working hypothesis of the structural organization appears reasonable. The mammalian skin collagen, with its intertwining fiber bundles is mainly stabilized by means of hydrogen bridges between oppositely located -CO-NH-groups; salt links of electrostatic nature supplementing the primary forces. In fish skin collagen, with parallelly grouped layers without any marked interlacing of fiber bundles, on the other hand, the low degree of stabilization of the structure is satisfactorily accounted for by the assumption that the hydrogen bonding is only weakly developed; electrovalent crosslinking on polar protein groups being responsible for the main part of the structural cohesion. The present finding of the extraordinary behaviour of this type of collagen is in harmony with such an explanation.

b. Reversible effects on collagen

Although bovine collagen is not, or only slightly so, attacked by SDS to an extent leading to solubilization, it undergoes certain changes in the treatment. Thus, it is markedly swelled and its hydrothermal stability is greatly impaired by its combination with the SDS, as shown by the considerable Ts decrease of the pelt taken *directly* from the SDS-solution. Also certain *irreversible* changes are imparted to the protein as shown by a permanent, lightly increased degree of swelling and a small permanent lowering of the *Ts* of the skin after the removal of the SDS in the subsequent washing. The following experiments illustrate these points.

10 g collagen, as calf skin pelt, was treated for 120 h in 100 ml of 5.0 % solution of SDS and further, the same quantity of protein in the form of hide powder in 150 ml of this solution. The hide powder swelled greatly and imbibed the solution almost completely. The substrate was separated from the solution by suction filtering under identical experimental conditions. From the total weight of the moist stock and analysis of part of the treated specimens for contents of dry substance and collagen, the amount of water

sorbed by the protein was ascertained. The degree of swelling or rather the degree of imbibition of water, is expressed in g water held by 1 g collagen. No determination of sorbed SDS was attempted, since such an evaluation appears meaningless under the present conditions. The residual solution was analyzed for total nitrogen and ammonia. No ammonia was found (no deamidation). The rest of the treated stock was extracted with 200 ml 60 % acetone for 2 h, three consecutive times. A part of this stock was dried and analyzed for collagen, sulphur and ash. The rest was washed further for 48 h in several changes of water (500 ml) in order to remove watersoluble matter. The *Ts* of the skin after the various treatments were determined. (*Ts* of original skin: 67° C) Tables 2 and 3 contain the data.

Table 2. Composition of substrates taken directly from the SDS-solution.

Substrate	Final pH	% Dissolved collagen	Degree of swelling	*Ts*	*ΔTs*
Hide powder	6.4	8.3	10.5	—	—
Calf skin pelt	6.6	0.9	2.8	52	– 15

The original skin contained 1.8 g water per g collagen and the treated skin after acetone extraction and subsequent hydration 2.0; the shrinkage temperature of the skin was then 65° C or 2° C lower than the *Ts* of the original skin. Hence, it is evident that the interaction of SDS with collagen brings about certain irreversible alterations of the protein. The very large *Ts* decrease, 15° C, induced by the association of SDS with collagen, determined on pelt directly from the treating bath, is probably due to the severance of saltlinks and a partial rupture of hydrogen bonds by the swelling induced by the association of anions of the detergent on the ionic protein groups, wedging them apart. The maximal disrupture of the collagen structure is generally approached in solutions containing one equivalent detergent on each equivalent of cationic protein groups or at a detergent/protein weight ratio of 0.25 (See, *e. g.*, Fig. 1). Hence, the peptide group are apparently not involved *directly* in the attachment of the SDS present in excess of the stoichiometric equivalent. The secondary effect of the additional swelling is probably caused by the electrostatic repulsion of the micellar layers as suggested by Lundgren [6].

Table 3. Composition of SDS-treated specimens after acetone-extraction.

Substrate	% Collagen (N × 5.55)	% Ash	% Organic S	% Fixed SDS
Hide powder	99.8	0.9	0.5	0.
Calf skin pelt	100.8	0.5	0.6	0

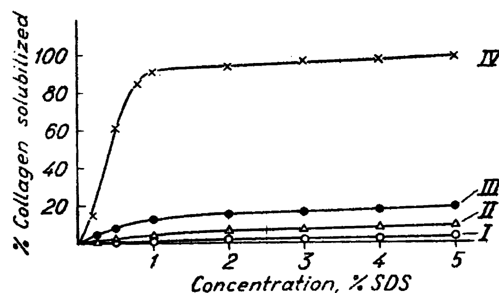


Fig. 1. The dispergation of collagen by SDS as a function of its concentration.

I = Calf skin pelt
II = Native hide powder (bovine)
III = Denatured hide powder (bovine)
IV = Collagen of cod skin

These data as well as those from a number of other series prove that no irreversible fixation of SDS by collagen takes place.

The previous data have shown that the increased degree of swelling and the lowering of the Ts of the pelt treated in moderately concentrated solutions of SDS are to some extent permanent. These changes do not affect the maximum acid binding capacity of collagen. The solubilization of the protein does not result in splitting of the protein and no deamidation takes place by the action of SDS. Hydrolysis of amide groups, however, is induced by 0.2 *M* solution of β-naphthalene sulphonic acid at pH 1.3—1.5.

It is illuminating in this connection to remind of an earlier finding [16] that heat denaturation of bovine collagen does not increase the binding capacity of the protein for mineral acid. However, it is important to note that denaturation leads to greater uptake of mineral acid (HCl) in the pH range 2.5 to 6, which has been explained as being due to weakening of the attraction between oppositely charged protein groups by the increased distance between these groups, forming the salt link, expected to result from the folding of the protein chains in the shrinkage. It was also shown that the main valency forces of collagen affected in the denaturation were the coordinate ones (rupture of hydrogen bonds); also a steric effect caused by the unfavourable spatial conditions created [16]. Comparing the titration curves of pelt in its original state and after SDS-treatment (washed), the latter showed on the whole a slightly greater fixation of acid in the pH range 2.5—6, although the values were quite irregular and hence are not included.

c. Irreversible effects on collagen

The final proof of the permanent alterations of collagen by the SDS-treatment is supplied by determination of the effect of this pretreatment on the reactivity of collagen towards tanning agents, reacting: 1) entirely with *ionic valency forces* and 2) to a large extent by means of *coordinate valency forces*, involving the groups serving as *loci* for the hydrogen bridges, primarily —CO—NH—. This method was also employed in the investigation of the mechanism of the heat denaturation of collagen [16].

The following chromium compounds were employed. The first type, mainly reacting ionically with collagen, contained only cationic chromium complexes: the 33 % basic chromic sulphate with its composition corresponding to the empirical formula:

$Cr_2(OH)_2(SO_4)_2 \cdot Na_2SO_4$ and the corresponding chromic chloride represented by the formula: $Cr_2(OH_2)Cl_4 \cdot 2NaCl$. The second type contained cationic as well as non-cationic (mainly uncharged) complexes of higher degree of aggregation than the first type. The interaction with collagen is in this instance governed by the ionic as well as by the coordinate potency of collagen: the 60 % basic chromic sulphate (made from the 33 % basic compound by soda-addition) and the 70 % basic chromic chloride, containing 80 % of its chromium in the form of uncharged complexes [17]. Finally a special complex chromium salt, the sulphito-sulphato-chromiate, mainly containing negatively charged complexes was studied [18].

Portions of 2.0 g collagen in the form of pelt and hide powder, both untreated and treated for 120 h in a 10 % solution of SDS, subsequently washed, were shaken for 96 h in 50 ml portions of solutions of the chromium compounds, containing 15 g/l Cr. The chromed stock was freed from uncombined electrolytes and analyzed for protein N and chromium (Table 4).

Table 4. Effect of pretreatment of collagen with 10 % solution of SDS on its affinity for chromium compounds.

	% Cr fixed by 1 g collagen from:				
Substrate	33 % basic Cr-sulphate	33 % basic Cr-chloride	60 % basic Cr-sulphate	70 % basic Cr-chloride	Sulphito sulphato chromiate
Hide powder	7.5	5.8	12.7	9.6	19.6
SDS-Hide powder	7.8	6.0	15.8	11.9	23.1
Calf skin pelt	7.6	5.2	10.0	6.8	—
SDS-Calf skin pelt	7.5	5.2	11.2	7.3	—

The following points are noteworthy. The treatment of collagen with SDS does only slightly affect its ionic groups as evident from the fact that the fixation of cationic chromium is not materially altered. Those agents which for their reaction with collagen require coordinate bonds and which reaction is governed by the state of the peptide groups (compensated or uncompensated intermolecularly) give considerably higher values of fixed chromium in the instance of the SDS-pretreated collagen. It is thus indicated that the main permanent changes of collagen caused by the treatment with the anionic detergent involve the coordination active groups (rupture of hydrogen bonds, indirectly by swelling).

It should also be of interest to ascertain the effect of SDS on deaminated collagen. The results of series with regular and deaminated hide powder and calf skin in the intact state and after 120 h treatment in 10 % solution of SDS (subsequently acetone and water extracted) are given in Table 5.

Table 5. Interaction of SDS with intact and deaminated collagen.

Substrate	% Collagen dissolved	Degree of swelling	Δ_0 Ts Directly	Δ_0 Ts After acetone wash	Δ_0 Ts After further water wash
Blank. Hide powder	9.6	9.8	—	—	—
Deaminated hide powder	1.7	4.9	—	—	—
Blank. Calf skin pelt	0.7	2.2	− 18	− 3	− 2
Deaminated calf skin pelt	0.5	2.3	− 18	− 16	− 16
Cod skin	96	—	—	destroyed	

In tanning experiments, employing deaminated hide powder in the intact state and after SDS-treatment (washed) as the substrates for the 70 % basic chromic chloride, the SDS-treated specimen showed about 30 % higher chrome fixation than the blank (untreated), proving a marked activation of the coordination *loci* of deamino-collagen as a permanent result of its interaction with the anionic detergent. This is in line with the data of the Ts of Table 5, showing that the impairment of the hydrothermal stability of deamino-collagen by SDS is *not* reversible whereas the Ts-decreasing effect of SDS on the intact skin collagen is to the largest part reversible.

d. Comparative data on the effect of other anionic agents

For a safer basis of discussion, some additional data from experiments with systems of various types of collagen and some other anionic agents will be briefly considered. The agents selected were β-naphthalene sulphonic acid (= NS) and polymetaphosphoric acid (= PMP), reacting with collagen under pH-conditions ensuring maximum fixation of these agents.

Some main points may be stressed. The strong NS is fixed by collagen entirely through its ionic groups; coordination being eliminated as a factor. The anion combines stoichiometrically with the basic groups; the combining equivalent being 1.0 meq. per g collagen (at final pH 1.3—1.4). Such solutions of NS have no swelling effect on collagen [19], which fact may be explained by the partly irreversible attachment of the anion on the cationic protein groups No Donnan effect is produced since the condition of a completely reversible system is not fulfilled. Complete inactivation of the ionic protein groups by means of NS, *i. e.*, breaking of salt links, will hence measure the extent to which the salt links contribute to the intermolecular stabilization [20]. It should also be noticed that NS is an *unifunctional* anionic agent.

The strong acid formed by acidifying a solution of sodium polymetaphosphate, 2.0 % solutions of pH values of 1.8—2.0 being employed in the present instance, is indicated to react as a *polyfunctional* electrolyte with proteins; its large anion being irreversibly fixed by the basic protein groups [21]. Also in this instance, the requirements for establishing of a Donnan effect are absent. The skin does not swell. Rather, it is dehydrated.

The results of the NS series are given in Table 6. 2.0 g portions of protein in the form of cod skin and calf skin pelt were treated two consecutive times, 24 h each time, in 50 ml. 0.2 *M* solution of NS. The experimental findings from the corresponding series of 2.0 % solutions of PMP, with pH values adjusted to pH 2.0, are contained in Table 7. Time: 24 h.

Table 6. Collagen-β-naphthalene sulphonic acid, 0.2 M.

Substrate	meq. acid fixed by 1 g collagen	Δ *Ts*	% collagen dissolved	% relative decrease of strength of treated skin
Calf skin	0.98	— 18	0.6	0
Cod skin	1.00	destroyed	1.2	90

Table 7. Collagen-polymetaphosphoric acid, pH 2.0.

Substrate	meq. acid fixed by 1 g collagen	Δ *Ts*	Degree of swelling	% collagen dissolved	% relative decrease of strength of treated skin
Calf skin	0.94	+ 1	1.6	0.4	10
Cod skin	0.96	± 0	1.8	3.2	90

The following points need to be accentuated. By complete inactivation of the cationic groups of bovine collagen by means of NS, the Ts is decreased 18° C, which figure may serve for evaluation of the degree of protein stabilization due to electrovalent crosslinks (saltlinks), since secondary effects on the other type of crosslinks (hydrogen bridges) are absent (no swelling). The character and mechanical strength of mammalian skin are not adversely affected; indicating non-ionic cohesive forces to be mainly responsible for the structural rigidity. Cod skin is not solubilized by NS. However, its tensile strength and structural features are completely lost. This behaviour fits in well with the conclusion drawn from other findings [13, 14] that the forces responsible for the stability of collagen of *teleostei* are mainly saltlinks; hydrogen bridges on —CO—NH-groups not being prominent. Cod skin is not disinte-

grated and dissolved by NS since electrostatic repulsion is lacking and also swelling. With SDS the secondary effect of the electrostatic forces promotes swelling and practically complete dispergation of fish collagen, as would be expected from the reaction mechanism postulated by Lundgren [2, 6].

The mechanism of the reaction of the PMP with the two types of collagen does also illuminate the present problem. The hydrothermal stability is not lowered, rather slightly elevated in both instances. The Ts-decrease of 18° C expected from complete rupture of the salt links by the irreversible attachment of an unifunctional agent to the cationic protein groups is not shown. Since the average molecular weight of the PMP was 7 600, about 75 PO_3-units are present in each chain. Evidently, the multifunctional PMP-anion reacts with adjacent protein chains under crosslinking. The extent of this stabilization seems about to outweigh the labilizing effect due to the breaking of the salt links of the intact collagen.

The mechanical strength of the cod skin was practically destroyed by the fixation of PMP. The strength of bovine skin was slightly lowered. Evidently, different cohesive forces control the hydrothermal stability and the mechanical strength of the collagen structures. It is indicated that the former involves the strength of the crosslinks of intramicellar units, whereas the latter is a function of the cohesive forces between the larger units (fibrils and fibers) [22]. Any marked solubilizing effect of PMP on cod skin is not shown in spite of its structural weakening. Since dehydration of collagen is effected by PMP, this fact is not surprising.

CONCLUSIONS

The results of this investigation bear upon two different aspects of protein behaviour: 1) The nature of the interaction of collagen and related proteins with anionic detergents and 2) The nature of the stabilizing forces of collagens of mammals and fishes.

It is demonstrated that the maximum dispergation of cod skin collagen, nearly complete, takes place by adsorption of SDS by collagen in amounts sufficient to allow complete interaction with the basic protein groups, or one tenth of the amount required for complete interaction of all nitrogen atoms of collagen with the detergent. Also, the degree of maximum swelling of bovine skin is approached under the conditions mentioned. These findings lend support to the view advanced by Lundgren for the explanation of the reaction mechanism of anionic detergents with globular proteins. The alterations of collagen, due to the swelling as a secondary effect of the association of SDS-anions on the cationic protein groups and the temporary destruction of the

crosslinks are to some extent permanent which is proved by the increased binding capacity of the SDS-treated collagen for agents involving coordinate valency for their reaction with proteins. The anionic detergents of the type of SDS are unifunctional, reducing the strength of the saltlinks and hence, the internal stability of the protein structure.

The interaction of fish skin with the unifunctional NS, partly irreversibly fixed and not possessing swelling power, leads to complete destruction of the polar links. However, hardly any collagen is solubilized since swelling and disorganization of the structure are not produced. Nevertheless, the mechanical strength as well as the hydrothermal stability of the collagen of *teleostei* are lost. It is pertinent to point out that solutions of hydrochloric acid of final pH values of 1.3—1.5, bring about complete discharge of the carboxyl ions of collagen, and hence a complete destruction of the saltlinks. Being a completely reversible system, the Donnan effect enters and the maximum degree of swelling is produced. In the case of cod skin, this will result in a nearly complete solubilization of collagen. In the presence of a swelling depressing neutral salt, for example sodium chloride, the acid does not dissolve fish collagen to any great extent and the Ts is unchanged or slightly elevated; proving the governing importance of swelling phenomena.

The multifunctional polymetaphosphoric acid, entirely ionic in its reaction, does not impair the heat resistance of the fish collagen. However, its tensile strength is practically lost. Since no swelling occurs, the collagen of fish skin is not turned into watersoluble products. These findings are satisfactorily explained by the supposition that *the hydrothermal stability of collagen is governed by the strength of the crosslinks of the ultimate protein units, whereas the mechanical strength concerns the forces between larger units* [22].

The various findings reported point to the importance of the anion affinity and the secondary effect of the anionic agents on the swelling of the protein. SDS is evidently such a strong competing agent, that it is capable of breaking up the internal interactions between cationic nitrogens and the other functional groups of the protein [23].

The diversity of properties and behaviour of bovine and fish collagens is probably due to different internal linking of protein chains and micellar units.

SUMMARY

The interaction of anionic detergents of the type of sodium dodecyl sulphate, in solutions of 5 % strength (micelles), with collagen of bovine skin results in: a) lowered shrinkage temperature, b) increased swelling and c) a minor solubilization. No SDS is irreversibly fixed. Permanent alteration of

the treated skin is shown by slightly lowered Ts and increased coordinate reactivity. The disruption of the protein initiated by its combination with the detergent is hence only partly reversible.

Cod skin collagen is largely solubilized. The different behaviour of the two types of collagen is in harmony with the view that mammalian collagen is stabilized by crosslinks on the peptide groups of adjacent chains (hydrogen bonds), supplemented by salt links. The latter type of cohesive forces contributes to the main organization and stability of collagen of *teleostei*.

Comparative experiments with naphthalene sulphonic acid and polymetaphosphoric acid give additional information on the organization of these collagens and the mechanism of the reaction between anionic detergents and collagens. The general concept advanced by Lundgren for the protein-detergent systems gives a satisfactory explanation of the findings.

It is indicated that the hydrothermal stability of collagen is a function of the degree of intermolecular linking of micellar units, whereas the mechanical properties are mainly governed by the cohesion between larger units, as fibrils and fibers.

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