Hydroxyproline and Stability of Collagens

K. H. GUSTAVSON

Garverinäringens Forskningsinstitut, Stockholm, Sweden

From investigations of the hydrothermal stability and the reactivity of various collagens from the corium of mammals and fishes (*Teleostei*), listed in a recent paper [1], it has been concluded that the interchain cross-linking by hydrogen bonds is mainly responsible for the intermolecular cohesion of mammalian collagen. The collagen of the skin of cold-water teleosts, for instance that of the cod fish, appears to have this cross-linking by hydrogen bonding less developed. The collagen of skins of warm-water fish, that of the pike for instance, is indicated to represent an intermediate degree of cross-linking of the protofibrils by hydrogen bond bridges. Neuman and Logan [2] found later that the principal difference between the amino acid composition of the two extreme types of collagens was the very much lower hydroxyproline (= hypro) content of fish collagen (halibut) than that of bovine collagen. There is somewhat more of aliphatic hydroxyamino acids and methionine in the teleost collagen also [3]. In the comprehensive investigation of Takahashi[4], a direct correlation between the hypro content of the three types of collagen mentioned and their degree of hydrothermal stability, measured by the temperature of instantaneous shrinkage of the fibers, *Ts*, has been demonstrated independently. The present author had earlier proved such a correlation for these collagens [1]. It appears as if the hypro residue of collagen should play the same governing role for its stability as the cystine residue does for keratin.

The values for hypro listed in the literature have been obtained by the method of Neuman and Logan [2]. The accuracy and reproducibility of this method is not as great as could be desired, and since indirect values only are obtained by this technique, a direct estimation of this imino acid was imperative. By a combination of the chromatographic procedures of Moore and Stein [5], and of Stein and Moore,[6] it has been possible to separate the hypro and proline (= pro) fractions of the hydrolysates from collagens of native bovine skin, skins of cod fish (*Gadus morrhua*) and of pike (*Esox lucius*). The first fractionation was accomplished on a column of starch, followed by the final separation on Dowex 50, operating in strongly acid solution, according to Åqvist's modification [7]. The estimation was kindly carried out by Dr. S. Åqvist. The collagens were in the native state (*not* alkali treated). The purification of the skins and the preparation of the collagens have been described in earlier publications [1]. For the final estimation of the pure imino acids, the micro-Kjeldahl method was applied. The data in the following table are given as g pro and hypro on 100 g collagen (fat- and ash-free dry substance) and as % of total nitrogen.

The hypro content of the collagen of the skin of cod, a typical cold-water fish, is appreciably lower than the figure of 9 % reported for the skin of halibut with T_s 40° [2]. However, Beveridge and Lucas [8] found a still lower value for the collagen (ichthyocol) from the swim bladder of hake, or about 4 %. The lowest hypro value in Takahashi's list [4] is 7.0 % obtained for the skin of the rockfish (*Sebastodes*) which also has the lowest T_s ever recorded for collagens, or 33—34 °C.

The sum of the pyrrolidine residues for the codskin collagen is hardly more than half of the value for the mammalian collagen. The great variations of the content of these imino acids in various collagens cannot be dismissed in framing of structural models of collagen. Approximately

Table 1.

Type of skin	T_s in°C	% total N	Pro		Hypro		Pro + Hypro	
			% on protein	% of total N	% on protein	% of total N	% on protein	% of Total N
Cod fish (*Gadus morrhua*)	40	18.3	10.8	7.2	5.8	3.4	16.6	10.6
Pike (*Esox lucius*)	55	18.4	13.3	8.8	7.9	4.6	21.2	13.4
Calf (*Bos taurus*)	65	18.3	14.1	9.5	12.7	7.4	26.8	16.9

every third amino acid residue of collagen is generally considered to be a pyrrolidine residue. This proportion is an integral part in recent models of collagen commencing with Astbury's classical model [9], and up to the helical structure principle of Pauling and Corey [10], although they have recently suggested occasional units of one residue of prolines of four residues [11].

The effect of the hypro content of collagen on its stability and reactivity was first examined in the light of Klotz's concept [12], according to which the hydroxy groups of the residues of aliphatic amino acids form hydrogen bonds of the type: $-OH\cdots\overline{O}CO-$ in some globular proteins. This linking should affect the reactivity of the carboxyl ion. The reactions of bovine and cod skin collagen with the cupric ion and with a number of cationic, anionic and non-ionic complex compounds of chromium, dyestuffs and non-electrolytes have been studied in the isoelectric zone. The results could not readily be reconciled with an extension of the Klotz concept to the collagens. Moreover, according to Bear [13], the electron-optical researches suggest the presence of the prolines in the interbands of the protofibrils, whereas the bands should contain the residues of the dicarboxylic amino acids. Hence, formation of the hypro-hydroxy—carboxyl bond appears improbable for steric reasons. Bear's concept has recently received strong experimental support chemically by Zahn's [14] isolation of dinitro-diphenyl-sulphone-*bis*-lysine derivatives and data of Schroeder *et al.* (15) provide some evidence that the peptides in partial hydrolysates of gelatin and collagen may contain the sequence: glycine-pro-hypro-glycine frequently. The nature of the effect of the hypro residues on the organization of collagen and the type of bond which the hypro forms have been studied on modified bovine collagens. A preliminary account of this work is given in the following note

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The Presence of Interchain Links Between Hydroxy and Keto-Imide Groups in Collagen

K. H. GUSTAVSON

Garverinäringens Forskningsinstitut, Stockholm, Sweden

The function of the hydroxyproline (=hypro) residue, the imino acid unique for collagen, has come to the forefront by the observation that the hypro content is directly related to the organization and reactivity of collagens [1]. Its possible participation in interchain links is obvious. An attempt of characterization of such cross-links has been made, primarily by the study of the effect of inactivation of the hydroxy groups of collagen on its properties and behaviour to certain reactants. The results of the investigation of collagen with its amino groups completely and its hydroxy groups to the maximum extent acetylated indicate that a part of the hydroxy groups forms a strong bond with the oxygen of the keto-imide groups (hydrogen bond). A preliminary account of the main findings forming the basis for this conclusion will be given in this note.