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Hydroxyproline and Thermal Stability of Collagen

GUSTAVSON[1] has recently directed attention to the importance of hydroxyproline residues in determining the hydrothermal stability of a collagen. He examined collagen samples from two broad sources, namely, mammalian and teleost connective tissue, and his findings demonstrated a direct relationship between the hydroxyproline content of a collagen and its shrinkage temperature. He considers that hydroxyproline will form interchain hydrogen bonds between its hydroxyl group and the carbonyl oxygen of an adjacent peptide grouping and may also be involved in the formation of ester links between polypeptide chains, thus playing a very important part in determining the stability and general reactivity of a collagen fibre. The evidence for assigning such a function to the hydroxyproline residue has, however, been derived from a study of many animal species. Furthermore, there is a growing body of evidence[2–4] which indicates that the reactivity of a collagen fibre depends on age and it still remains to be established whether variations in the hydroxyproline content can account for the differing degrees of reactivity with age, shown by collagen obtained from a single animal species.

We now wish to report some findings which bear on this question. In a study of the action of collagenase on samples of dermal collagen from sixty individuals, Keech[3] found that fifty of these responded normally to the enzyme, the response being a function of age. Eight samples, however, failed to show any digestion as assessed by the electron microscope, that is, were collagenase-resistant, while two other samples, taking into account their age, reacted excessively. As part of a more detailed investigation into those collagen preparations which show an abnormal response towards collagenase, hydroxyproline content and shrinkage temperature were determined. In all, fourteen samples of the same preparations used in previous work were examined, namely, human abdominal skin from persons of different age, purified by the method of Neuman[5] as shortened by Keech[3]. These collagen samples were classified into groups: group 1, ten showing normal response towards collagenase; group 2, two which were collagenase-resistant; and group 3, two which were hypersensitive towards collagenase.

Hydroxyproline content was determined using the method of Martin and Axelrod[6], to an accuracy of ± 5 per cent. The range of temperature over which the samples shrunk was determined by the microscopic method of Borasky and Nutting[7]. Small fibres were teased from the samples and rehydrated in distilled water for one hour, before mounting in the heating chamber. Heat was applied at a constant rate (2 deg. C./min.) and, so far as possible, fibres of similar size were used. Following Borasky and Nutting, the range of shrinkage was determined by recording both temperatures at which the fibres started to shrink and also the final one at which no further change in dimensions occurred.

Table 1

	No.	Age (years)	Hydroxyproline (gm./100 gm. dry collagen)	Range of shrinkage (° C.)
Group 1 (control)	1	7	14·3	65–69
	2	9	13·6	64·5–68·5
	3	19	13·7	65–66
	4	23	13·6	65·5–69
	5	27	14·2	64–67·5
	6	36	13·6	62–69
	7	42	13·1	65·5–69·5
	8	43	12·5	63–68
	9	57	13·1	70·5–74
	10	89	12·8	62–68
Group 2 (collagenase resistant)	11	18	14·0	72–77
	12	51	12·3	72–79·5
Group 3 (collagenase hypersensitive)	13	20	14·0	60–67
	14	52	12·5	60·5–65

The results are shown in Table 1. The hydroxyproline levels all lie within the range 12·3–14·3 per cent and within the accuracy of the determination these are reasonably constant, though there is a tendency in all three groups for the values to decrease with age. Until more accurate methods of determination become available, all that can be said, therefore, is that the hydroxyproline content of collagen preparations from one animal species does not vary much with age. On the other hand, the temperature-ranges of shrinkage shown by the three groups are quite distinct. With the exception of sample 9, the collagens in the control group (group 1) shrank over the range 62–69·5° C., the collagenase-resistant samples in group 2 over 72–79·5° C., while those in group 3, which are hypersensitive towards collagenase, shrank over a lower temperature-range, namely, 60–67° C. Furthermore, even with samples of similar age, although the hydroxyproline levels lie fairly close together, the temperature-ranges of shrinkage are again quite distinct. It seems probable, therefore, that the views of Gustavson may require amending since the thermal shrinkage of collagens from one animal species may be independent of the hydroxyproline content and that other factors, which depend on age, may be more important in determining the stability and general reactivity of such protein systems.

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