ISOLATION OF AN ALDEHYDE-CONTAINING PEPTIDE FROM TROPOCOLLAGEN[#]

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Ichthyocol and other tropocollagens contain 1-2 equivalents (per tropocollagen of m.w. 300,000) of a component which reacts directly as an aldehyde in several specific aldehyde tests (Blumenfeld et al., 1963; Gallop, 1964). Although the exact nature of this component and its mode of attachment to the protein have not been established as yet, the spectral behavior of its derivatives made with 2, 4-dinitrophenylhydrazine or N-methyl benzothiazolone hydrazone resembles that of similar derivatives of unsaturated aldehydes. After treatment of ichthyocol with $NaBH_4$ under conditions which cause aldehydes to be reduced to alcohols, the specific tests for aldehyde indeed become negative; this suggests that the aldehyde function is initially free or in hemiacetal form on the protein. Other evidence shows that the aldehyde is covalently attached to the protein. This communication describes the isolation and preliminary characterization of a pure aldehyde-containing peptide from a collagenase digest of ichthyocol.

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About 4.3 grams of ichthyocol parent gelatin were dissolved in 60 ml of 0.005 M $CaCl_2$ and digested for 24 hours at 37° and pH 8.0 with 186 units of collagenase. Proteolysis was complete as shown by determination of free amino groups by ninhydrin analysis. One gram portions of the digestion mixture were chromatographed on a Sephadex G-25 column using 1 N acetic acid as eluant. The results obtained are shown in Figure 1. Of the amounts placed on the column initially, zones II and III

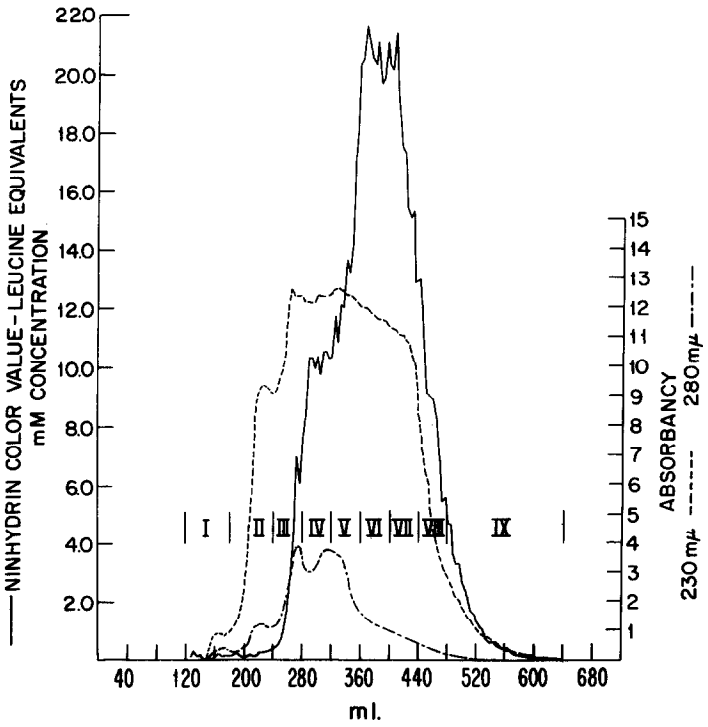

Figure 1. Chromatographic pattern of a collagenase digest of ichthyocol gelatin on a 4.25 x 50 cm Sephadex G-25 column at 25°. Absorbancy at 230 mμ and 280 mμ expressed in arbitrary units.

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contained respectively 4.8% and 21.1% of the total nitrogen, 10.5% and 22.1% of the total tyrosine, 2.9% and 4.8% of the total hexoses and 14.1% and 24.5% of the total aldehyde as determined by a modified Sawicki reaction (Blumenfeld et al., 1963 a). From the position of emergence of zones II and III it is probable that they contain relatively large peptides. The fractions constituting individual zones were

evaporated to dryness in a rotary evaporator, and each residue was dissolved in 5 ml of water and kept frozen until needed. Samples of 1 or 2 ml of zones II or III, containing respectively 0.98 and 7.22 mg of nitrogen, were treated at room temperature for 5 minutes with equal volumes of 0.5% 2, 4-dinitrophenylhydrazine in 2 N HCl. The excess 2, 4-dinitrophenylhydrazine was removed by gel filtration on a 1.5 x 17.5 cm column of Sephadex G-25, using 1 N acetic acid as eluant. The yellow fraction emerging between 12.5 and 25 ml (λ_{max} 390 mμ) was concentrated to a small volume in vacuo and rechromatographed on a 1.5 x 17 cm column of carboxymethyl Sephadex C-25 using 0.5 N acetic acid as eluant. The peak fraction, emerging between 12 and 25 ml and containing in an 80% yield the material absorbing at 390 mμ, was evaporated to dryness in vacuo and dissolved in 1 ml of water. Nineteen % of the total ninhydrin-reacting material placed on the column was recovered in this peak fraction, and this represented 0.25% of the ninhydrin-reacting equivalents in the total collagenase digest. The yellow absorbing material (λ_{max} 390 mμ) could not be extracted from the aqueous phase into any of a variety of solvents used, e.g., ethyl acetate or chloroform, suggesting that it was bound to the peptides.

The spectra of this material, at pH values 2.8 and 12.0 are shown in Figure 2. λ_{max} shifts from 390 mμ (pH 2.8 to 11.0) to 448 mμ at pH 12.0. At more alkaline

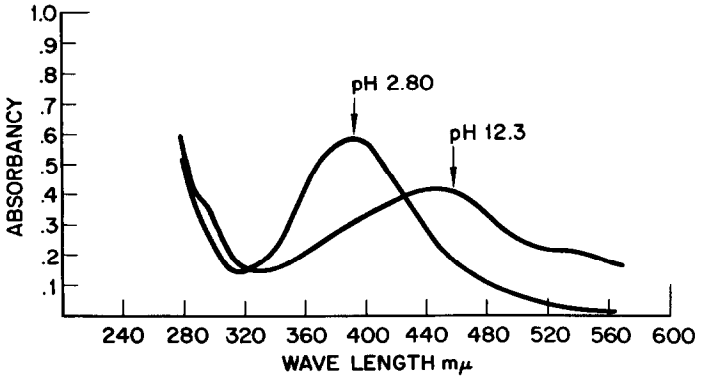

Figure 2. Spectrum of the 2, 4-dinitrophenylhydrazine derivative of the aldehyde-containing peptide at pH values of 2.8 and 12.3.

pH values, λ_{max} shifts to 436 mμ. These spectral characteristics at acidic, neutral and alkaline pH values are similar to those of 2, 4-dinitrophenylhydrazine derivatives, in aqueous media, of unsaturated aldehydes containing one double bond in conjugation with the carbonyl group. However, at pH values higher than 12 the spectrum resembles that of 2, 4-dinitrophenylhydrazones prepared from simple, saturated aldehydes*.

One-half ml aliquots of the samples obtained from carboxymethyl Sephadex were subjected to descending chromatography in butanol-acetic acid-water (4:1:5) for 16 hours. A yellow spot, migrating as a broad band 6-9 cm from the origin, was eluted from the paper with water and concentrated to 0.5 ml. It was then subjected to electrophoresis on Whatman 3MM paper for 3 hours at 10 volts/cm in 0.1 M pyridine-acetate buffer, pH 5.0, or 0.1 M Veronal buffer, pH 8.6. In either solvent system the yellow spot moved toward the anode and was ninhydrin-negative. Three other ninhydrin-positive spots had very low mobilities and were displaced towards the cathode. These did not contain any 2, 4-dinitrophenylhydrazone.

The yellow, ninhydrin-negative spot was eluted from paper with water, concentrated to a small volume, hydrolyzed with 6 N HCl in vacuo at 100° for 18 hours. Amino acid analyses were then carried out on an IR 120 column by an automatic technique (Spackman et al., 1958). The results are shown in Table I.

The close agreement in amino acid composition of the peptide material obtained after electrophoresis at two different pH values, and its reproducibility in different preparations of ichthyocol, point to the fact that it represents a single peptide of good purity. The absence, in this peptide, of many amino acids otherwise present in whole collagen also is of significance in this regard. The molar ratios of the residues suggest that the peptide is of a large size, i.e., about 30 residues. This molecular size is

*We have observed that acidic and neutral aqueous solutions of 2, 4-dinitrophenylhydrazones of aldehydes show a 10-15 mμ shift in their λ_{max} toward the visible spectral region as compared with solutions made in chloroform or ethanol (Jones et al., 1956).

Table I

Amino Acid Composition of the Aldehyde-Containing Peptide of Ichthyocol

Component	Residues per 100 Residues					Approx. No. of Residues in Peptide[e]
	Preparation Number				Average	
	I[c]	II[c]	II[d]	IV[c]		
Aspartic Acid	9.4	7.0	6.5	6.6	7.4 ± 1.1	2
Serine[a]	13.1	9.7	10.5	7.6	10.2 ± 1.5	3
Glutamic Acid	12.7	12.0	14.0	12.7	12.8 ± 0.6	4
Proline	12.2	14.6	9.9	12.9	12.4 ± 1.4	4
Glycine	34.2	31.5	31.8	30.8	32.0 ± 1.0	10
Alanine	11.6	8.8	13.2	12.5	11.5 ± 1.4	4
Valine	3.8	3.6	3.7	3.1	3.6 ± 0.2	1
Methionine[b]	6.8	7.3	7.3	8.3	7.4 ± 0.4	2
Tyrosine	3.7	3.8	1.8	4.0	3.3 ± 0.7	1
Leucine	trace	1.0	0.7	0.9	0.9 ± 0.3	0.3
Aldehyde	2.7	4.0	---	2.8	3.2 ± 1.2	1

a Not corrected for losses after hydrolysis.

b Calculated as methionine sulfoxides.

c Electrophoresis performed in 0.1M pyridine-acetate buffer, pH 5.0.

d Electrophoresis performed in 0.1M Veronal buffer, pH 8.6.

e Assuming that the peptide contains one molar equivalent of aldehyde.

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consistent with the position of emergence of the peptide from the Sephadex G-25 column. Table I also shows that the peptide contains one aldehyde equivalent, assuming a molar extinction coefficient of 20,000, as found with model 2, 4-dinitrophenylhydrazones of unsaturated aldehydes. There are no basic amino acids present, and hydroxyproline, threonine, isoleucine, and phenylalanine are also absent. Glycine

contributes about one-third of the total residues, as is also the case with "non-dialyzable" peptides isolated from collagenase digests of various collagens (Franzblau et al., 1964).

The peptide, since it contains tyrosine, most likely arises from an α2 chain of tropocollagen. It has recently been shown in our laboratory that α1 chains contain less than 0.4 residues of tyrosine (Smith et al., to be published).

The presence of trace quantities of aldehydic components in commercial gelatins and collagen has been previously reported (Landucci, 1954; Levine, 1962; Blumenfeld et al., 1963; and Gallop, 1964). The isolation from a collagenase digest of ichthyocol of a pure peptide which contains a covalently bound aldehydic substance in stoichiometric proportions, indicates that the aldehyde is indeed an integral component of tropocollagen.

Little is known about the structure and mode of attachment of the aldehyde to the protein. This problem is now under active investigation. Also being studied is the possible role of this aldehydic substance as an inter- or intramolecular crosslinking agent in collagen.

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