THE NATURE OF THE COLLAGEN DEFECT IN LATHYRISM[+]

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Collagen obtained from lathyritic animals is considered to be both deficient in intramolecular cross-links (Martin et al., 1961; 1963) and unable to form intramolecular cross-links when incubated in vitro at 37°C and neutral pH (Gross, 1963). Following the isolation of an aldehyde-containing peptide from ichthyocol collagen (Rojkind et al., 1964; 1966) and ratskin collagen, (Bornstein et al., 1966), several hypotheses implicating aldehyde constituents have been proposed to explain the action of certain lathyrogens (Bornstein et al., 1966; Tanzer et al., 1966; and Bensusan et al., 1966), but precise mechanisms of action have yet to be established. This communication describes the changes, with respect to aldehyde components, in lathyritic ratskin and chick embryo collagens, and in their separate α components.

MATERIALS AND METHODS

Twenty male rats, each weighing 50-60 gms, received a daily subcutaneous injection of 25 mg of β-aminopropionitrile (βAPN) fumarate (Abbott Laboratories) for 30 days. The rats were sacrificed by decapitation and collagen extracted from the skin and from tail tendon. Skins were extracted twice overnight with 5 volumes of

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1 M NaCl, and the collagen purified as described by Gross (1958). Acid extractable collagen from skin or tail tendon was prepared by two successive extractions for about 18 hours with 10 volumes of 0.5 M acetic acid. The collagen was then purified as described by Gallop (1955). In other experiments, groups of 50 to 100 developing fertilized chicken eggs, each fourteen days old, received either 20 mg of βAPN or 5 mg of thiosemicarbazide (TSC) (Eastman Organic Chemicals) by injection into the chorioalantoic membrane. On the 17th day of embryonic development, the chicks were removed. Collagen from skin and bones was then extracted (about 18 hours) with 2 volumes of 1 M NaCl and purified as described by Gross (1958). Aldehyde determinations were performed by the method of Paz et al. (1965). Individual component chains of tropocollagen were separated on a 2.5 x 20 cm column of carboxymethyl cellulose (CMC) by the method of Piez et al. (1961). Five ml aliquots were collected and read at 230 mμ in a Beckman DU spectrophotometer. Contents of every fifth or tenth tube was dialyzed against distilled water, concentrated to dryness and resuspended in a small volume of 20% sucrose. An aliquot (0.2 ml) of this was used for acrylamide gel electrophoresis by the method of Nagai et al. (1964). Fractions obtained using CMC were then pooled in accordance with the gel electrophoresis patterns. The combined fractions were then dialyzed against distilled water and lyophilized. Protein was determined by the Kjeldahl method.

RESULTS AND DISCUSSION

The aldehyde contents of tropocollagens obtained from different animal species are very similar (Harper and Seifter, unpublished results) and appear to be independent of the relative concentrations of different α components. The value found is approximately 0.5 to 0.6 μmoles of acetaldehyde equivalents per 100 mg of protein. In the experiments presented here, both lathyritic ratskin and chick embryo tropocollagens showed a decrease of more than 30% from the control value. This decrease in aldehyde

occurred in collagen of both species with use of either lathyrogen (βAPN or TSC) (Table I). It was of interest then to explore whether the change in aldehyde affected all chains.

TABLE I

Aldehyde Contents of Normal and Lathyritic Collagens
(Neutral Salt-Extracted)

(μmoles acetaldehyde equivalents/100 mg of protein)

	Total*	α1	α1+	α2
Control				
Ratskin	0.50	0.88	-	0.90
Lathyritic				
Ratskin (βAPN)	0.34	0.46	-	0.88
Chick Embryo (βAPN)	0.35	0.44	0.63	0.86
Chick Embryo (TSC)	0.32	0.48	0.66	++

\+ Second component of the α1 peak
++ Not determined
* The total aldehyde is not the sum of that measured in separated chains

Using the method of separation described, very pure α chains were obtained, but pure β components could not be isolated in good yields. The α1 peak of lathyritic chick embryo collagen always showed a second component; this was the case no matter whether βAPN or TSC was used as the lathyrogen. Under the conditions used for separation (Fig 1) the second component chromatographed as a shoulder of the α1 peak. Its mobility on acrylamide gel electrophoresis was identical to the other α chains. When a less steep gradient was used, the separation was improved but no complete resolution was achieved (Fig. 2). The two fractions were pooled as indicated in Figs. 1 and 2, and prepared for aldehyde determination as described under methods.

The aldehyde contents of the isolated chains in the neutral salt-extracted collagen were quite constant and of the order of 0.8 to 0.9 μmoles per 100 mg of protein

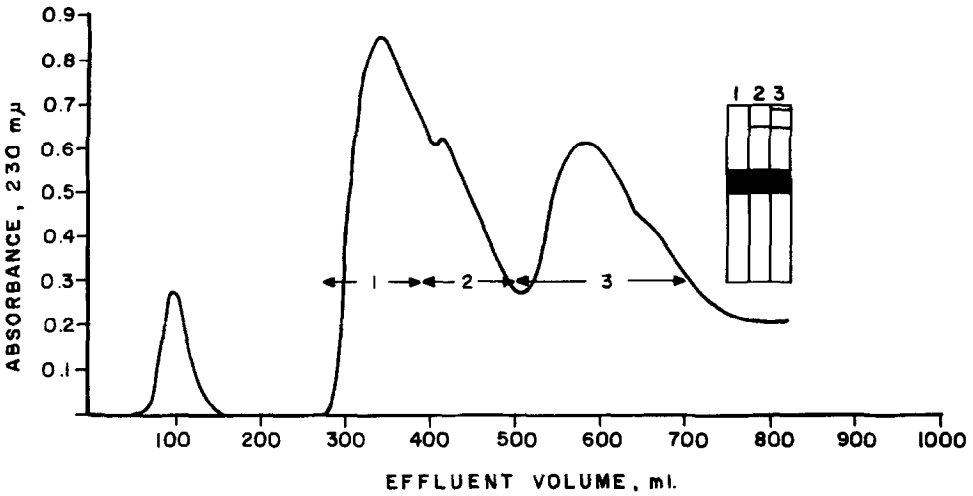

Fig. 1. CMC chromatography of 100 mg of denatured (βAPN) lathyritic chick embryo collagen. The eluting buffer was 0.06 ionic strength sodium acetate buffer, with a superimposed linear gradient from 0.0 to 0.12 M NaCl over a total volume of 800 ml. The diagram represents the electrophoretic behavior of each component on acrylamide gels, at high protein concentration (> 200 μg).

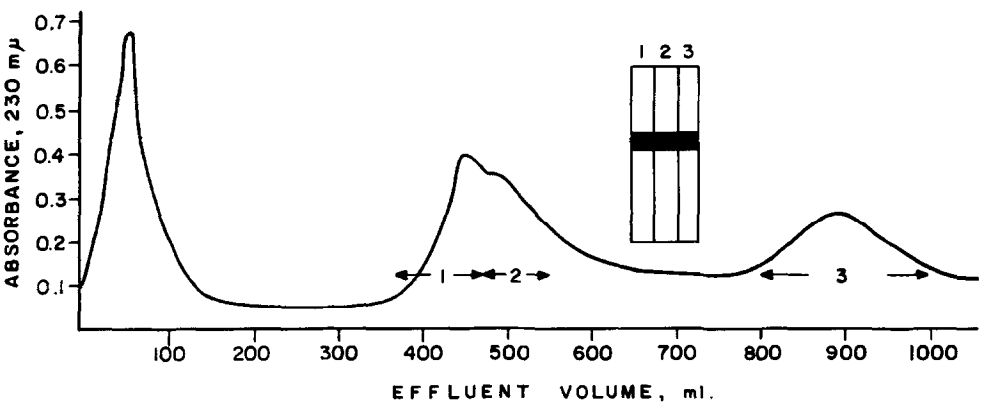

Fig. 2. CMC chromatography of 70 mg of denatured (TSC) lathyritic collagen. The eluting buffer was 0.06 ionic strength sodium acetate buffer, with a superimposed linear gradient from 0.0 to 0.10 M NaCl over a total volume of 1000 ml. The diagram represents the electrophoretic behavior of each component on acrylamide gels, at high protein concentration (> 200 μg).

(Table I). In acid extracted collagen the values were slightly higher, being 1.0 μmole per 100 mg of protein; thus the presence of one aldehyde group per chain was indicated.

As can be seen from the data presented in Table I, the α2 chains from control and lathyritic ratskin collagens contained identical amounts of aldehyde. This observation was also true for the collagen of chick embryos made lathyritic with either βAPN or TSC. The α1 chain from lathyritic ratskin showed a 50% decrease in aldehyde as compared with the controls, and in the chick embryo important differences were observed when the two α1 components were analyzed separately. The first half of the α1 peak always gave values below 0.5 μmoles of acetaldehyde equivalents per 100 mg protein, and the second half contained more than 0.6 μmoles.

The above results reinforce an earlier suggestion (Rojkind et al., 1964; 1966; Gallop et al., 1965; and Bornstein et al., 1966) that aldehydes are important in cross-linking and maturation of collagen. The present experiments also suggest that aldehyde formation in α1 may be the major rate-limiting step in the overall process of cross-linking and maturation. Current evidence suggests that each of the 3 α components of ratskin collagen contain one aldehyde function (Kang et al., 1966), and our results indicate that only a sub-fraction of the α1 component is deficient in aldehyde in lathyrism. Work is in progress to determine if chick embryo tropocollagen consists of 3 different α chains, as has been shown for codfishskin tropocollagen (Piez, 1965), and to establish whether the alteration in aldehyde is specific for only one of the 3 components.

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