THE PRESENCE IN ELASTIN OF POSSIBLE CYCLIC PRECURSORS OF DESMOSINE AND ISODESMOSINE*

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Summary

Mass spectral evidence is presented that the elastin of bovine ligamentum nuchae contains, in addition to desmosine and isodesmosine, larger levels of their respective cyclic dehydrodesmopiperidine precursors. In addition to being precursors of the desmosines, such compounds are most likely cross-links in themselves. In fact, the occurrence of these compounds, now isolated as their reduced derivatives, may clarify the discrepancies in the stoichiometry of the conversion of lysine residues to cross-linking components as noted by several workers (1,2,3). With this study the occurrence of all of the tetrafunctional amino acid cyclic structures related to the desmosines is now confirmed by mass spectrometry.

Introduction

Elastin is presently considered to be synthesized as a linear polypeptide, tropoelastin, that subsequently undergoes chemical modification leading to formation of cross-links within the polypeptide chain or between several polypeptide chains. The proposed reactions for the formation of cross-links are shown in Figure 1a. Desmosine and isodesmosine were isolated as polyfunctional pyridinium amino acids (4), and schemes for their formation were postulated by Partridge et al. (5) and elaborated on by Davis and Anwar (6). The latter authors hypothesized cyclic intermediates preceding the final fashioning of the pyridinium

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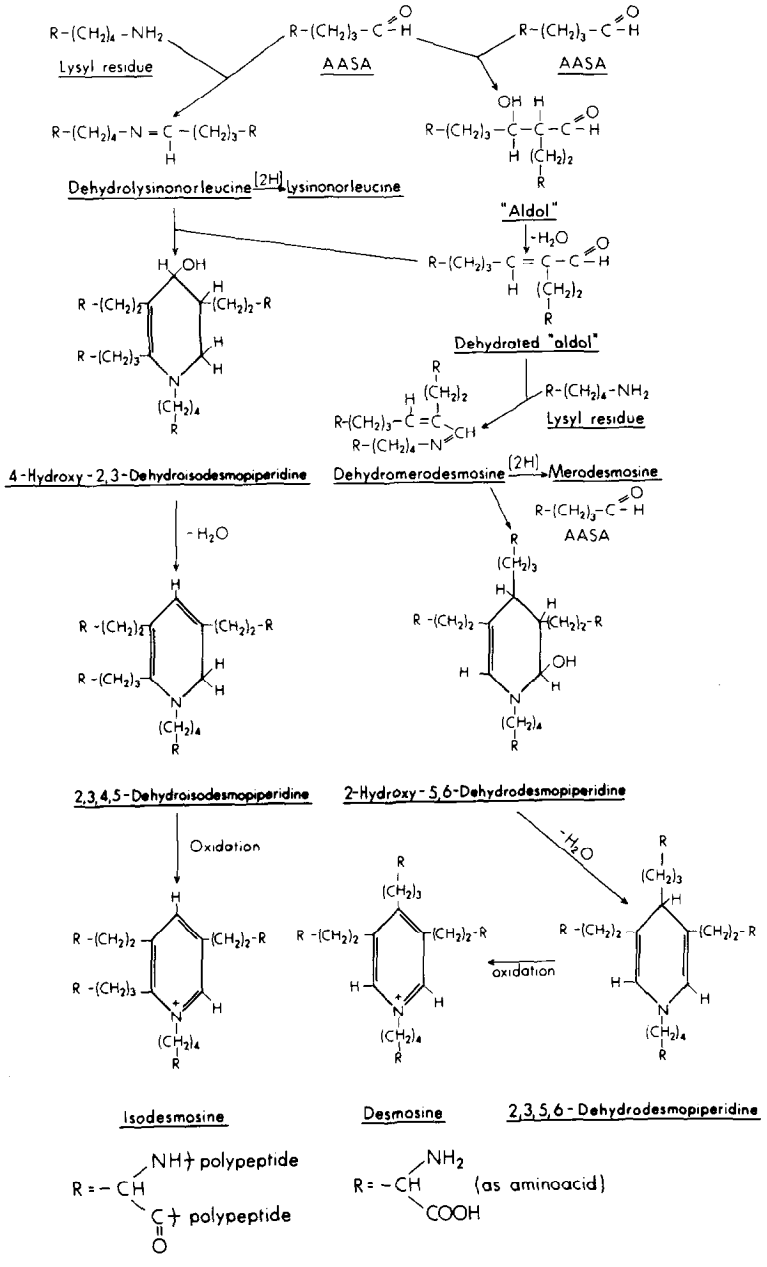

Fig. 1a Proposed reactions for the formation of cross-links in elastin. The abbreviation, AASA, represents α-amino adipic acid δ-semialdehyde.

rings of the desmosines; of necessity such intermediates must contain rings in a higher state of reduction than the pyridinium ring. The precursors were designated as dihydrodesmosines by Davis and Anwar who then reasoned that these could be oxidized spontaneously to the desmosines.

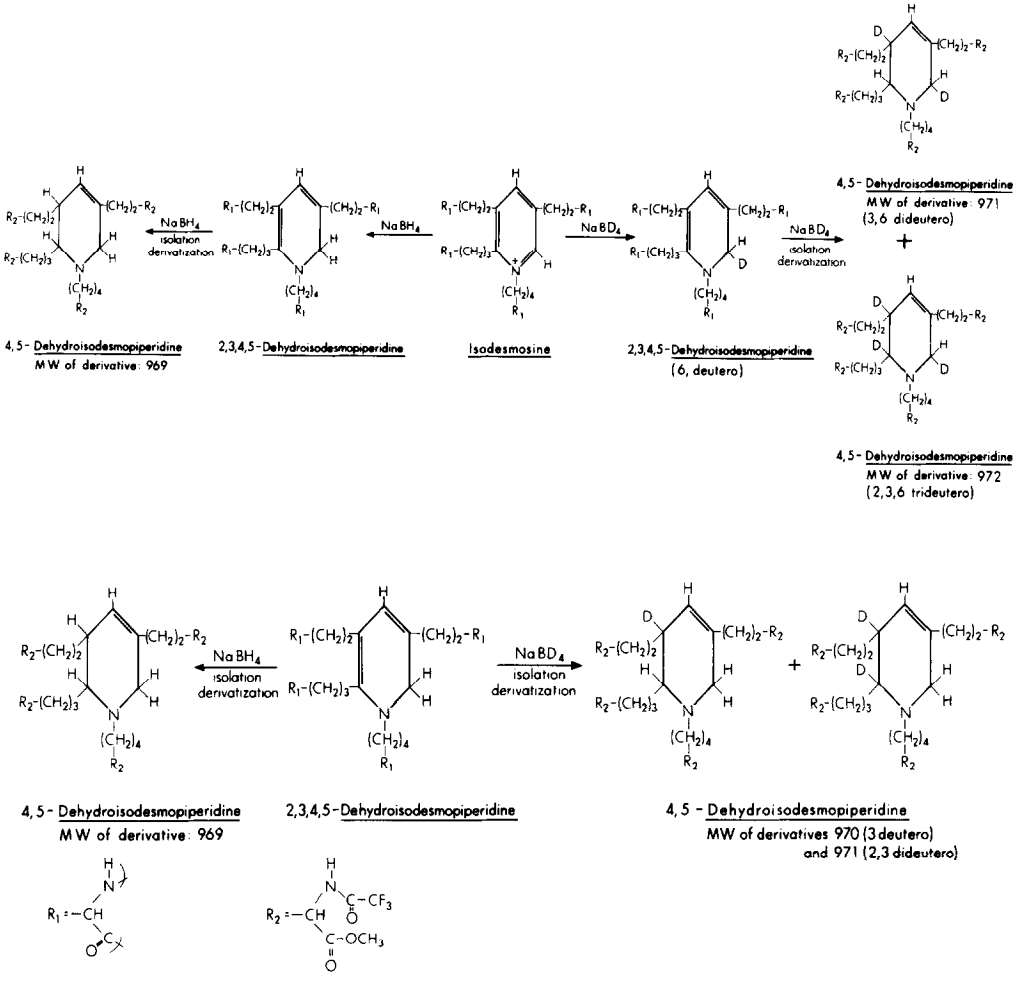

Fig. 1b Expected pathways of reduction of isodesmosine and of 2,3,4,5-dehydroisodesmopiperidine.

In the course of experiments with reduction of elastin with tritiated sodium borohydride, $^{3}[H]NaBH_4$, and tritiated sodium borodeuteride, $^{3}[H]NaBD_4$, we have now been able to isolate compounds with the characteristics of dehydropiperidine derivatives. These compounds resulted both from saturation of two of the double bonds of the desmosine components

and from reduction of one double bond of the postulated dehydrodesmopiperidine precursors.

Figure 1b shows the expected pathways of reduction of isodesmosine and of its postulated precursor, 2,3,4,5-dehydroisodesmopiperidine. The figure also shows the molecular weights of the N-trifluoroacetyl methyl ester derivatives that would be obtained if various isotopic borohydrides were used in the reduction. In the experiments to be described, mass spectrometry of the products demonstrated that these could not have originated solely by reduction of polypeptidyl isodesmosine and must also have originated from the postulated polypeptidyl 2,3,4,5-dehydroisodesmopiperidine. One could write a similar scheme for the pathways of reduction of desmosine and its postulated precursor.

Experimental

Elastin was prepared from bovine ligamentum nuchae by the method of Partridge et al. (7). Elastin (200 mg) was then suspended in 10 ml of distilled water and the pH adjusted with NaOH to 9-9.5. Two types of experiments were then performed. In the first, the suspension of elastin was mixed with approximately 50 mg of tritiated $NaBD_4$, and in the second the elastin was mixed with 50mg of tritiated $NaBH_4$*. The tritiated $NaBD_4$ and $NaBH_4$ were calibrated as described previously (8). Each of the elastin mixtures was incubated with continuous stirring for 30 minutes at room temperature. The reaction was stopped by addition of glacial acetic acid to pH 5. The suspension was centrifuged, the supernate discarded, and the residue washed 5 times with distilled water followed by centrifugation. Approximately 20 microequivalents of tritium were incorporated into 100 mg of elastin. The elastin was then hydrolyzed with 6N HCl at 105° for 22 hours. The HCl was removed in vacuo and the hydrolysate washed 5 times with repeated evaporations with water. Aliquots of the redissolved hydrolysate were used for radioactive counting, determination of nitrogen, and for fractionation by chromatography using the short column of

* The tritium levels compared to the hydrogen and deuterium levels respectively were such as not to be observed in the mass spectrometer but provided specific activities in the range of 12×10^6 dpm/μ mole of reduced compound.

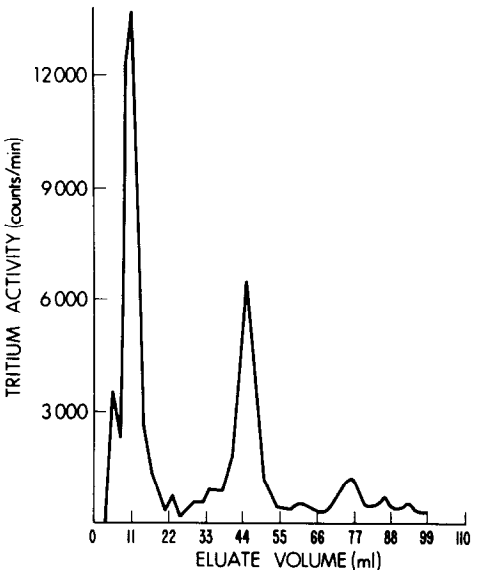

Fig. 2 Pattern of elution of reduced and acid-hydrolyzed elastin; the material contained in the peak between 38 and 55 ml includes the reduced products of the desmosines and their cyclic precursors.

a Beckman amino acid analyzer model 120B. Aliquots of 20-30 mg were used for each fractionation and elution was performed with 0.35M K-acetate buffer, pH 4.65. Fractions of 1 ml were collected and radioactivity determined. All of the acidic and neutral amino acids appeared in the first 20 ml, and this fraction contained about 54% of the total counts. The reduced products which eluted between phenylalanine and lysine appeared as a peak containing about 26% of the counts placed on the column. This peak had radioactivity corresponding to the incorporation of 5-6 microequivalents of tritium per 100 mg of elastin. The other radioactive peaks such as lysinonorleucine had relatively much less radioactivity. The pattern of elution is shown in Figure 2.

Peaks of reduced material obtained in 5 separate runs were pooled and the substance purified further by chromatography on a BioGel P-2 column (86 x 1.5 cm) using 0.1M acetic acid as eluant. Fractions of 1.2 ml were collected and monitored by radioactivity. The purified material was next converted to the N-trifluoracetyl methyl ester by the procedure of Bailey and Peach (9).

Desmosine and isodesmosine were isolated from unreduced elastin after acid hydrolysis. Isodesmosine was reduced with $^{3}[H]NaBH_4$ and $^{3}[H]NaBD_4$ and then derivatized as described above. All the derivatives were then studied in the Hitachi RMU6E mass spectrometer. Tris(pentadecafluoroheptyl)-S-triazine (Peninsular Chemresearch, Inc.) was used to

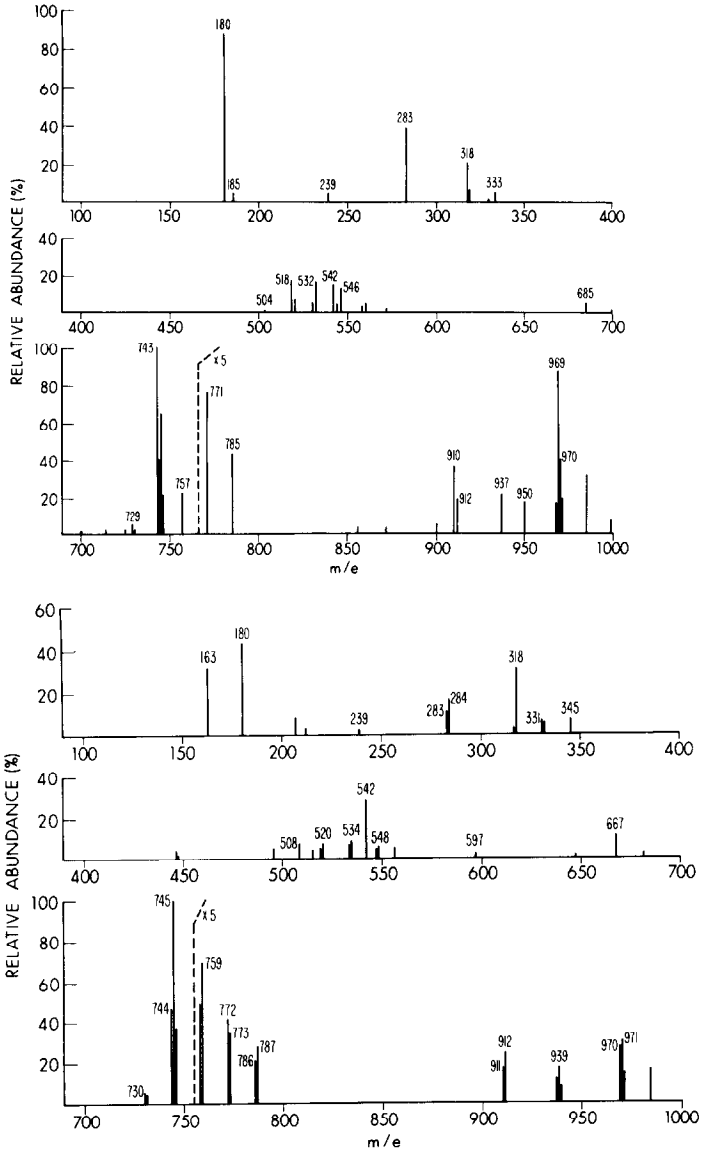

Fig. 3a Mass spectra of the N-trifluoroacetyl methyl ester derivatives of the reduced products isolated from elastin. The spectrum at the top was obtained when the reduction was with tritiated sodium borohydride, and that at the bottom when the reduction was with tritiated sodium borodeuteride.

facilitate the counting of the peaks representing high masses since this material gives ions at 771, 866, 916, 928, 966, 1166 and 1185.

Results and Discussion

Figure 3a shows the mass spectra obtained with the N-trifluoracetyl methyl ester derivatives of the isolated compounds from reduced elastin. Figure 3b presents the mass

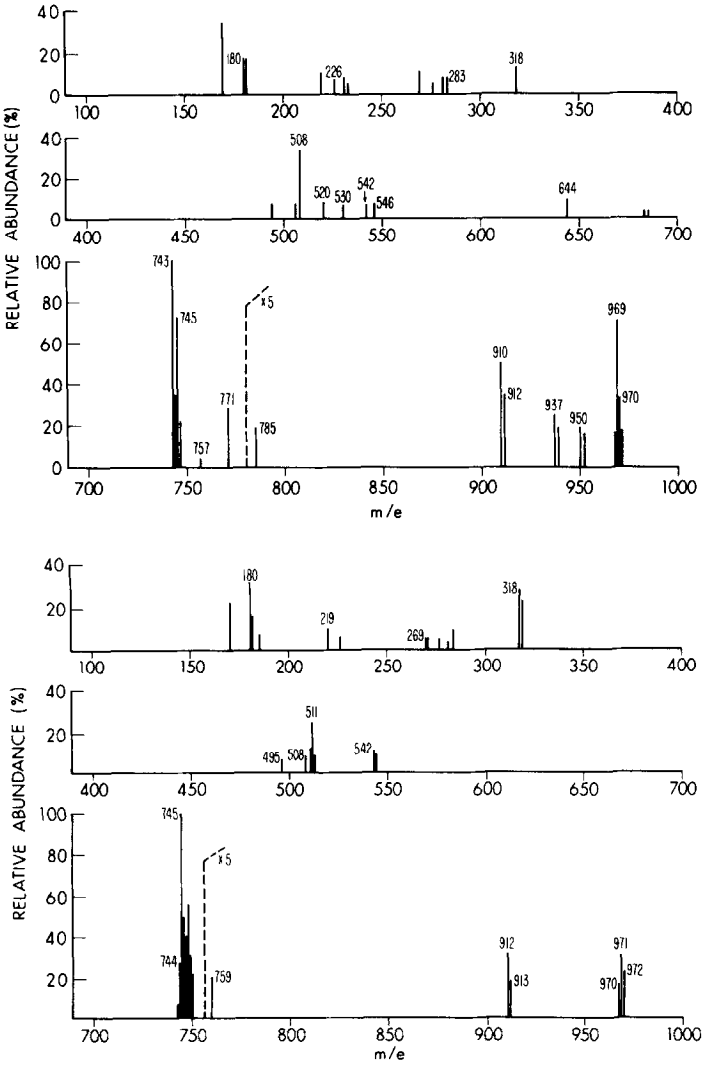

Fig. 3b Mass spectra of the N-trifluoroacetyl methyl ester derivatives of the isolated isodesmosine that had been reduced. The spectrum at the top was obtained when the reduction was with tritiated sodium borohydride, and that at the bottom when the reduction was with tritiated sodium borodeuteride.

spectra of similar derivatives of isolated isodesmosine that had been reduced. In each case the parent odd electron ion is present together with strong ions indicating radical losses from the sidechains of 184, 198, 212 and 226. The loss of 226 results in the base ion with m/e at 743. Furthermore, cyclization leads to a loss of N-trifluoracetyl pipecolic acid methyl ester as a neutral molecule of 239, leaving an odd electron ion of m/e at 730; the latter, in turn undergoes radical losses of 184, 198, 212 and 226 giving rise to strong ions at 546, 532, 518 and 504 respectively. The parent ion can also split off a neutral molecule of N-trifluoracetyl proline methyl ester by cyclization to give an odd electron ion at 744, which then also can lose sidechains as the radicals enumerated above.

The experiment using $NaBD_4$ was designed to distinguish between dehydrodesmopiperidines originating from the reduction of two double bonds of the desmosines as opposed to reduction of one double bond in the postulated precursor (see Figure 1b). Mass spectra of the derivatized compounds obtained from reduced elastin show parent ions at 970 and 971. If one corrects for natural isotope abundance, these are the masses anticipated if indeed the precursor 2,3,4,5-dehydroisodesmopiperidine were deuterated. After correction for natural isotope abundance, a peak at 972 is also evident, as would be expected if the desmosines were deuterated; however this peak is relatively small (see Table I). If elastin is not treated with sodium borohydride, but is acid hydrolyzed, desmosine and isodesmosine can be isolated mixed with much less precursor material. After correction, a higher percentage of the 972 ion is evident in the mass spectrum of the derivative obtained from the product of reduction of isodesmosine with $NaBD_4$ (Fig. 3b and Table I). Furthermore, relatively less of the peak (mass 970) characteristic of the precursor is found. One may conclude that the elastin studied here contained more of dihydrodesmosine and/or dihydroisodesmosine than of desmosine plus isodesmosine per se.

The occurrence of these compounds may clarify the discrepancies in the stoichiometry of the conversion of lysine residues to cross-linking components in insoluble elastin as noted by others (1,2,3).

Table I

The Corrected Heights of Critical m/e Peaks Obtained in the Mass Spectral Analysis of Compounds in Elastin and of Isodesmosine after Reduction with $NaBD_4$

	Corrected Peak Heights of Parent Ions -%	
m/e	Compounds from Reduced Elastin	Reduced Isodesmosine
970	57	33
971*	38	46
972*	5	21

* Corrections are for natural abundance of isotopes

References

1) Partridge, S.M., in Chemistry and Molecular Biology of the Intercellular Matrix, 1, 593 (1970), (E.A. Balazs, ed.), Academic Press, London.
2) Sandberg, L.B., Weissman, N. and Gray, W.R., Biochemistry 10, 52 (1971).
3) Franzblau, C. and Lent, R., in Structure, Function and Evolution in Proteins, Brookhaven Symposia in Biology 21, 358 (1968).
4) Thomas, S., Elsden, D.F. and Partridge, S.M., Nature, 200, 651 (1963).
5) Partridge, S.M., Elsden, D.F., Thomas, J., Dorfman, A., Telser, A., Ho, P.L., Nature 209, 399 (1966).
6) Davis, N.R. and Anwar, R.A., J. Amer. Chem. Soc. 92 (12), 3778 (1970).
7) Partridge, S.M., Davis, H.F. and Adair, G.S., Biochem. J. 61, 11 (1955).
8) Paz, M.A., Henson, E., Rombauer, R., Abrash, L., Blumenfeld, O.O. and Gallop, P.M., Biochemistry 9, 2123 (1970).
9) Bailey, A.J. and Peach, C.M., Biochem. Biophys. Res. Communs. 33, 812 (1968).