STUDIES ON CROSS-LINKED REGIONS OF ELASTIN

R.A. Anwar, G.E. Gerber and K.M. Baig

Department of Biochemistry
University of Toronto
Toronto, Canada M5S 1A8

ABSTRACT

Elastin, a protein of unique elasticity and tensile strength, is a connective tissue component. Like most elastomers, it consists of randomly coiled polymer chains, joined together by cross-links into an extensible, three dimensional network. The major cross-links of elastin are formed as the result of the deamination of three out of four lysine side chains which subsequently condense to give desmosine or isodesmosine cross-links. We made a novel use of Edman degradation in the study of desmosine and isodesmosine containing elastolytic peptides of mature elastin. This permitted the isolation and sequence studies of peptides C-terminal to the desmosine cross-links in bovine, porcine and human aortic elastin as well as bovine ligamentum nuchae elastin. This identifies the lysines in the tropoelastin (soluble precursor of elastin) which give rise to the desmosine cross-links. The sequences of the C-terminal peptides were found to fall into two distinct classes, one starting with hydrophobic residues, the other starting with alanine. The study of lysine sequences of tropoelastin from a lathyritic calf, with the use of Myxobacter AL-I Protease II, suggests that essentially all lysines occur in pairs separated by two or three small amino acid residues. The majority of the lysines occur in the sequence -Lys-Ala-Ala-Lys- and -Lys-Ala-Ala-Ala-Lys-. It is proposed that two such pairs meet to form desmosine or isodesmosine cross-link and that the hydrophobic residue at the carboxyl end of lysine prevents the enzymic oxidative deamination of the adjacent lysine ε-amino group and this then contributes the nitrogen to the pyridinium ring of the cross-link.
INTRODUCTION

The protein elastin is concentrated in those tissues (ligaments, alveoli, large blood vessels) which require rapid extension and complete recovery, but is only a minor component of skin, tendons and loose connective tissue (Partridge, 1962). It is insoluble in all non-hydrolytic solvents. Hydrated elastin fibers exhibit the unusual properties of a typical elastomer (Hoeve et al., 1958; Weis-Fogh and Anderson, 1970); thus they stretch rapidly under a small load and retract rapidly and completely to their original dimensions (with little loss of energy as heat) upon removal of the load. All typical synthetic elastomers consist of long polymeric chains which are freely mobile with respect to one another except at points of cross-linking; these cross-links must be sufficiently widely spaced to allow considerable extension without breaking covalent bonds, but must be sufficiently close to give the fiber its high tensile strength and modulus when fully stretched. Therefore, it was argued (Partridge, 1962) that elastin must consist of an amorphous system of peptide chains covalently cross-linked at intervals, to account for its elastic properties and insolubility. This realization led to the search for elastin cross-links.

ELASTIN CROSS-LINKS

The search for elastin cross-links culminated in the isolation of two polyfunctional amino acids named desmosine and isodesmosine from bovine ligamentum nuchae elastin (Thomas et al., 1963). The structures of these compounds are shown in Fig. 1. Each compound has a pyridinium nucleus and 4 α amino and carboxylic groups. Desmosine is 1,3,4,5 substituted pyridine and isodesmosine is 1,2,3,5 substituted pyridine. Thus these compounds are capable of cross-linking four polypeptide chains. The isolation of these two new amino acids was later extended to elastin preparations from different tissues and species (Anwar, 1966). After the isolation of these compounds, the attention naturally turned to the problem of their biosynthesis.

BIOGENESIS OF DESMOSINE CROSS-LINKS

The structures of desmosine and isodesmosine suggested that each could be derived from four lysine molecules. In support of this suggestion, it was shown that $^{14}$C label was incorporated into desmosine and isodesmosine isolated from aortas of rats given $[^{14}$C$]$-lysine (Partridge et al., 1964, 1966) and chicken aortas grown in a medium containing $[^{14}$C$]$-lysine (Miller et al., 1964, 1965). The degradation of isolated desmosine and isodesmosine