A NEW ADHESIVE PROTEIN MEDIATING THE INTERACTION BETWEEN MESENCHYMAL CELLS AND ELASTIN FIBERS: ELASTONECTIN

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An inducible adhesive protein was demonstrated in smooth muscle cells and fibroblasts which mediate the adhesion of mesenchymal cells to elastic fibers. It is proposed to designate it elastonectin. This protein plays probably an important role in the morphogenesis of elastic tissue and its degradation is probably involved in the formation of the atherosclerotic plaque.

Smooth muscle cells of the vascular wall and fibroblasts in the skin are often in close contact with elastic fibers. Previous studies have shown that the most often observed biological role of structural glycoproteins such as fibronectin, laminin, chondronectin and others, is to mediate the adhesion of differentiated cells to specific fibrous elements of the extracellular matrix. (For a review see ref.[1].)

Examples which were well studied comprised the adhesion of fibroblasts to collagen fibers of type I or III mediated by fibronectin, the adhesion of epithelial or epidermal cells, to collagen type IV mediated by laminin, and of chondrocytes to collagen II mediated by chondronectin. We wanted to explore the possibility of the presence of a specific protein mediating the adhesion of mesenchymal cells to elastic fibers. Our results clearly point to the existence of a specific inducible membrane protein which plays this role as an interface between mesenchymal cells and elastic fibers.

EXPERIMENTAL

The methods used comprise the addition of highly purified insoluble elastic fibers obtained from *ligamentum nuchae* and labelled with [^{3}H]borohydride to confluent cultures of human skin fibroblasts and pig aorta smooth muscle cells. After a lag period of about 30 min, the adhesion of the elastic fibers can be quantitated by decanting the supernate with the non-adherent fibers and counting the adherent and the non-adherent radioactivity.

Biosynthetic experiments were also carried out by adding [^{35}S]methionine to cell cultures together with soluble and fibrous elastins. Control experiments were carried out by adding first the labelled precursor and only after 6 h (at the end of the incubation period), the fibrous elastin, just before stopping both incubations. Adhering proteins were then eluted with a series of solvents such as 1M-NaCl, 1M urea, 4M guanidinium, and 4M guanidinium with 0·1M dithiothreitol. This last eluent showed the strongest increase in radioactivity in induced cultures as compared to the controls.

RESULTS

Figure 1 shows the adhesion curve of elastic fibers to human skin fibroblasts. Videotapes were also prepared in culture chambers under the inverted microscope to follow the details of this procedure. It could be shown that the elastic fibers adhere to the cell surface and not to the cell free spaces of the culture dish (Fig. 2). The kinetics of this adhesion could be strongly accelerated by adding soluble elastin peptides (kappa-elastin) to the culture medium half an hour before the addition of fibrous elastin. The adhesion could be inhibited when protein biosynthesis inhibitors such as cycloheximide were added to the cultures. Adherent elastic fibers could be removed using trypsin or elastase but much less or not at all with collagenase.

The nature of this adhesive protein for which we propose the name elastonectin was studied by incubating the cells with [^{35}S]methionine during the induction of the synthesis of the adhesive protein. This could then be eluted from the elastic fibers using guanidinium and dithiothreitol after previous extraction of the less strongly adhesive proteins with 1M urea and 4M guanidinium (without dithiothreitol).

The final extract contained a strong band at 120 000 and a weaker band at 45 000. Further studies are necessary for the detailed characterization of this adhesive

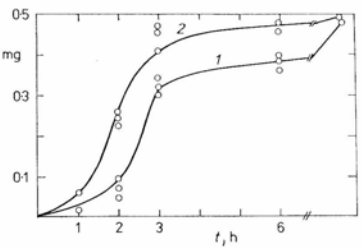

FIG. 1

Binding of elastic fibers to cells. Abscissa: time of incubation at 37°C, hours; ordinate: mg elastin bound per 10^5 cells. *1* porcine aorta smooth muscle cells, *2* human skin fibroblasts

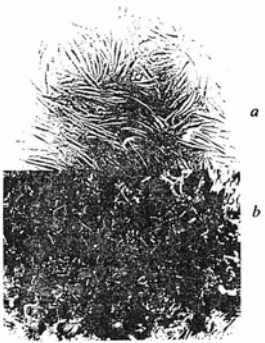

FIG. 2

Adhesion of elastic fibers to human skin fibroblasts. *a* Human skin fibroblasts in culture at the moment when elastic fibers were added. *b* After 1 h of incubation; elastic fibers are adhering to the surface of the cells

protein. Its susceptibility to elastase-type enzymes shows that the induction of such enzymes which were shown to be present on the membrane of smooth muscle cells (a serine protease[2-4]) and in the fibroblast (a metallo-protease[5,6]) could play a role in detaching the cells from their matrix enabling them to migrate and divide. This kind of phenomena are observed during the formation of the atherosclerotic plaque and were designated as phenotypic modulation of the smooth muscle cells which from sessile and contractile become biosynthetic, mobile and form the atherosclerotic plaque[7,8]. For more details on elastonectin see ref.[9].

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B. Kratochvíl, L. Jenšovský: **Úvod do krystalochemie (Introduction to Crystal Chemistry).** SNTL, Prague 1987. 240 pp; price Kčs 23,—.

The monograph by B. Kratochvíl and L. Jenšovský, *Introduction to Crystal Chemistry*, SNTL (Prague, 1987), in spite of its relatively small size, provides the maximum amount of information on the preparation and properties of crystals, their symmetry, structural research and relationships between the structure and properties of crystalline substances. A brief summary of structural types is also given, primarily to define the importance and position of crystal chemistry as a field lying between chemistry and physics, mineralogy, geology, metallurgy, biology and some technical disciplines. The book is intended mainly as a study text for students in the natural sciences and technical fields but can also be recommended to scientific and research workers who wish to become acquainted with the basis of crystal chemistry. It forms a basis for more intensive utilization of the great deal of information available on crystal structure and parameters in the literature, to solve scientific and technical problems wherever a relationship is sought between the structure, chemical composition and properties of solid substances and also to predict chemical composition and methods for preparation of solid substances with defined properties.

The careful preparation of the text and content of the monograph require no comment. On p. 209, the 5th place on the Mohs scale should be "apatite". Pearsons' valence laws tend to be rather neglected in the literature and also in this book.

The limited size of the book prevented elaboration in great detail; nonethless, however, the importance of Burger's vector for description of dissociation types, dislocation growth and formation of dislocation steps should be mentioned.

This book can be considered to make a positive contribution to the scientific literature.

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