The Elastin Receptor: A Galactoside-Binding Protein

ALEKSANDER HINEK, DAVID S. WRENN, ROBERT P. MECHAM,* SAMUEL H. BARONDES

The elastin receptor complex contains a component of 67 kilodaltons that binds to a glycoconjugate affinity column containing β-galactoside residues and is eluted from this column with lactose. This protein component is also released from the surface of cultured chondroblasts by incubation with lactose, and its association with immobilized elastin is inhibited by lactose. Since lactose also blocks elastic fiber formation by cultured chondroblasts, the galactoside-binding property of the elastin receptor is implicated in this process.

MANY VERTEBRATE TISSUES contain galactoside-binding proteins that are on the cell surface or in the extracellular matrix (*1*). These endogenous lectins are confined to specific cell types, but their functions have not been established. A 14.5-kD β-galactoside–binding lectin was suggested to be a participant in elastic fiber formation in developing rat lung, since developing lung tissue shows concomitant increases in synthesis of this lectin and elastin (*2*). In addition, immunohistochemical studies show that this endogenous lectin apparently accumulates in elastic fibers (*3*).

In studies of the possible role of endogenous lectins in the organization of elastic fibers we have identified an additional β-galactoside–binding protein in developing bovine lung and in primary cultures that synthesize elastin. We show here that this 67-kD protein is a component of the recently characterized elastin receptor (*4*). We also present evidence that the carbohydrate-binding function of this protein is important for elastic fiber assembly.

Evidence that lung contains a 67-kD galactoside-binding protein comes from asialofetuin-Sepharose affinity chromatography of extracts of immature lung. When the lung protein that bound to this column was eluted with lactose, we obtained a 67-kD protein in addition to the prominent endogenous lectin of 14 kD (Fig. 1). This 67-kD protein has the same apparent subunit molecular weight as the largest component of the elastin receptor complex purified by affinity chromatography on a column of immobilized elastin peptides (Fig. 1, lane B). To determine if the two proteins are the same, we reacted each with a monoclonal antibody specific for the purified elastin receptor (*5*). Both proteins reacted strongly with this monoclonal antibody on immunoblots (Fig. 1, lanes C and D). Surprisingly, this monoclonal antibody also reacted with the 14-kD lectin purified from lung (Fig. 1, lane C). Furthermore, a polyclonal antiserum (*6*) to highly purified 14.5-kD rat lung lectin (anti-RL-14.5) that reacts with the bovine 14-kD lung lectin showed a strong cross-reaction with the 67-kD protein purified by either asialofetuin affinity chromatography or elastin affinity chromatography (Fig. 1, lanes E and F). This immunological cross-reactivity raises the possibility that some of the antigen shown to be associated with elastic fibers in prior immunohistochemical studies (*3*) might be the 67-kD protein.

In immunohistochemical studies, we found that anti-RL-14.5 bound to the surface of cultured auricular chrondroblasts that were producing elastin. To evaluate the nature of the immunoreactive material we eluted the surface of the chondroblasts with phosphate-buffered saline containing 50 m*M* lactose and passed the extract, after dialysis, over an asialofetuin column. After elution with lactose, we found a prominent band in the eluate at 67 kD but no 14-kD protein, although extracts of the whole cells had substantial amounts of the latter protein on immunoblots. The 67-kD protein prepared from asialofetuin also bound to elastin and could be eluted with lactose. These results indicated that it was the 67-kD carbohydrate-binding protein that was present on the surface of elastin-producing chrondroblasts and that it could be dissociated from the surface by lactose.

From these experiments it appeared that a function of the carbohydrate-binding site of the 67-kD protein was to promote association of this protein with the cell surface, presumably by interaction with glycoconjugates at this site. But, in further studies, another function for the carbohydrate-binding site was suggested. In these experiments, the association of the 67-kD protein with an immobilized elastin peptide column was examined by addition of a series of sugars including lactose, galactose, glucose, and fucose. Lactose (1 m*M*) eluted this protein from the immobilized elastin column, and 100 m*M* galactose was also effective in releasing it, whereas 100 m*M* glucose or 100 m*M* fucose had no effect. Bovine tropoelastin has no sites for *N*-glycosylation (*7*), and in studies with radioactive sugars we have found no evidence for glycosylation of bovine tropoelastin (*8*). Therefore it is unlikely that the association between 67-kD protein and tropoelastin is mediated by binding of the carbohydrate-binding site of the former with a glycoconjugate on the latter. Furthermore, purified tropoelastin does not bind to immobilized asialofetuin, confirming that tropoelastin is not a galactoside-binding lectin. Therefore, it seems likely that the association between elastin and the 67-kD protein is by a tight (K_d = 8 n*M*) protein-protein interaction (*4*), which is allosterically inhibited by binding lactose to the 67-kD protein. These findings are con-

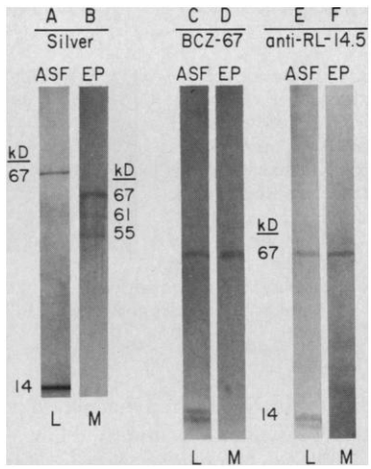

Fig. 1. Eluates from affinity columns containing either immobilized asialofetuin (ASF) (*6*) or immobilized elastin peptides (EP) (*4*). Extracts of lung (L) from a 250-day bovine fetus prepared as described (*3*) and detergent extracts of plasma membranes (M) from auricular chondroblasts (*4*) were applied to the columns and eluted with 0.1*M* lactose in 0.1*M* sodium bicarbonate buffer, *p*H 8.0. The eluates were dialyzed against water at 4°C, concentrated by lyophilization, and electrophoresed on polyacrylamide gels under reducing conditions in the presence of SDS, as described (*4*). Gels were reacted with silver stain (lanes A and B), or immunoblotted and reacted with monoclonal antibody (*5*) to the 67-kD component of the bovine elastin receptor (BCZ-67, lanes C and D), or with antiserum (*6*) raised to rat lung 14.5-kD β-galactoside–binding lectin (anti-RL-14.5, lanes E and F). Gel A is 10% polyacrylamide. Gels B through F are 5 to 12.5% polyacrylamide gradient gels that resolve the single 14-kD band visible on the nongradient gel into a doublet. Identical molecular weight standards were run on both gradient and nongradient systems.

A. Hinek and R. P. Mecham, Department of Cell Biology and Physiology, and Respiratory Disease and Critical Care Division, Department of Medicine, Jewish Hospital at Washington University Medical Center, St. Louis, MO 63110.
D. S. Wrenn, Department of Pathology, Memorial Hospital, Pawtucket, RI 02860.
S. H. Barondes, Langley Porter Psychiatric Institute, Department of Psychiatry, University of California, San Francisco, CA 94143.

*To whom correspondence should be addressed.

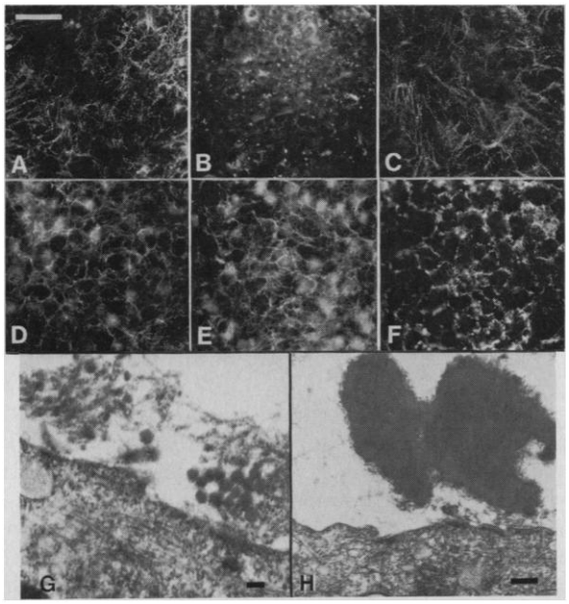

Fig. 2. Effects of sugar on elastic fiber formation by cultured chondroblasts. Auricular chondroblasts from a 150-day bovine fetus were grown to confluency in eight-well multichamber slides (Miles Scientific) in Dulbecco's modified Eagle's medium (containing 9 m*M* glucose) supplemented with 5 m*M* lactose, 5 m*M* fucose, or 150 m*M* glucose. Elastin fibers, microfibrils, and fibronectin were visualized by immunohistochemical staining with antibodies to bovine elastin (*14, 15*), bovine MAGP (*16*), and human plasma fibronectin (*15*), respectively. (**A**) The elastin network in control cultures grown without added sugar. The addition of lactose (**B**) disrupted deposition of elastin in the extracellular matrix but fucose (**C**) and glucose (not shown) had no effect. Extracellular organization of MAGP or fibronectin was not disrupted by concentrations of lactose that inhibited elastin fiber formation. (**D**) MAGP without sugar. (**E** and **F**) MAGP and fibronectin, respectively, with 5 m*M* lactose. (**G** and **H**) Electron micrographs of extracellular elastin in auricular chondroblasts grown in the presence or absence, respectively, of 5 m*M* lactose. Cultures were fixed in cacodylate-buffered 2% glutaraldehyde containing 1% tannic acid, postfixed in 1% osmium tetroxide in cacodylate buffer, dehydrated, and embedded in Spurr's low-viscosity resin (*17*). Thin sections were stained with uranyl acetate and lead citrate. In cultures containing lactose (G), elastin was detected as small discrete globules that did not coalesce to form larger fibers characteristic of normal, mature elastin (H). Bars, 100 μm (A to F); 100 nm (G and H).

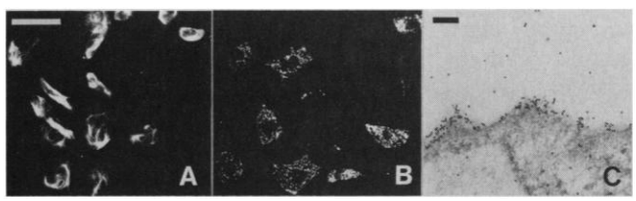

Fig. 3. Distribution of 67-kD antigen on the surface of chondroblasts grown in the absence (**A**) or presence (**B**) of lactose. Unfixed and unpermeabilized cells were stained with 50 μg/ml of anti-67-kD monoclonal immunoglobulin and then by fluorescein-labeled second antibody. (**C**) Localization at the plasma membrane of cultured chondroblasts of 15-nm gold particles coated with BCZ-67 antibody is shown. Bars, 100 μm (A and B); 200 nm (C).

sistent with the observation that elastin peptides and the synthetic peptide Val-Gly-Val-Ala-Pro-Gly, a chemotactic sequence found in elastin (*9*), also block the association between the 67-kD protein and elastin (*10*), presumably by interfering with a protein-protein interaction. A bifunctional lectin that participates in both protein-protein and protein-carbohydrate interactions has been described (*11*).

We then determined if the carbohydrate-binding site on the 67-kD protein played a role in elastic fiber organization by cultured chondroblasts. Addition of 5 m*M* lactose to the culture medium blocked the formation of elastic fibers in the extracellular matrix, whereas comparable concentrations of fucose or high concentrations of glucose had no effect (Fig. 2). In contrast, lactose did not alter the extracellular distribution of fibronectin or microfibrillar-associated glycoprotein (MAGP) (*12*). The principal effect of lactose on elastic fiber formation appeared to be on fiber assembly since tropoelastin synthesis was not altered (*13*).

Further analysis with a monoclonal antibody to elastin showed that, in the presence of lactose, some elastin accumulated in the extracellular matrix as numerous, small, irregularly distributed globules. Electron microscopy confirmed the globular nature of elastin in the sugar-treated cultures (Fig. 2G), and it is evident from these images that elastin globules in lactose-containing cultures did not coalesce to form the large elastin structures characteristic of normal extracellular matrix (Fig. 2H). The inhibitory effect was specific since extracellular elastic fiber formation was not altered by glucose or fucose.

In addition to perturbing elastin accumulation in the extracellular matrix, the inclusion of lactose in cell culture medium altered the distribution of elastin receptor on the membrane of chondroblasts and smooth muscle cells. Immunofluorescence staining with a monoclonal antibody to the 67-kD protein showed the receptor to be organized in a fibrillar pattern on the surface of control cells (Fig. 3A) and in a punctate distribution on cells treated with lactose (Fig. 3B). Immunoblot analysis demonstrated 67-kD antigen in culture medium from lactose-treated cells but not control cells, confirming that the 67-kD lectin is released from the cell surface by lactose. Localization of 67-kD protein to the cell surface was obtained by using immunogold particles coated with BCZ-67 antibody (Fig. 3C).

Our results indicate that the 67-kD component of the elastin receptor complex has a biologically significant galactoside-binding site, which is involved in the macromolecular assembly of elastin into elastic fibers. To our knowledge this is the first example of a role for a protein-carbohydrate interaction of this type in macromolecular assembly. How the 67-kD protein functions in this assembly process is not presently clear. One possibility is that it presents tropoelastin on the cell surface in an orientation that leads to fiber formation. Another possibility is that the carbohydrate-binding site of the 67-kD protein interacts with the highly glycosylated microfibrillar proteins that are critical for elastic fiber assembly (*12*). The change in association between the 67-kD protein and tropoelastin in the presence of lactose suggests allosteric regulation of this interaction, which could be important at the cell surface and in the matrix where elastic fibers are being formed. The precise way in which the 67-kD protein acts in fiber formation and the function of 14-kD galactoside-binding lectin synthesized by cells that are making elastic fibers remain to be determined.

REFERENCES AND NOTES

1. S. H. Barondes, *Science* **223**, 1259 (1984); in *The Lectins: Properties, Functions, and Applications in Biology and Medicine*, I. E. Liener *et al.*, Eds. (Academic Press, Orlando, FL, 1986), pp. 437–466.
2. J. T. Powell and P. L. Whitney, *Biochem. J.* **188**, 1 (1980).
3. R. F. Cerra, P. L. Haywood-Reid, S. H. Barondes, *J. Cell Biol.* **98**, 1580 (1984).
4. D. S. Wrenn *et al.*, *J. Biol. Chem.*, **263**, 2280 (1988).
5. R. P. Mecham *et al.*, *Biochem. Biophys. Res. Commun.*, in press.
6. R. F. Cerra, M. A. Gitt, S. H. Barondes, *J. Biol. Chem.* **260**, 10474 (1985).
7. K. Raju and R. A. Anwar, *ibid.* **262**, 5755 (1987); H. Yeh *et al.*, *Collagen Relat. Res.* **7**, 235 (1987).
8. Glycosylation of tropoelastin was investigated by incubating a mince of 270-day fetal bovine ligamentum nuchae in Earle's balanced salt solution containing 3% fetal bovine serum and 0.1 mCi/ml each of [2-^{3}H]mannose (22 Ci/mmol), [6-^{3}H]galactose (25 Ci/mmol), [5,6-^{3}H]fucose (44 Ci/mmol), or [1-^{3}H]glucose (25 Ci/mmol). A second tissue mince was incubated similarly with 8 μCi/ml of [^{3}H]leucine (133 Ci/mmol). Tropoelastin in the medium or tissue extract was immunoprecipitated with monoclonal antibody BA-4 as described by D. S. Wrenn *et al.* [*J. Biol. Chem.* **262**, 2244 (1987)]. Precipitated products were washed thoroughly and a portion was taken for scintillation counting. The remaining sam-

ple was analyzed by SDS–polyacrylamide gel electrophoresis and visualized by autofluorography. Although there was considerable incorporation of radioactive leucine into tropoelastin, there was no detectable incorporation of sugar into this protein.

9. R. M. Senior *et al., J. Cell Biol.* **99**, 870 (1984); D. S. Wrenn, G. L. Griffin, R M. Senior, R. P. Mecham, *Biochemistry* **25**, 5172 (1986).
10. R. P. Mecham, D. S. Wrenn, A. Hinek, in preparation.
11. W. R. Springer, D. N. W. Cooper, S. H. Barondes, *Cell* **39**, 557 (1984).
12. E. G. Cleary and M. A. Gibson, *Int. Rev. Connect. Tissue Res.* **10**, 97 (1983); M. A. Gibson, J. L. Hughes, J. C. Fanning, E. G. Cleary, *J. Biol. Chem.* **261**, 11429 (1986).

13. Chondroblasts in 100-mm culture dishes were grown to confluency in Dulbecco's modified Eagle's medium containing 10% calf serum with or without 100 m*M* lactose. For tropoelastin determination, the cells were incubated for 18 hours in culture medium supplemented with β-aminopropionitrile (100 mg/ml). Tropoelastin levels in the collected medium and in an acetic acid extract of the cell layer were determined by competitive enzyme-linked immunosorbent assay (*4*). Values were normalized to DNA content and expressed as nanograms of elastin per microgram of DNA per 18 hours. There was no difference in total tropoelastin production between control and lactose-treated cultures.

14. R. P. Mecham, *Methods Enzymol.* **144(D)**, 232 (1987).
15. ———, J. Madaras, J. A. McDonald, U. Ryan, *J. Cell. Physiol.* **116**, 282 (1983).
16. E. G. Cleary, in *Diseases of Connective Tissue: The Molecular Pathology of the Extracellular Matrix,* J. Uitto and A. J. Perejda, Eds. (Dekker, New York, 1986), pp. 55–81.
17. A. Hinek, J. Thyberg, U. Friberg, *Cell Tissue Res.* **172**, 59 (1976).

18. Supported by NIH grants HL-26499 (R.P.M.), HL-29594 (R.P.M.), and HL-38627 (S.H.B.), and a grant to S.H.B. from the National Science Foundation. We thank T. Cleary and J. A. McDonald for providing antisera to MAGP and fibronectin, respectively.

13 November 1987; accepted 29 January 1988

Selection of Amino Acid Sequences in the Beta Chain of the T Cell Antigen Receptor

STEPHEN M. HEDRICK, ISAAC ENGEL, DAVID L. MCELLIGOTT, PAMELA J. FINK, MEI-LING HSU, DANIEL HANSBURG, LOUIS A. MATIS

The induction of an immune response in mammals is initiated by specifically reactive T lymphocytes. The specificity of the reaction is mediated by a complex receptor, part of which is highly variable in sequence and analogous to immunoglobulin heavy- and light-chain variable domains. The functional specificity of the T cell antigen receptor is, however, markedly different from immunoglobulins in that it mediates cell-cell interactions via the simultaneous recognition of foreign antigens and major histocompatibility complex–encoded molecules expressed on the surface of various lymphoid and nonlymphoid cells. The relation between the structure of the receptor and its functional specificity was investigated by analyzing the primary sequences of the receptors expressed by a series of T lymphocyte clones specific for a model antigen, pigeon cytochrome c. Within this set of T lymphocyte clones there was a striking selection for amino acid sequences in the receptor β-chain in the region analogous to the third complementarity-determining region of immunoglobulins. Thus, despite the functional differences between T cell antigen receptors and immunoglobulin molecules, analogous regions appear to be important in determining ligand specificity.

ANTIGEN RECOGNITION BY T LYMPHOCYTES is mediated by a membrane-bound receptor complex, the T cell receptor (TCR), comprising at least eight polypeptide chains. Two of the chains, termed α and β, manifest a diversity characteristic of immunoglobulin (Ig) molecules (*1, 2*), and these two chains transmit the T cell specificity for antigen and major histocompatibility complex (MHC) molecules (*3*). The α-chain gene contains six exons, the second resulting from the somatic rearrangement of a variable gene segment encoding approximately 95 amino acids (V-region), and a joining gene segment encoding between 18 and 21 amino acids (J-region) (*4, 5*). The β-chain is also encoded by six exons, the second resulting from the somatic rearrangement of a variable gene segment encoding about 98 amino acids, a diversity gene segment encoding from 0 to 4 amino acids (D-region), and a joining gene segment encoding about 16 amino acids (*6, 7*). Additional amino acid residues, encoded by N-regions, are often present between each of the rearranging gene segments and originate from a template-independent addition of nucleotides (*8*).

Primary sequence analyses of Ig V-regions have shown three regions of sequence hypervariability resulting from evolutionary divergence and antigen-selected somatic hypermutation (*9, 10*). Crystallographic studies have shown that these hypervariability regions can roughly define the complementarity-determining regions (CDRs) that constitute the antigen contact residues (*11*). Several distinct characteristics of the TCR make similar studies problematic. A comparison of TCR sequences has not clearly revealed hypervariable regions (*5, 7*), and this is due, in part, to a lack of somatic mutations found in TCR genes (*12, 13*). In addition, T cells are always specific for MHC molecules, in contrast to the diverse specificity of Ig molecules, and neither the TCR nor its MHC ligand can be readily isolated in quantities necessary for x-ray crystallographic studies.

In this report we present an extensive sequence comparison of α- and β-chains expressed by T cells specific for pigeon cytochrome c. The absence of somatic mutations implies that it is difficult to determine by primary sequence analyses which of the amino acids in the α- and β-chains are important in determining specificity. However, if the TCR is analogous to Ig, then the highly variable V-(D)-J-region junction is predicted to encode the third CDR, and within a set of clones with a similar receptor specificity there may be a distinct selection of particular amino acids in forming a combining site. We show that for three different clonal phenotypes defined by antigen-MHC specificity, the V-D-J-region junction of the β-chain is selected for distinct amino acid sequences, whereas for the α-chain no such sequence selection is observed.

Information concerning the clones to be examined in this report is tabulated in Table 1. The T cell clones are arranged into three groups based on specificity for cytochrome c in association with either B10.A (MHC allele recognized is E^k), B10.A(5R) (E^b), or B10.S(9R) (E^s) antigen-presenting cells (APCs), and all but clone V1.9.2 respond to the carboxyl-terminal fragment of pigeon cytochrome c (amino acids 81 to 104).

The α- and β-chain sequences from nine clones that express the $V_\beta 3$ gene segment were analyzed together, and Fig. 1a shows the α-chain V-, J-region junction sequences of eight of the clones (*14*). The junctions of six of the eight clones are similar, with few N-region nucleotide additions, and this reflects the average α-chain junctional diversity (*15*). Among those expressing $V_\alpha 11$, one of the clones, C.F6, has a proline substituted for the more common alanine or threonine amino acids, and this substitution would be expected to alter the conformation of the CDR loop. Furthermore, quite a different junctional sequence was found in the α-chain of 2B4, including two additional ami-

S. M. Hedrick, I. Engel, D. L. McElligott, P. J. Fink, M.-L. Hsu, Department of Biology and Cancer Center, University of California, San Diego, La Jolla, CA 92093.
D. Hansburg, Department of Pathology, Fox Chase Cancer Center, Philadelphia, PA 19111.
L. A. Matis, Division of Biochemistry and Biophysics, Center for Drugs and Biologics, Food and Drug Administration, Bethesda, MD 20892.