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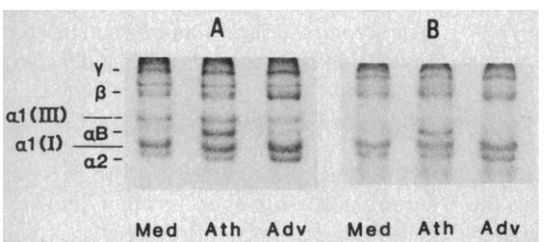
Collagen αB Chain: Increased Proportion in Human Atherosclerosis

Abstract. *In a study of human atherosclerotic plaques, the relative abundance of α chains in pepsin-solubilized collagens from 28 human aortas was estimated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The ratio of αB, a component of the α chain in type V collagen, to α1(I) was markedly increased in the atherosclerotic plaques compared to the nonsclerotic intact media and adventitia. It is suggested that proliferating smooth muscle cells in the sclerotic lesion were transformed to synthesize a larger amount of collagen αB chain during the process of human atherogenesis.*

Fibrous thickening of the intima with lipid deposition is the main feature of human atherosclerosis. Collagen is a major component of human atherosclerotic plaques, constituting as much as 30 percent of the dry weight (*1*). At present at least five genetically distinct species of collagen are known (2). Type I collagen, designated $[\alpha1(I)]_2\alpha2$, is the most common collagen (*3*). Type II (*4*) and III collagens (*5*) consist of three identical α chains and are designated as $[\alpha1(II)]_3$ and $[\alpha1(III)]_3$, respectively. Type II collagen is found exclusively in cartilaginous tissue. Type III collagen, observed as fine reticulin fibers, is found in several tissues such as skin, aorta, and uterus. Type IV collagen is the basement membrane collagen and a molecular formula $[\alpha1(IV)]_3$ has been popular (*6*), but there still remains some controversy (*2*). Type V collagen is made up of αA and αB (*7*) or A and B chains (*8*). This collagen is synthesized by a variety of cells including smooth muscle cells (*9*, *10*) and tends to be localized pericellularly (*10*). Blood vessels contain types I, III, IV, and V collagens. Type III collagen is the predominant collagen of nonsclerotic media of the human aorta, comprising approximately 70 percent of the total collagen (*11*). However, only a few reports are available concerning the change of collagen types in human atherosclerosis (*10*–*12*). The present study was undertaken to determine the compositional change of collagen types by estimating relative proportions of collagen α chains. Here I report that the αB chain of type V collagen is proportionately increased in human atherosclerotic plaques.

Twenty-eight aortas were obtained at autopsy from humans (ages 54 to 68 years) who had died of various diseases including stroke, myocardial infarct, aortic aneurysm, and neoplasms. Aortas were removed within 4 hours after death, samples being collected from portions with sclerotic lesions. (All of the aortas showed atherosclerotic lesions at some stage of development.) Samples of adjacent intact media and adventitia were used as controls. Fragments of all of the samples were fixed in 10 percent Formalin for histological study. Minced tissues were washed in cold distilled water overnight and freed of blood. Tissues were homogenized with a Polytron ST-10 in 50 volumes of 0.5*M* acetic acid containing pepsin (Sigma, twice crystallized) at a concentration of 1 mg/ml. Collagen was extracted with constant stirring for 24 hours at 4°C. The solutions were centrifuged at 39,000*g* for 1 hour at 4°C. Collagen was reextracted from the pellets under the same conditions for 48 hours. The supernatants were then combined and collagen was precipitated by adding 4.0*M* NaCl to a final concentration of 2.0*M*. The precipitate was dissolved in 0.5*M* acetic acid and dialyzed against 0.02*M* Na_2HPO_4. Precipitated collagen was redissolved in 0.5*M* acetic acid, then



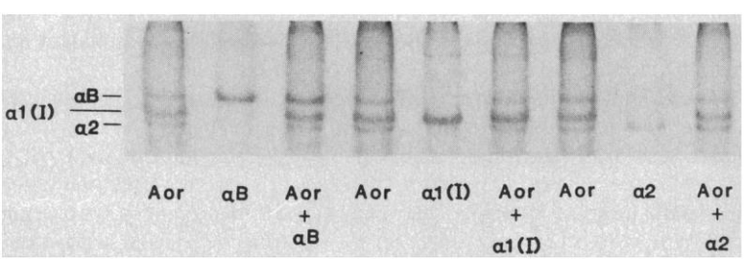


Fig. 1 (left). Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis of pepsin-solubilized collagens from the intact media (*Med*), atherosclerotic plaque (*Ath*), and adventitia (*Adv*) of the human aorta. (A) Samples of heat-denatured collagen (20 to 30 μg) were electrophoresed on the SDS–polyacrylamide gel slab for 2.5 hours. One hour after reduction in situ with β-mercaptoethanol the electrophoresis was resumed for another 2.5 hours. (B) Electrophoresis of the same samples as above for 5 hours without reduction with β-mercaptoethanol. Since reduction in situ did not change the electrophoretic behavior of αB, α1(I), and α2, these chains were not held by disulfide bonds. In this study the α chains derived from type IV collagen could not be detected because of their paucity in the sample. Fig. 2 (right). Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis of the aortic collagen (*Aor*) and its component α chains. Samples (20 to 30 μg) of aortic collagen, purified α chains, and a mixture of the two were electrophoresed on the SDS–polyacrylamide gel slab for 5 hours without reduction with β-mercaptoethanol. Note that purified αB, α1(1), and α2 comigrate with corresponding α chains in the aortic collagen. See the text and legend to Fig. 1 for details.

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dialyzed against 0.05*M* acetic acid exhaustively and finally lyophilized. The solubility of the tissue collagen in each sample was estimated by comparing the hydroxyproline content as measured (*13*) in the initial homogenate and in the final collagen solution. It was found that 68 to 76 percent of the tissue collagen was extracted. In these experiments, the extracted collagen was taken to be representative of the total collagen deposited in the tissue. In some instances, whole aortas or pooled tissues collected from separate regions of the aorta were used for isolation of types I, III, and V collagens by the differential salt precipitation (*7*). Component α chains in types I, III, and V collagens were separated by carboxymethyl cellulose chromatography as described (*7*).

The procedure for the estimation of the relative abundance of α1(III) chain was the same in principle as the interrupted gel electrophoresis developed by Sykes *et al.* (*14*). Electrophoresis was performed in a 6 percent polyacrylamide gel slab measuring 160 by 135 by 2 mm, with 12 parallel tracks. Gel and electrode buffers were 0.1*M* phosphate buffer, *p*H 7.2, containing 0.1 percent sodium dodecyl sulfate (SDS). Lyophilized sample collagens were dissolved at a concentration of 2 mg/ml and denatured in the gel buffer containing 1 percent SDS at 60°C for 30 minutes. Portions of 10 to 15 μl of denatured collagen were applied to the gel and electrophoresed at 80 mA. After 2.5 hours the current was switched off and sample wells were filled with 20 percent β-mercaptoethanol (reduction in situ) and allowed to diffuse into the gel for 1 hour to cleave intramolecular disulfide bonds of type III collagen, $[\alpha 1(III)]_3$. The electrophoresis was then resumed for another 2.5 hours. As shown in Fig. 1, bands of α1(I), α2, and α1(III) stained with Coomassie blue were separated. A band located between α1(III) and α1(I) was identified as αB on the basis of the elution profile on carboxymethyl cellulose chromatography (not shown), electrophoretic mobility (Fig. 2), and amino acid analysis (Table 1). In this manner each collagen α chain could be separated and quantitated by densitometry. The relative abundance of αB and α1(III) chains was calculated by dividing their optical densities by the optical density of α1(I).

The most striking finding was a significant increase ($P < .01$) in the ratio of αB chains in the atherosclerotic plaque (0.56 ± 0.32) to αB chains in the media (0.28 ± 0.13) and adventitia (0.05 ± 0.04) (Fig. 3B). In contrast, there was little change between α1(III) in the atherosclerotic plaque (0.79 ± 0.18) and in the media (0.73 ± 0.18) (Fig. 3A), although these two values were far greater than the value for α1(III) in the adventitia (0.29 ± 0.10). Thus it may be concluded that fibroblasts, which are the major collagen-producing cells in the adventitia, synthesize predominantly type I collagen and a lower amount of type III and type V collagens.

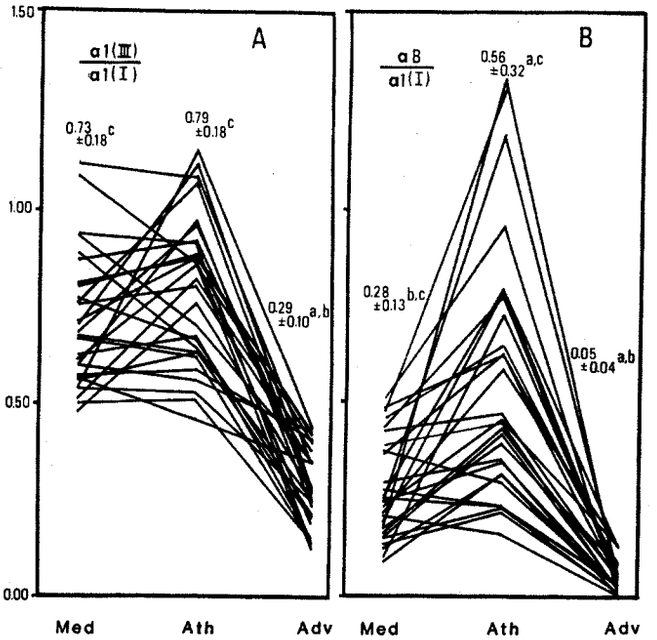

Fig. 3. Relative abundance of collagen α1(III) and αB chains compared with α1(I) in the intact media (*Med*), atherosclerotic plaque (*Ath*), and adventitia (*Adv*) of the human aorta. Numerical data represent the mean ± standard deviation. Values were analyzed for statistical significance among three groups (intact media, atherosclerotic plaque, and adventitia) by multiple *t*-test; a, b, and c show the statistically significant difference ($P < .01$) from the values of the intact media, atherosclerotic plaque, and adventitia, respectively.

Table 1. Amino acid composition of αB and αl(I) chains isolated from human aortic collagen. Collagen αB and αl(I) chains purified by carboxymethyl cellulose chromatography (*7*) were hydrolyzed in 6*N* HCl at 110°C for 20 hours. Amino acid composition was determined in a Hitachi amino acid analyzer, model 835-50. The data are expressed as residues per 1000 amino acid residues. The values presented are averages obtained in two separate determinations. Note the lower content of alanine and larger content of hydroxylysine in αB compared to αl(I). The amino acid compositions of these α chains are very similar to previously published values of αB and αl(I) (*7, 8*) (N.D., not detected).

Amino acids	αB	αl(I)
3-Hydroxyproline	5	N.D.
4-Hydroxyproline	114	111
Aspartic acid	44	35
Threonine	21	14
Serine	28	32
Glutamic acid	95	88
Proline	118	112
Glycine	321	328
Alanine	54	121
Half cystine	N.D.	N.D.
Valine	19	18
Methionine	8	7
Isoleucine	22	11
Leucine	37	24
Tyrosine	N.D.	2
Phenylalanine	12	12
Hydroxylysine	35	9
Lysine	18	29
Histidine	6	3
Arginine	43	44
Total	1000	1000

McCullagh and Balian (*11*) reported that the atherosclerotic plaque contained approximately 35 percent of type III collagen and 65 percent of type I collagen. McCullagh *et al.* (*12*), using an immunofluorescent method with specific antibodies to human collagens, recently showed that there is an increased immunoreaction to type I collagen in the advanced atherosclerotic plaque. I found that 32 percent of samples (9 out of 28) showed a decrease in α1(III) in atherosclerotic plaques, but some of the remaining cases, on the contrary, showed an increased proportion (Fig. 3A). The reason for inconsistency between their results and mine may reside in the samples taken from atherosclerotic plaques. I noticed that even individual samples used in the present study included a variety of sclerotic lesions, the severity of the lesions ranging from early intimal plaque to complicated atheroma of the late, advanced stage. Gay and Miller (*10*) demonstrated by immunofluorescence that the intimal fibrillar network produced by proliferating smooth muscle cells at an early stage of atherosclerosis is largely type III collagen with αA and αB chains; as the disease progresses the vascular lesions become fibrous and are finally replaced with scar tissue that contains mostly type I collagen. I also found histopathologically that at an initial stage

of atherosclerosis fine reticulin fibers stained with silver impregnation, which were considered to be type I collagen, were increased, and tissues of advanced sclerotic lesions were replaced mostly with thick bundles of fibers that stained blue by Masson's trichrome stain and that appeared to be type I collagen. It is likely that smooth muscle cells in an early atherosclerotic plaque produce a higher proportion of type III collagen and with progression of the lesion these cells are switched to synthesize predominantly type I collagen in response to functional requirements of the tissues.

The molecular composition of type V collagen has been a matter of controversy [that is, $\alpha A(\alpha B)_2$ (*7*) or $(\alpha A)_3$ and $(\alpha B)_3$ (*8, 15, 16*)]. I found that the type V collagen separated from whole aortas by the differential salt precipitation (*7*) was composed predominantly of αB chain. The ratio of αB to αA (7.6:1) was much larger than the reported ratio of 2:1 (*7*), suggesting that αA and αB are components of two separate collagen molecules (*8, 15, 16*). In the present study I noted that the αA chain comigrated with α1(I) on the SDS–polyacrylamide gel electrophoresis. Therefore, the ratio of α1(III) and αB calculated in terms of α1(I) are likely to be somewhat underestimated. Nevertheless, the marked increase in the proportion of αB is quite evident in the atherosclerotic plaque (Fig. 3B). Judging from the weights of lyophilized collagens separated by differential salt fractionation (*7*), pooled tissues from atherosclerotic plaques yielded a higher ratio of type V collagen to type I (0.41) compared to tissues from nonsclerotic intact medias (0.22).

It was observed by Stenn *et al.* (*17*) that cultured epithelial cells synthesize type V (AB_2) collagen. These workers suggested that the AB_2 collagen is necessary for the continual migration and movement of these epithelial cells (*16*). It is well known that in atherosclerosis, smooth muscle cells proliferating in the intimal layer are derived from the media through fenestrae of the internal elastic lamina (*18*). Type V collagen, therefore, may be related to such migration and movement of vascular smooth muscle cells. Finally, the increased proportion of αB or type V collagen provides at least a biochemical basis for transformation of smooth muscle cells proliferating in human atherosclerotic plaques.

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Bulk Solute Extrusion as a Mechanism Conferring Solute Uptake Specificity by Pinocytosis in *Amoeba proteus*

Abstract. *A variety of positively charged solutes induce pinocytosis in* Amoeba proteus, *ranging from metabolically useful material to solutes that may prove harmful, such as Alcian blue. Alcian blue is taken up by pinocytosis and then a fraction of the accumulated dye is expelled in bulk form through "extrusion channels." This response is not elicited by other solutes taken up by pinocytosis in the amoeba, implying that if any selectivity is associated with this process, it is by specific solute extrusion mechanisms in the cytoplasm.*

Pinocytosis, a mechanism by which cells can accumulate solute from the external medium, involves the uptake of bulk medium and membrane-bound solute by cell surface invagination and vesiculation. The initial step in pinocytosis in the amoeba is the binding of an inducer to the cell surface (*1*). This displaces a fraction of the surface calcium (*2*), which may in turn influence the permeability of the plasma membrane (*3*) and trigger the pinocytotic cycle. Inducers of pinocytosis in the amoeba, all cations, have been classified into three groups on the basis of how tightly they bind to the cell surface (*4*). The first group of inducers includes inorganic cations (such as Na^+) and amino acids that bind very loosely to the cell surface and which must be constantly present in the external medium to induce pinocytosis. The second category includes proteins that bind more tightly to the cell surface but can be removed from the cell by changes in the *p*H of the external medium. The third category includes basic dyes, such as Alcian blue, which bind irreversibly to the cell surface. A great variety of external solutes are taken up by pinocytosis in the amoeba, including solutes that may be beneficial to the cell as well as solutes that may prove harmful. This study is concerned with the fate of the three types of solute molecules ingested during pinocytosis in *Amoeba proteus* (*5*).

Under normal conditions, *A. proteus* streams about in a directed fashion along the substratum (Fig. 1a). When the cell is briefly exposed to 0.01 percent Alcian blue, the dye is bound to the cell surface in localized areas (Fig. 1b) and the cell ceases streaming and begins to withdraw its pseudopods. Five to ten minutes after the dye is added, pinocytotic channels can be observed in the cell (Fig. 1c) and membrane-bound solute (dye) uptake begins. After 20 to 30 minutes, the dye originally bound to the cell surface has been internalized and the pinocytotic channels have disappeared (Fig. 1d). The internalized dye, which presumably is still membrane-bound, appears to collect inside specific areas of the cell. During the next half hour, the cell begins to expel a portion of the dye, along with what appears to be some membrane and cytoplasm, through a distinct channel leading from the mass of dye in the cytoplasm to the cell surface (Fig. 1e and Fig. 2).

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