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Two-dimensional high-resolution electrophoresis of elastin-derived peptides

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Abstract

Degradation of human aortic elastin in vivo yields a restricted number of differentially sized and charged peptides. Elastin-derived peptides (EDP) can be analyzed by two-dimensional electrophoresis after their extraction from human abdominal aortic tissue by 0.2 *M* sodium chloride. The peptides were separated according to charge by acetic acid–urea–PAGE and then according to molecular mass by SDS–PAGE. The identity of these peptides as EDP was continued by immunoprecipitation and Western blots. The two-dimensional electrophoretic system can resolve desmosine-like cross-linked EDP of the similar molecular configuration but differing in the number of positively charged amino acid residues. The new separation technique of EDP has the capacity to identify defects in desmosine-like cross-links and may be useful in characterizing abberations in elastin structures.

Keywords: Peptides; Elastin

1. Introduction

Mature elastin, found predominantly in the aortic media, is very inert and insoluble, due to extensive cross-linking between the polypeptide chains of tropoelastin by desmosine and other cross-linkages derived from the oxidation of lysyl residues by the copper-requiring enzyme lysyloxidase [1]. The half life of elastin probably exceeds that of the life of the individual, which permits a continuous adaptation to the mechanically dynamic changes in the aorta wall. Elastin, however, is partially degraded in several pathological conditions affecting the lung and blood vessels [2–5]. Elastin degradation products (EDP) can be extracted from tissues by 0.2 *M* NaCl and these peptides exhibit hydrophobic properties [6]. Elastin peptides derived in vivo [6] and in vitro [2] have been fractionated by SDS–PAGE into 5–6 bands. The homogeneity of these bands using other electrophoretic systems in a second dimension is complicated to analyze because of the difficulties in eliminating SDS from the proteins. This emphasises the need for a simple and rapid procedure for separation of individual peptides from mixtures of proteins extracted from aorta tissues by 0.2 *M* NaCl. During the maturation of elastin from tropoelastin, not all lysines are used for desmosine-like cross-linking, thus EDP are not only hydrophobic but should also be highly positively charged at low pH. It has been shown that in acetic acid–urea–PAGE,

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basic chromosomal proteins–histones, differing in one charged lysine residue, can easily be separated [7]. The other advantage of this electrophoretic system is that acetic acid and urea in the gel can easily be replaced by any buffer required for the electrophoretic system in a second dimension. Finally, due to the high hydrophobicity of EDP, their resolution can be improved by the addition of a non-ionic detergent to the acidic acid–urea–PAGE system [8].

In this study we demonstrate that a 2D electrophoretic system, in which the proteins are separated according to their positive charge in the first dimension, can be separated in the second dimension according to molecular mass, and such a technique is useful for the analysis of EDP.

2. Experimental

2.1. Chemicals and solvents

Bovine neck ligament elastin, oxalic acid, PMSF, Tris, SDS, Tween-20, Triton X-100, DTT, 2-mercaptoethanol, glycine, urea, TCA and glycerol were obtained from Sigma. Acrylamide, BIS, TEMED and CBB G-250 were from Bio-Rad. Protein molecular mass markers and Protein A-Sepharose 6MB were from Pharmacia. Peroxidase-conjugated goat-Ig to rabbit-Ig was purchased from DAKOPAT and the enhanced chemiluminescence Western blot detection system was from Amersham. Other chemicals used in this work were of analytical grade.

2.2. Preparation of antibodies

To get soluble α-elastin, insoluble bovine neck ligament elastin was treated with 0.25 *M* oxalic acid at 90°C according to the procedure of Partridge et al. [9]. The α-elastin was dissolved in PBS to a concentration of 2 mg/ml, followed by emulsion in an equal volume of Freund's complete or incomplete adjuvant. New Zealand White rabbits were immunized by multiple subcutaneous injections using complete Freund's adjuvant. All of the rabbits were boostered fortnightly over a period of 3 months. The rabbits were bled by ear venipuncture 10 days after the last injection, and serum was collected by centrifugation. Immunoglobulins were purified using protein-A affinity chromatography and dialyzed against PBS and stored in a concentration of 2 mg/ml at 4°C.

2.3. Aortic samples

Full thickness aorta was dissected from associated fat and mural thrombs and stored at −50°C. The tissue was diced into 5 mm^2 squares and agitated in 0.15 *M* NaCl–1 m*M* PMSF at 4°C twice for 30 min to remove blood, and crushed in liquid nitrogen to a fine powder.

2.4. Tissue extraction

Tissue powder was extracted twice each time for 24 h with a solution containing 0.2 *M* NaCl, 5× diluted PBS, 5 m*M* EDTA and 0.1 m*M* PMSF. For 1 mg of aortic tissue 1 ml of sodium chloride solution has been taken [6]. After the extraction, the tissue suspension was centrifuged at 30 000 *g* for 30 min and the clear protein solution was frozen at −50°C.

2.5. Preparation of samples for electrophoresis

To prepare samples for SDS–PAGE 12.5 μl 0.5 *M* Tris–HCl, pH 6.8 containing 0.001% of bromphenol blue, 30 μl 20% SDS, 20 μl 0.5 *M* DTT and 20 μl glycerol were added to 100 μl of the 0.2 *M* NaCl extract of aortic proteins and the mixture was boiled for 5 min. Samples for acetic acid–urea–PAGE were prepared as follows: the 0.2 *M* NaCl extract of aortic proteins were dialyzed against 6 *M* urea. To 500 μl of protein solution in 6 *M* urea, 28 μl of glacial acetic acid containing 0.001% of Rhodamine 6G, 35 ml of 0.5 *M* DTT or 30 μl of 2-mercaptoethanol and two drops of 99% glycerol were added. The sample then was kept at room temperature for 2 h and used for electrophoresis or freezed at −70°C [7].

2.6. SDS polyacrylamide gel electrophoresis

SDS–PAGE was performed on pore gradient polyacrylamide gel slab (1.5 mm thickness, 55 mm length, 80 mm width) composed of 7.5%–15% running gel and 4% stacking gel according to Laemmli [10]. Electrophoresis was carried out using

a voltage of 30 V/cm until the free bromphenol blue had left the gel. During electrophoresis the gel was cooled by running tap water. The gel was then fixed for 2 h in 12.5% TCA and stained by colloidal CBB G-250 [11].

2.7. Acetic acid–urea polyacrylamide gel electrophoresis

Acetic acid–urea–PAGE was performed in 12% polyacrylamide gel slab (1.5 mm thickness, 80 mm length, 80 mm width) containing 0.9 *M* acetic acid and 6 *M* urea [7]. The gel was subjected to pre-electrophoresis during 5 h with a starting current of 20 mA. During pre-electrophoresis the sample wells for the samples were loaded with 0.9 *M* acetic acid–6 *M* urea. Before electrophoresis the system was filled with new 0.9 *M* acetic acid. A constant current at 6 mA/gel was maintained during the electrophoresis. Following electrophoresis, the gel was frozen at −50°C or fixed for 2 h in 12.5% TCA and stained by colloidal CBB G-250 [11].

2.8. Acetic acid–urea–Triton X-100 polyacrylamide gel electrophoresis

The samples of proteins extracted from aortic tissues by 0.2 *M* NaCl were prepared as for the acetic acid–urea–PAGE system and loaded across the top of a 12% polyacrylamide gel slab (1.5 mm thickness, 80 mm length, 80 mm width) containing 0.9 *M* acetic acid–6 *M* urea and a transverse gradient of 0 to 12 m*M* Triton X-100 [8]. The gradient of Triton X-100 was made by filling the gel chamber 2 cm from the bottom with gel solution without the Triton X-100 and then with a gradient of 0 to 12 m*M* Triton X-100. To the gel solution not containing Triton X-100 sucrose to 10% final concentration was added. For electrophoresis the gel slab was turned by 90°. All conditions of pre-electrophoresis and electrophoresis itself was the same as in the acetic acid–urea–PAGE system.

2.9. 2D electrophoresis

The first dimension was carried out in 12% polyacrylamide gel slab (1.5 mm thickness, 80 mm length and 80 mm width) containing 0.9 *M* acetic acid–6 *M* urea. For the second dimension, 3 mm of wide gel strips were equilibrated for 30 min in Millipore Q water, for 1.5 h in a solution containing 10% glycerol, 50 m*M* DTT, 2.3% SDS, 62.5 m*M* Tris–HCl, pH 6.8 and 0.001% bromophenol blue. The first dimension gel was then tightly fixed at the top of the second dimensional gel and covered by 1 mm layer of equilibration solution. Agarose was not used for gel fixing. The second dimension electrophoresis was carried out on a pore gradient polyacrylamide gel slab (2.25 mm thickness, 55 mm length, 80 mm width) composed of 7.5–15% acrylamide containing 0.1% SDS, 50 m*M* DTT, 375 m*M* Tris–HCl, pH 8.83 using a voltage of 30 V/cm until the free bromophenol blue had left the gel. The gel was then stained by colloidal CBB G-250 [11].

2.10. Immunoblotting with anti-α-elastin antibodies

To transfer proteins to the PVDF membrane (Bio-Rad), the polyacrylamide gel was incubated for 1 h in transfer buffer (12.5 m*M* Tris, 96 m*M* glycine, 0.1% SDS, 10% methanol) and proteins were transferred to the PVDF membrane (Bio-Rad) using the semi-dry Semiphor (Hoefer) and a current of 0.8 mA/cm^2 for 1.5–2 h. Following transfer, the gel was fixed in 12.5% TCA for 2 h and stained by colloidal CBB G-250. The membrane was blocked with 2.5% BSA dissolved in PBS–Tween 20 (PBS solution containing 0.05% Tween 20) by incubation overnight at 0°C and washed five times with PBS–Tween 20 solution containing 0.12% Tween 20. The membrane was then incubated at room temperature for 1 h with 1:4000 dilution of anti-α-elastin antibodies in the latter PBS–Tween 20 solution washed five times during 30 min with the same PBS–Tween 20 solution, then further incubated with a 1:2000 dilution of peroxidase conjugated goat-Ig to rabbit-IgG antibodies in PBS-Tween 20 solution for 1 h at room temperature and washed again in the same manner as above. Immunoreactive proteins were visualized using enhanced chemiluminescence Western blotting reagents.

2.11. Immunoprecipitation

Immunoprecipitations were performed by mixing, in a microcentrifuge tube, 100 μl aortic tissue

proteins extracted by 0.2 *M* NaCl, 500 μl of 2× immunoprecipitation buffer (1×=1% Triton X-100, 150 m*M* NaCl, 10 m*M* Tris, pH 7.4, 1 m*M* EDTA, 1 m*M* EGTA, 0.2 m*M* PMSF and 0.5% NP-40), 400 μl H_2O and 2.5 μl anti-α-elastin antibodies. The mixture was incubated for 1 h at 4°C under constant agitation. 50 μl washed Protein A-Sepharose 6MB were then added and the suspension incubated for a further hour at 4°C at constant agitation. The immunocomplexes were collected by centrifugation at 13 000 *g* for 5 min and the unbound fraction in the supernatant was retained. For SDS gel electrophoresis, resin pellets were washed three times with immunoprecipitation buffer by centrifugation for 5 min in a microfuge and resuspended in 30 μl of sample solution containing 4.6% SDS, 0.125 *M* Tris, pH 6.8, 20 m*M* DTT and 10% glycerol, boiled for 5 min, centrifuged and used for electrophoretic analysis. For acetic acid–urea gel electrophoresis resin pellets were washed with immunoprecipitation buffer, deionized water and four times with 5 m*M* Tris–HCl, pH 7.6. Resin pellets were resuspended in 30 μl solution consisting of 1.8 *M* acetic acid–6 *M* urea at 4°C for 2 h and then centrifuged for 5 min in microfuge. To 25 μl of supernatant 9 mg urea, 2 μl 2-mercaptoethanol and 1 drop of glycerol were added and the mixtures were used for electrophoretic analysis.

3. Results and discussion

3.1. Specificity of anti-α-elastin antibodies

In order to identify EDP from human abdominal aortic tissues rabbit antibodies were prepared against soluble α-elastin, obtained by solubilization of bovine neck ligament elastin with 0.25 *M* oxalic acid [9]. It has been shown that antisera to soluble bovine ligamentum elastin has the ability to cross-react with elastins from other species [12,13]. Molecular mass markers (phosphorylase *b*, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, α-lactalbumin (Pharmacia)), cytoplasmic proteins of HL-60 cells differentiated in vitro to granulocytes, proteins extracted from human abdominal aortic tissues by 0.2 *M* NaCl and bovine neck ligamentum α-elastin solubilized by oxalic acid treatment [9] were separated by SDS–PAGE and immunoblotted with anti-α-elastin antibodies which were diluted 1:4000 (Fig. 1). At this concentration antibodies raised against bovine α-elastin only react with a different molecular mass degradation products of bovine neck ligament elastin and with some proteins extracted from human abdominal aortic tissues by 0.2 *M* NaCl (Fig. 1, lanes 3′, 4′). These data confirm the cross-reactivity of rabbit antibodies raised to bovine α-elastin, against proteins from human abdominal aortic tissue, because the intensities of the immunoreactive signals in the blots (Fig. 1, lanes 3′, 4′) are proportional to the concentrations of the human aorta and bovine ligamentum antigens in the electrophoretic patterns (Fig. 1, lanes 3, 4).

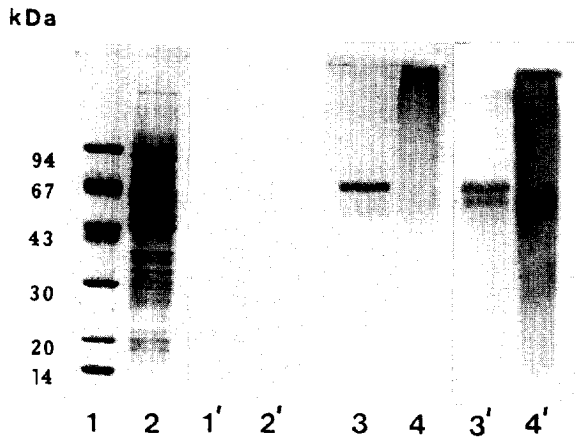

Fig. 1. Separation of proteins by SDS–PAGE. Lanes: (1) low molecular mass markers (Pharmacia); (2) cytoplasm proteins of promyelocytic leukemia HL-60 cells differentiated in vitro to granulocytes; (3) protein extracted by 0.2 *M* NaCl from human abdominal aortic tissue; (4) bovine neck ligament elastin solubilized by oxalic acid (α-elastin). Immunoblotting of proteins. Lanes: 1′,2′,3′,4′-proteins separated in SDS–PAGE (lanes 1, 2, 3, 4). Proteins were transblotted to PVDF membrane and probed with anti-α-elastin antibodies as described in Section 2.

3.2. Electrophoretic fractionation, immunoblotting and immunoprecipitation of proteins extracted by 0.2 M NaCl from human abdominal aortic tissues

The main goal of this study was to separate peptides extracted from aortic tissues by 0.2 *M* NaCl into individual polypeptides and in the mixture identify the polypeptides as elastin degradation products. The possibility to isolate EDP from 0.2 *M* NaCl

extracts of sheep arterial wall by hydrophobic affinity chromatography has been demonstrated, but electrophoretic separation of individual EDP has not earlier been described [6]. For fractionation of these proteins four different electrophoretic systems were used, i.e., (I) SDS–PAGE; (II) acetic acid–urea–PAGE; (III) acetic acid–urea–Triton X-100–PAGE; (IV) two dimensional electrophoresis (acetic acid–urea and SDS electrophoretic systems). The improved resolution compared to previous SDS gel electrophoresis techniques [3,6] is demonstrated in Fig. 2A. The number of bands detected from proteins extracted from NAA and AAA was at least 25 in comparison to the separation to 5–6 bands in previous studies. There are seventeen bands of proteins with molecular masses in the region of 70–14 kDa numbers, which are lower than the molecular mass of the mature elastin precusor – tropoelastin [1].

Fig. 2B, demonstrates separation of proteins extracted by 0.2 *M* NaCl from NAA and AAA tissues in the acetic acid–urea–PAGE system. This electrophoretic system, in contrast to the isoelectrofocusing method usually used for 2D electrophoresis [14], has the advantages of simplicity, reproducibility and high resolution of proteins, such as elastin, which contain several basic amino acid residues and possess hydrophobic properties. Since this technique is capable of distinguishing proteins differing by a single lysine residue [7] it permits to identifying elastin derived peptides with defects in desmosine-like cross-links. It can be seen that proteins are resolved into 16–18 bands, which is similar to the resolution in SDS–PAGE system. High resolution and the ease with which acetic acid and urea can be switched to Tris–SDS buffer makes this electrophoretic system useful for 2D electrophoresis. The qualitative protein fraction patterns separated in both electrophoretic systems are more or less typical for NAA and AAA. The relative amounts of proteins vary, however, in some bands. The reason for this variability is not yet clear (eight specimens of AAA and three of NAA have been analyzed), but the patterns are independent of such morphological properties of aorta as wall thickness, hardness and blood contamination.

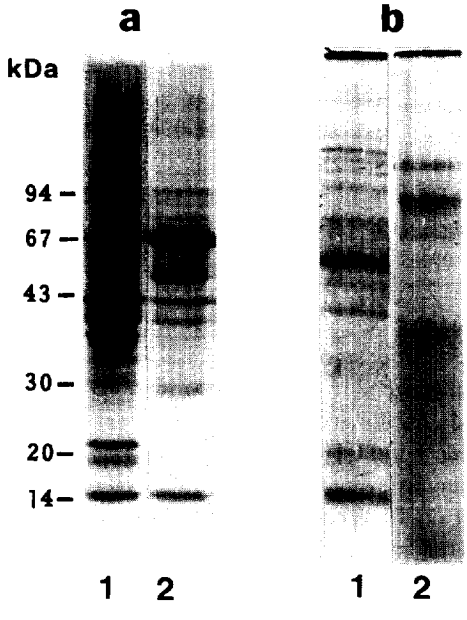

Fig. 2. Electrophoretic separation of human abdominal aortic tissue proteins extracted with 0.2 *M* NaCl. (A) SDS–PAGE and (B) acetic acid–urea–PAGE. Lanes: (1) proteins of normal human abdominal aortic tissue; (2) proteins of human aneurysmal abdominal aortic tissue.

Fig. 3d represents the results of the 2D electrophoresis. Proteins extracted with 0.2 *M* NaCl from normal human abdominal aortic tissues were separated in acetic acid–urea–PAGE system in the first dimension and then by SDS–PAGE system in the second dimension. The corresponding one-dimensional electrophoretic separations and their immunoblots are presented in Fig. 3a, a′ and b, b′. In immunoblots of the proteins separated in acetic acid–urea–PAGE, there are two diffuse bands denoted by number 1 and numbers 2, 3, 4, which are immunoreactive with anti-α-elastin antibodies (Fig. 3a′). In immunoblots of proteins separated in SDS–PAGE four major bands 1, 2, 3 and 4 with molecular mass 70–55 kDa, which have been identified by their electrophoretic mobilities, can also be seen (Fig. 3b′). Most of the proteins in the 2D gel (Fig. 3d, e) were observed on a diagonal line. This diagonal protein distribution appear if the average charges of the proteins are approximately proportional to their molecular mass in both the first and the second dimension of 2D electrophoresis. This means that the positively charged proteins in acetic acid–urea–PAGE and the negatively charged proteins in SDS–PAGE, should have been distributed according to their molecular masses. Proteins located at the right

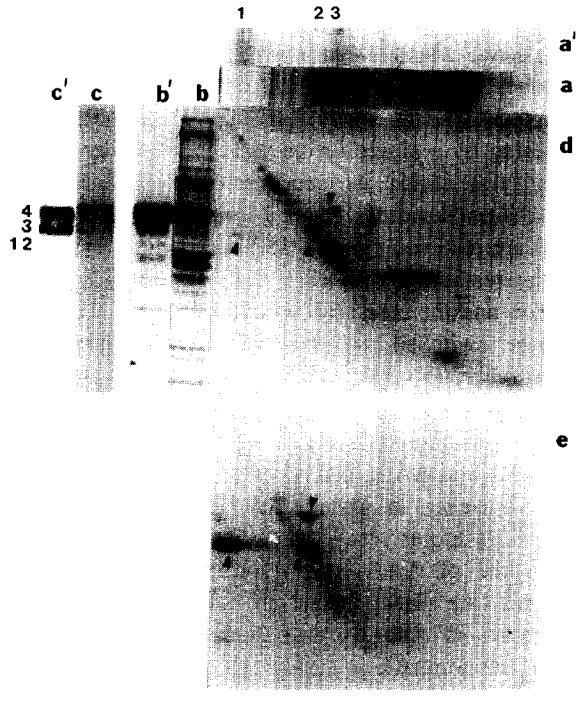

Fig. 3. Electrophoretic separation and immunoblots of proteins extracted from human abdominal aortic tissues by 0.2 *M* NaCl. (a) acetic acid–urea–PAGE; (b) SDS–PAGE; (c) separation of proteins immunoprecipitated from 0.2 *M* NaCl extract of normal human abdominal aortic tissues by anti-α-elastin antibodies and fractionated in SDS–PAGE; 2D-PAGE of normal human abdominal aortic (d) and human abdominal aortic aneurysm (e) proteins extracted with 0.2 *M* NaCl: the first dimension acetic acid–urea–PAGE and the second dimension SDS–PAGE. a′,b′,c′-immunoblotting of proteins separated in (a, b, c) electrophoretic systems. Proteins were transblotted to PVDF membrane and probed with anti-α-elastin antibodies as described in Section 2. Protein bands in a′ and c′ indicated as 1, 2, 3 and 4 are projections of four spots (denoted by arrows) in 2D-PAGE, which are immunoreactive with anti-α-elastin antibodies.

side of the diagonal should contain more diamino amino acids bearing positive charges at acidic pH than proteins of the same molecular mass positioned to the left of the diagonal. Immunoblotting of the 2D gel revealed that there are four minor spots of polypeptides (denoted by arrows) immunoreactive with anti-α-elastin antibodies (results not shown) and their electrophoretic mobilities in the 2D gel correspond to the mobilities of bands 1, 2, 3 and 4 in the one-dimensional gels and immunoblots. These data indicate, that the polypeptides 1, 2, 3 and 4 belong to EDP. In Fig. 3c the protein fractions immunoprecipitated with anti-α-elastin antibodies from 0.2 *M* NaCl extract of normal aortic tissues are separated in SDS–PAGE. There are three immunoprecipitated peptides with electrophoretic mobility coinciding with the electrophoretic mobility of the minor spots positioned outside of the diagonal in 2D electrophoresis. As can be seen from immunoblotting of the immunoprecipitated proteins (Fig. 3c′) these three peptides are highly immunoreactive with anti-α-elastin antibodies. The differences in the intensity of the distinct EDP bands on immunoblots (Fig. 3b′ and c′) may be due to different dilutions of anti-α-elastin antibodies during the immunoblot (1:4000) and immunoprecipitation (1:400) procedures. These data again confirm that the three minor polypeptides positioned outside the diagonal in the 2D electrophoresis are EDPs. It should be pointed out that the 2D gel electrophoresis of the immunoprecipitated proteins (results not shown) revealed that the band with the highest electrophoretic mobility in SDS–PAGE (Fig. 3c) did consist of two polypeptides (indicated as numbers 1 and 2) and the polypeptide with the higher mobility in acetic acid–urea–PAGE (band 2) appears on the diagonal. The difference in electrophoretic mobility according to the positively charged amino acids of these EDPs, still with the same molecular configuration and with the same molecular mass, suggests that they contain different numbers of lysines, involved in desmosine-like cross-links of elastin. It can be suggested that polypeptide 1 (EDP1) is a degradation product of normally cross-linked elastin, while the origin of polypeptide 2 (EDP2) and polypeptides 3 and 4 (EDP3, EDP4) are derived from mature elastin which is defective in desmosine-like cross-linking. The possibility that the slow electrophoretic mobility of EDP 1 depends on dimerization of two elastine peptides by disulfide bond is unlikely due to high concentration of reducing agents (5% of 2-mercaptoethanol or 50 m*M* of DTT). When proteins of NAA and AAA were compared in both samples approximately similar or slightly higher amount of EDP1 than EDP2 were found, however, the amounts of peptide EDP4 more often was the highest in AAA (Fig. 3d,e). It should be pointed out that the relative total amount of the polypeptides EDP1, EDP2 and EDP4 in comparison to the total amount of the rest proteins in 0.2 *M* NaCl

extract is much higher in AAA than in NAA (Fig. 3d,e). This is consistent with data describing a marked decrease in insoluble elastin content in aneurysmal aortic media [4,5,15].

Similar qualitative, but not quantitative 2D electrophoretic pattern of EDP isolated from NAA and AAA suggest that: (1) there are specific, elastase sensitive sites in the mature elastin polypeptide network forming EDPs with different desmosine-like cross-link content; (2) the mature elastin in AAA tissues is more sensitive to the action of elastases at these sites generating EDP with reduced content of cross-links (polypeptides EDP2 and EDP4). To check the hydrophobicity of the proteins giving the positive signal with anti-α-elastin antibodies, the peptides extracted from the aorta tissues by 0.2 *M* NaCl were fractionated in acetic acid–urea–Triton X-100–PAGE system. As can be seen in Fig. 4 the mobility of the major part of the separated peptides, especially those with lower molecular mass, decreases with increasing content of Triton X-100 in the gel. By changing the concentration of Triton X-100 in the gel it is possible to find the best conditions for separation of the proteins required. There are three protein bands (indicated by arrows), displaying decreased electrophoretic mobility more suddenly in the low concentration range of detergent in the gel. This fact suggests that these three bands contain polypeptides which are the richest in hydrophobic amino acids among the peptides separated. Immunoblotting of this gel with anti-α-elastin antibodies demonstrates that the two bands with the higher molecular mass give positive signals in ECL Western blot detecting system (results not shown).

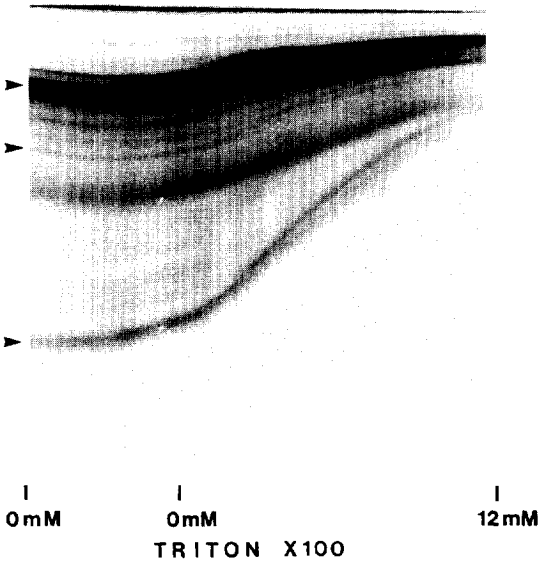

Fig. 4. Differential change in the electrophoretic mobility of proteins extracted by 0.2 *M* NaCl from human abdominal aortic tissues in the presence of increasing concentrations of nonionic detergent. Proteins were loaded across the top of a 12% polyacrylamide slab containing 0.9 *M* acetic acid–6 *M* urea and a transverse gradient of 0–12 m*M* Triton X-100. Two cm of the left side of the gel was free of Triton X-100. Arrows indicate most hydrophobic and immunoreactive bands in Western blot when probed with anti-α-elastin antibodies.

The high hydrophobicity of the polypeptides, which are immunoreactive with anti-α-elastin antibodies, also proves their identity as degradation products of elastin. It should be pointed out that human lung EDP generated in vitro and in vivo revealed the main fractions of EDP to be in the range of molecular mass of 70–55 kDa [2,3]. The predominant fraction of EDP with molecular masses about 50 kDa has been isolated from 0.2 *M* NaCl extract of sheep arterial tissues by hydrophobic affinity chromatography [6]. The estimated molecular mass and molecular configuration of EDP separated by 2D SDS–PAGE needs further discussion. Most SDS–protein complexes have a constant charge per unit mass and rod-like conformation, therefore, differences in their electrophoretic mobilities in SDS–PAGE are due to the differences in their size [16]. Since, EDP are cut out by elastases from three-dimensional, desmosine-like cross-linked network of the mature elastin, there are two possibilities: (1) EDP are cut out as linear peptide between two desmosine-like cross-links and in this case the molecular masses judged from SDS–PAGE are true; (2) EDP are cut out as a two- or three dimensional network of peptides and the electrophoretic mobilities of such structures in SDS–PAGE are defined by the configuration of the peptide concerned. Therefore, the real molecular mass of the EDP can be higher than the apparent one, at least due to parallel peptides cross-linked. The second possibility is suggested by the facts, that in the molecule of tropoelastin the largest peptide between two, theoretically possible, desmosine-like cross-links consists of about 75–80 amino acid residues which corresponds to approx. 8 kDa [17] and even

EDP with a molecular mass of 12–14 kDa obtained in vitro by digestion of pancreatic or leukocyte elastases have also been shown to contain desmosines [18]. The biological significance of the suggested difference in non-cross-linked lysine content between EDP1 and EDP2 or EDP4 is presently investigated.

4. Abbreviations

AAA	abdominal aortic aneurysm
BSA	bovine serum albumine
CBB G-250	Coomassie Brilliant Blue
DTT	ditiothreitol
ECL	enhanced chemoluminescence
EDP	elastine derived peptide
BIS	N,N′-methylene-bis-acrylamide
NAA	normal abdominal aorta
PBS	phosphate buffered saline (0.2 *M* NaH_2PO_4, 0.8 *M* Na_2HPO_4, 0.1 *M* NaCl, pH 7.5)
PAGE	polyacrylamide gel electrophoresis
PVDF	polyvinylidene fluoride
SDS	sodium dodecyl sulfate
TEMED	N,N,N′,N′-tetramethylethylenediamine;
TCA	trichloracetic acid
2D	two-dimensional

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