PRECIPITATING ANTIBODIES DIRECTED AGAINST SOLUBLE ELASTIN – THE BASIS OF A SENSITIVE ASSAY

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1. Introduction

We have been interested in the biosynthesis of elastin, a major component of mammalian blood vessel walls, and required a sensitive and specific assay for soluble, non-crosslinked elastin in tissue extracts of lathyritic chickens. As this protein has no easily demonstrable specific biological activity and contains no unusual amino acid in large amounts, as does collagen for example, we attempted to raise a specific antiserum to it. Elastin was, for some time, thought of as non-immunogenic [1–3]. Recent studies have demonstrated that soluble peptides derived from partial hydrolysis of bovine *ligamentum nuchae* elastin are weakly immunogenic in rabbits [4–5]. We hoped to elicit a strong response by injecting, into a phylogenetically remote host, elastin as a suspension of purified insoluble fibres rather than as a partial hydrolysate.

We hoped, further, that the soluble, non-crosslinked protein would have a structure sufficiently similar to that of the injected elastin to provide common antigenic determinants and so give a visible cross-reaction. Although purified soluble elastin from lathyritic chick aortas was available [6,7] it was rather too scarce for use in the production of antisera. However, there was a plentiful supply of insoluble elastin from the same source which, this letter reports, could be used to produce an apparently monospecific antiserum.

2. Experimental

Chickens were raised on a lathyrogenic diet and the major arteries dissected as previously described [6]. Soluble elastin was purified from neutral salt extracts of these vessels by DEAE-cellulose chromatography, salt precipitation and isoelectric focusing. Purity was judged by amino-acid analysis and polyacrylamide gel electrophoresis [7]. Insoluble, crosslinked elastin was prepared from the neutral salt insoluble residue by four 10 min extractions in 0.1 N NaOH at 98°C followed by successive washings in hot water, ethanol and acetone After drying with air, the material was suspended in sterile physiological saline at 20 mg^{-1} and allowed to swell for two days before homogenisation to a fine suspension. Sheep received fortnightly subdermal injections of an inoculum prepared by mixing 1 ml of this suspension with an equal volume of Freund's complete adjuvant. Blood was taken from the jugular vein every four weeks. Serum was tested for the presence of precipitating anti-elastin antibodies by double diffusion in gel and by immunoelectrophoresis.

Fig. 1 shows a single precipitin line developed against

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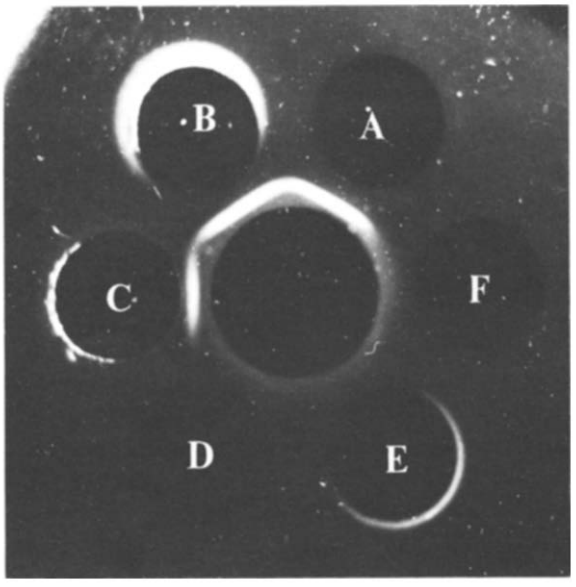

Fig. 1. Immunodiffusion in 1% agarose: 0.5 M NaCl, 0.02 M sodium phosphate pH 7.2. The central well contained antiserum raised in sheep against insoluble elastin fibres from lathyritic chicks. The outer wells contained: (A) Purified salt-soluble elastin (1 mg ml^{-1}). (B) Neutral salt extract of lathyritic chicken aortas. (C) Neutral salt extract of control chicken aortas. (D) Blank. (E) Oxalic acid solubilised elastin (1 mg ml^{-1}). (F) Chick skin tropocollagen (0.5 mg ml^{-1}). Samples were dissolved, where appropriate, in the gel buffer. The gel was developed for 16 hr at 4°C and photographed in diffuse light.

purified soluble elastin which was continuous with a strong line developed against a neutral salt extract (0.5 M NaCl, 0.02 M sodium phosphate pH 7.2, 0.02% w/v β-aminopropionitrile fumarate, 0.01% w/v sodium azide) of lathyritic chicken aortas. This, too, was continuous with a weaker line which developed against an extract from the aortas of normal birds prepared in the same way. No visible precipitin line developed against purified chick skin tropocollagen (1.0 mg ml^{-1} in neutral, 0.5 M NaCl) or, perhaps surprisingly, against oxalic acid solubilised elastin (5 mg ml^{-1} in physiological saline) prepared from the same batch as was used for antiserum production. There were no qualitative differences in the pattern when lines developed against serum from a sheep immunised with elastin from normal chickens. In another plate (not illustrated) there was no visible reaction between the antiserum

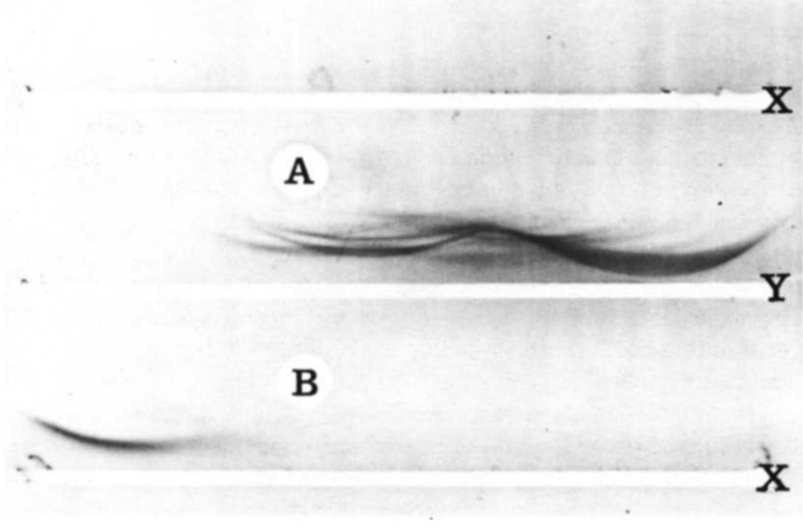

Fig. 2. Immunoelectrophoresis in 1% agarose. Wells contained: (A) Serum from a normal 14 day-old chicken. (B) Purified salt-soluble elastin. (1 mg ml^{-1} in 0.05 M barbitone buffer pH 8.6). Troughs contained: X. Antiserum raised in sheep against insoluble elastin fibres purified from lathyritic chicken aortas. Y. Commercial anti-chicken antiserum. (Wellcome Research Laboratories, Beckenham, Kent, UK.) Electrophoresis (15 V/cm^{-1}) continued for 180 min at room temperature. Precipitin arcs were developed for 48 hr at 4°C and stained with 0.5 w/v amido-black in 7% acetic acid.

and soluble non-crosslinked elastin purified from copper deficient calf *ligamentum nuchae* [8].

Immunoelectrophoresis (15 V/cm^{-1} for 180 min) in 1% agarose [9] of a sample of purified salt-soluble elastin in 0.05 M barbitone buffer pH 8.6 gave a single precipitin arc when reacted against the sheep antiserum (fig. 2). Electrophoresis of normal chicken serum on the same plate located the position of serum components under identical conditions. Moreover, the observed pattern provided evidence not only that the sample of salt-soluble elastin contained no components that were normally present in chicken serum but also that the sheep anti-elastin preparation was not contaminated with antibodies directed against normal chicken serum proteins.

We have employed radial immunodiffusion [10] to measure the concentration of soluble elastin in neutral salt extracts of aortic tissue from both lathyritic and control chickens and also from lathyritic chick embryos. The technique has proved satisfactory for the determination of antigen concentrations down to 0.05 mg ml^{-1}. The specific assay has been very useful improving preparative procedures for soluble elastin, fuller details of which appear elsewhere [7].

3. Discussion

The serological cross-reaction provides strong evidence, additional to the similarity of amino acid composition and some physical properties [7], that there is a close structural relationship between the soluble protein isolated from the aortas of lathyritic chickens and crosslinked elastin purified from the same tissue. It has been suggested [11] that the antigenic determinants of elastin are located in the cross linking amino acids desmosine and isodesmosine which are unique to insoluble elastin. However, the cross-reaction observed in the experiments described here cannot be explained on this basis as the salt-soluble elastin is devoid of these amino acids. The cationic behaviour of the antigen at pH 8.6 effectively discounts the possibility that antibodies had been produced, not against elastin but against the small residue of acidic glycoprotein(s) with which all samples of 'purified' elastin are likely to be contaminated [12] and that these were combining with similarly small amounts of the same protein(s) which possibly contaminated the soluble elastin preparation.

The availability of a specific antibody provides an opportunity for quantitative experiments in elastin biosynthesis and may permit, among other things, the intracellular localisation of the molecule by antibody-labelling techniques.

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