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Some Modifications to the Technique of Two-Dimensional Immunoelectrophoresis

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The technique of two-dimensional immunoelectrophoresis as described by Minchin-Clarke & Freeman (1968) is very effective for the quantitative analysis of a mixture of proteins. The method consists of running the mixture first in a strip of agarose on a 10 cm × 10 cm glass plate, when a partial linear separation of the proteins occurs. The strip is then transferred to a second plate of similar size, the empty portion of the plate being filled with agarose containing an appropriate mixture of antibodies. The current is run at right-angles to the previous direction and the antigens are driven into the bed of antiserum. This results in the formation of antigen-antibody curves, the area under which is proportional to the amount of the protein present.

The choice of 10 cm × 10 cm glass plate appears to limit the use of the method, since it conditions the volumes of expensive reagents and the size of equipment. However, the size of the plate can be reduced to one-quarter of the original size without loss of clarity or detail. As a result, the electrophoretic tank can be completely redesigned to produce a more compact and flexible unit in which the volume of barbitone buffer is reduced to 10% of that originally used.

Redesigning the tank necessitates a change in the preparation of the plate, in that the two dimensions have to be prepared before commencing electrophoresis. This involved the construction of a new pattern of carrier and wick-electrode system.

A further improvement, which involved superimposition of plates one on top of the other, made it possible to increase the number of plates developed in a single run three- or four-fold.

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The Low-Molecular-Weight Components of Bovine Skeletal Myosin

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Low-molecular-weight components (light components) released from rabbit skeletal myosin by chemical modification, urea and alkali treatment have been well characterized and more recently implicated in the functioning of the intact myosin molecule (Stracher, 1969). The presence of light components in myosins of other species has been demonstrated (Locker & Hagyard, 1967; Perrie, Perry & Stone, 1969) and an examination of those of bovine skeletal myosin was therefore made.

Myosin was prepared from muscle in rigor and was free of nucleoprotein and adenylate kinase activity. Low activities of adenylate deaminase present could be removed by batch treatment with DEAE-cellulose. No cathepsin-like proteolytic activity (Gordon & Zak, 1963) could be detected. On chromatography on DEAE-cellulose, Sephadex G-200 and Sephadex G-100 and on ultracentrifugation the preparation appeared as a single component. Amino acid analyses, molecular weight (540 000, by sedimentation-diffusion) and enzymic characteristics closely correspond to those of rabbit (J. Thomas, unpublished work).

Acetylation, maleylation or treatments with 0.1 M-Na₂CO₃, 1% sodium dodecyl sulphate or 8 M-urea were shown to release the light components. The acetylated and alkali-treated forms (best characterized in rabbit) and the maleylated form were examined in more detail. Light components obtained by acetylation and maleylation treatments were separated from the high-molecular-weight material (heavy components) by (NH₄)₂SO₄ fractionation, whereas the light components from alkali treatment were separated by dilution. In no case was the separation complete: the light components were contaminated by the heavy component. Further purification on Sephadex G-100 gave a component representing about 10% of the total protein that had consistent amino acid analyses and gave a single peak ($s_{20,w}^0$ 1.6S) on ultracentrifugation. The results of polyacrylamide-gel (7.5%) electrophoresis at pH 9.0 were complex, but in light components prepared with thiol groups protected by *N*-ethylmaleimide three major bands were detected.

The acetylated, maleylated and alkali-treated light components behaved differently on Sephadex G-100 calibrated for molecular weight and Stokes radius (the latter derived from diffusion coefficients of standard proteins): each gave a single peak, though at different elution volumes. In view of the sedimentation data and the work of Habeeb (1967)

on the succinylation of proteins, this was attributed to an increase in Stokes radius caused by chemical modification. The molecular weight and the Stokes radius of the alkali-treated light component were 24000 and 21.6×10^{-8} cm respectively; Stokes radius for the maleylated light component was 27.5×10^{-8} cm and 22.7×10^{-8} cm for the acetylated light component.

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Localization and Chemical Composition of Elastin in Ox Lung

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The protein elastin occurs in elastic fibres in the connective tissues of animals. In most tissues elastin is a minor component, but a few tissues such as ligaments and blood vessels contain considerable amounts. The elastin from the ligamentum nuchae of ruminants has been extensively purified, its chemical composition established and is generally taken as standard elastin (Partridge, 1962, 1966).

Despite the elastic nature of lung relatively few biochemical studies have been made on its elastin content and composition (Fitzpatrick & Hospelhorn, 1962). In the present work adult ox lung has been dissected into the main components, major bronchi, parietal and visceral pleuras and the parenchyma. The trachea and ligamentum nuchae were also taken. The elastin contents of these tissues expressed as g/100g of dried tissue and determined by the method of Lansing, Rosenthal, Alex & Dempsey (1952) were as follows: visceral pleura, 27%; parietal pleura, 15%; parenchyma, 13%; bronchi, 1.4%; trachea, 1%; ligamentum nuchae, 70%.

In view of the low elastin content no further studies were made on bronchi and trachea. The elastins obtained from the other lung tissues were analysed for nitrogen, carbohydrate and amino acid content and gave values similar to those for ligament elastin. Carbohydrate was present in small amounts only. Nitrogen content was 16.6%, and almost 96% of this could be recovered as amino acids. The characteristic features of the amino acid pattern were the high content of proline, glycine, alanine

and valine; the low content of amino acids with polar side chains; and the presence of the three cross-linking amino acids desmosine, isodesmosine and lysinonorleucine, which are specific for elastin.

Further characterization of lung elastin was confined to that from visceral pleura. The number and type of *N*-terminal residues in this material, as determined by the fluorodinitrobenzene method of Sanger (1945), were virtually identical with those of ligament elastin. It has long been known that elastin has a characteristic bluish-white fluorescence (Blomfield & Farrar, 1967). Elastins from ligamentum nuchae and visceral pleura solubilized by proteolytic digestion had three fluorescent peaks with activation/fluorescence maxima of 250/374 nm, 280/312 nm and 330/384 nm. The 280/312 nm peak is due to tyrosine, but the others are due to unidentified substances.

Since elastin is an insoluble protein it is generally prepared by harsh methods such as autoclaving, treatment with formic acid or alkali to remove all other materials. It can also be purified by a comparatively mild method using guanidine and collagenase (Miller & Fullmer, 1966). Elastins purified from ligamentum nuchae by these four methods gave a protein of almost constant chemical composition. However, the elastin from visceral pleura was more difficult to purify, the best preparations being obtained from the alkali and formic acid methods.

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