The use of acetate sheets as records for chromatographic patterns

Taxonomists are often faced with marking and analysing chromatographic patterns of large samples of taxa. If sheets, 46 × 57 cm are used, the recording of the position of compounds, their fluorescence and color under U.V. light and their color changes with different chromogenic sprays is often cumbersome, if done on separate sheets.

The recording upon the original chromatogram is not advisable, since these can repeatedly be exposed to different vapor phases for color change, and can then be subjected to a chromogenic spray to find further color reactions. If, in such a case, the position of compounds on the original chromatogram has been marked, the previously drawn lines may suggest compounds that are not in fact visible.

My work of recording chromatographic patterns was greatly facilitated by superimposing a thin sheet of clear acetate (Grumbacher Tuffilm 196–150) upon the original chromatogram. With wax pencil or china marker the exact outline of the compounds can be recorded on the acetate and their coloration noted. This can also be done in a vapor chamber or under U.V. light. The original can again be subjected to NH_3 or other vapors, U.V. light and finally be sprayed, without being cluttered with notations, and without shadow spots produced by finger marks. In two-dimensional chromatography the exact position and relation of the compounds to each other in the pattern to be recorded is greatly facilitated. Since the application point and front line are also noted on the acetate sheet, several of these can be superimposed and deviating patterns immediately recognized. The acetate is inexpensive, resistant to many vapors, easily stored and can be used as a permanent record.

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"Crossing" paper electrophoresis for the detection of complexes of enzymes with their inhibitors

The inhibition of lysozyme activity occurs in the presence of high molecular weight acidic polymers. It was found that the following substances effectively inhibited lysozyme activity: heparin[1–3], ribo-nucleic acid, deoxyribonucleic acid, hyaluronic acid, pneumococcus polysaccharide, Vi antigen, glutamyl polypeptide[4]. The inhibition was of the competitive type. The lysozyme–trypsine complex was isolated[5] and the precipitation and aggregate formation of deoxyribonucleic acid with lysozyme was examined[6].

A similar type of inhibition can be observed with ribonucleic acid, which is the inhibitor of deoxyribonuclease[7,8]. The reaction between these inhibitors belonging to the group of macromolecular acid compounds and lysozyme or deoxyribonuclease has an electrostatic character. In our previous work, we have demonstrated with the help of "crossing" electrophoresis that inhibitors of macromolecular acidic polymer nature

inhibit the activity of ribonuclease by forming stable enzyme–inhibitor complexes[9]. The principle of "crossing" electrophoresis is that by applying two substances as two lines that form an angle, the substances can be made to cross each other during paper electrophoresis. If the substances possess chemical affinity, then an addition product is formed at the crossing point[10].

The aim of this work was the investigation of complexes of lysozyme with its inhibitors and a complex of deoxyribonuclease with ribonucleic acid by "crossing" paper electrophoresis.

Materials and methods

The substances employed were: (a) lysozyme (Mann Research Laboratories Inc., New York), (b) crystalline deoxyribonuclease (Mann Research Laboratories Inc., New York), (c) yeast ribonucleic acid (BDH, England), (d) deoxyribonucleic acid (Light, England) and (e) heparin (Polfa, Poland).

The substances to be tested were applied to the filter paper as lines drawn obliquely to each other. The paper used was Whatman No. 1, 12 × 40 cm or 18 × 40 cm. Electrophoresis was carried out for 20 to 60 min, at 700 V with acetate buffer, pH 4.0, and ionic strength 0.05, 0.1 or 0.25. Lysozyme and deoxyribonuclease were stained with bromphenol blue[11], ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) with Victoria Blue B[12] and heparin with toluidine[13] or Victoria Blue. In addition the nucleic acids were examined under a U.V. lamp.

Results

The enzymes migrate at pH 4.0 towards the cathode, while the inhibitors examined migrate towards the anode. Fig. 1 shows an electrophoregram on which lysozyme has been obliquely applied to the lines of RNA, DNA or heparin. During the electrophoresis as a result of the formation of an enzyme–inhibitor complex, the direction of the

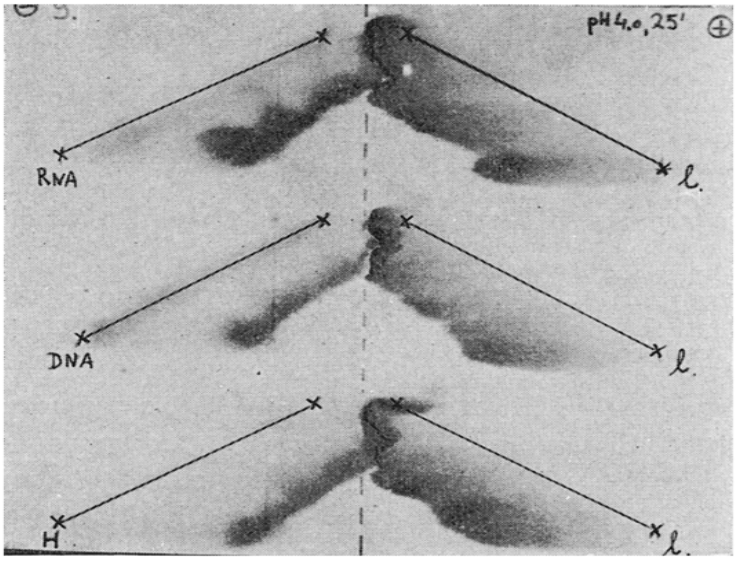

Fig. 1. Detection of lysozyme complexes with the inhibitors. ×——× l = 20 µl/8 cm of 2% lysozyme; ×——× RNA = 20 µl/8 cm of 10% ribonucleic acid; ×——× DNA = 20 µl/8 cm of deoxyribonucleic acid; ×——× H = 20 µl/8 cm of 2.5% heparin; — — — — = the middle of the paper strip. Stained with bromophenol blue and Victoria Blue.

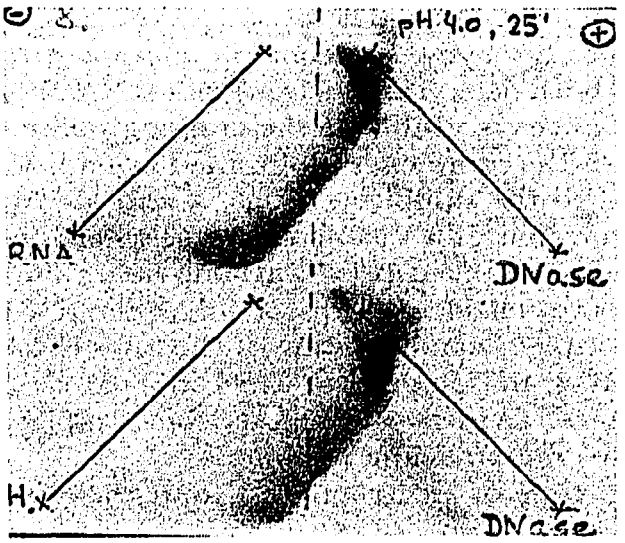

Fig. 2. Detection of deoxyribonuclease complexes with ribonucleic acid and heparin. ×——× DNase = 15 μl/6 cm of 2% deoxyribonuclease; ×——× RNA = 15 μl/6 cm of 10% ribonucleic acid; ×——× H = 15 μl/6 cm of 2.5% heparin; — — — — = the middle of the paper strip. Stained with bromophenol blue and Victoria Blue.

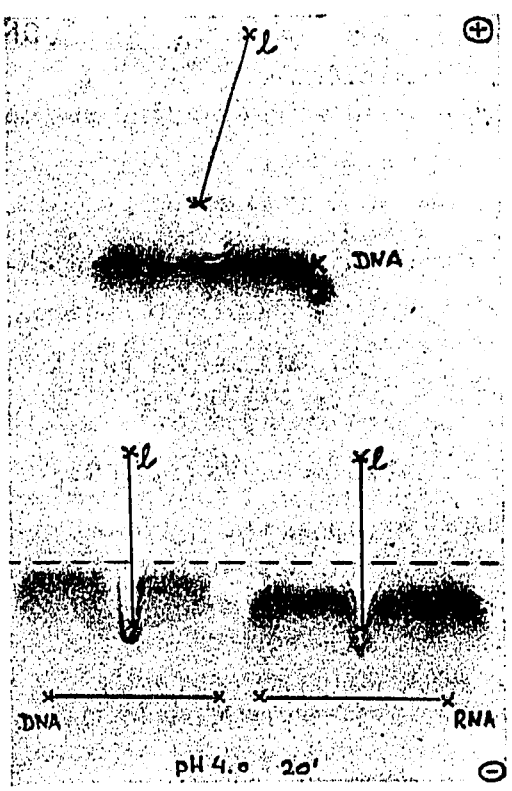

Fig. 3. Detection of lysozyme complexes with ribo- and deoxyribonucleic acids. ×——× l = 12.5 μl/5 cm of 2% lysozyme; ×——× RNA = 10 μl/4 cm of 10% ribonucleic acid; ×——× DNA = 10 μl/4 cm of 10% deoxyribonucleic acid; — — — — = the middle of the paper strip. Stained with Victoria Blue.

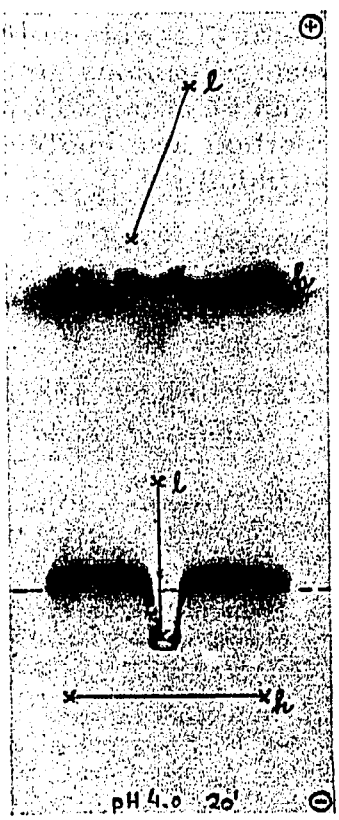

Fig. 4. Detection of lysozyme complex with heparin. ×——× l = 12.5 μl/5 cm of 2% lysozyme; ×——× h = 12.5 μl/5 cm of 2.5% heparin; — — — — = the middle of the paper strip. Stained with toluidine blue.

lines changes at the crossing point. These complexes can be seen as dark-staining bands in the places where lysozyme has come in contact with its inhibitors.

In the experiments with deoxyribonuclease and RNA or heparin a distinct change of direction of the substances is visible at the crossing point (Fig. 2).

A different picture of the complexes is obtained when lysozyme is applied perpendicularly or at an angle to the inhibitors (Figs. 3 and 4). Application nearer to the anode results in the formation of a visible complex where the enzyme and inhibitor meet. Application of the substances in the cathode field, however, in addition to the complex formation, leads to considerable distortion of the RNA, DNA or heparin lines.

The formation of complexes and bending of the lines is caused by a reaction of an electrostatic nature between the enzyme, of which the pH is in the alkaline range, and the macro-acidic polymers. Evidence of this is the complete crossing without any mutual interaction of two macro-acidic polymers.

The influence of the time of electrophoresis and the ionic strength of the buffer was also examined. Neither prolonging of the time even to 60 minutes nor change in the ionic strength had any effect on the stability of the complex, once formed. It should be remembered that the complexes obtained are sometimes visible even before the staining of the electrophoregrams. After the strips are dried, a yellowish band corresponding to the complex can be seen at the crossing point of the two substances. This can also be seen under ultraviolet light.

The results of the above experiments indicate that in inhibition of the competitive type between polyanions and the substrate of the enzyme, stable enzyme–inhibitor complexes can be obtained by means of "crossing" electrophoresis.

This method also enabled us to obtain positive results in experiments on the action of antibiotics[14].

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