CHROM. 4434

The demonstration of tryptophan, tyrosine and carbohydrate-containing proteins in disc electrophoresis gels

The application of histochemical techniques for the localization of proteins in disc electrophoresis gels has been primarily limited to nonspecific protein-staining dyes, for example Amido Black, and to methods for the demonstration of enzymatic activity. Specific histochemical methods are available for the localization of amino acid components of proteins[1,2]. Certain proteins are known for their relative abundance of specific individual amino acids. The present study was initiated in order to localize in disc electrophoresis gels the components of amyloid, a pathologic protein which is unique among fibrous proteins in containing trytophan as a prominent amino acid constituent. Several other proteins are known to be either devoid of tryptophan, *e.g.* collagen, or to have a high tryptophan or tyrosine content, *e.g.* lysozyme. The purpose of the present paper is to describe the adaptation of two histochemical techniques, *i.e.* methods for detecting tryptophan and tyrosine on disc electrophoresis gels. In addition, proteins containing carbohydrates are also demonstrable by histochemical methods, for example, by the periodic acid Schiff (paS) reaction. The methods for this reaction that have been previously described, however, have not been found to be consistently reproducible.

Materials and methods

Human serum was used in all experimental techniques, and the sample was dissolved in 10% glucose in saline immediately before application to the gel. The electrophoretic macrotechnique was that described by DAVIS[3] and ORNSTEIN[4] and the microtechnique was that described previously (ref. 5)*. To improve diffusion conditions, the gels were placed on a shaking machine during each reaction step. The gels are made up in the following manner: concentration gel (3.5 cm long) contains 3.5% acrylamide, 0.875% N,N'-methylenebisacrylamide (Bis), 0.5 mg% riboflavin, and 59 m*M* Tris–phosphate buffer (pH 6.9); separation gel (7.0 cm long) contains 6.5% acrylamide, 0.175% Bis, 0.0575 vol. % TEMED, 3.75 mg% $K_3Fe(CN)_6$, 70 mg% ammonium persulfate and 0.375 *M* Tris–HCl buffer (pH 8.8); and electrode buffer contains 49.5 m*M* Tris–glycine (pH 8.4). Densitometric recording of stained gels is as described[6].

Staining procedures

Tryptophan. 100 μl of human serum are applied to standard gels 5 mm in diameter. After electrophoresis the gels are stained by treatment for 1 h at 20° using 1 g *p*-dimethylaminobenzaldehyde, 30 ml of glacial acetic acid, 10 ml of conc. HCl; then for 15 min at 20° using 40 ml of glacial acetic acid, 0.3 ml of 1 *N* HCl, 0.7 ml of freshly prepared 1% sodium nitrite; and finally for 30 min at 20° using 1.2 g picric acid, 10 ml of glacial acetic acid and water was added up to 100 ml. If crystallization occurs on the surface, gels are placed in glacial acetic acid until the crystals are dissolved.

* Combined macro–micro disc electrophoresis equipment: Boskamp, 5304 Hersel/Bonn, G.F.R.

Thereafter the gels are rinsed in running water for 5 h. Densitometric recording was made at 626 mμ.

Tyrosine. 1.5 μl of human serum are applied to micro columns 1 mm in diameter. After electrophoretic separation, the gel threads are treated for 16 h at 4° in a mixture of 12.5% acetic acid and 13.8% sodium nitrite (1:1); for 3 × 30 min in degassed distilled water at 20°; for 30 min at 20° using a freshly prepared solution containing 1 g 1-amino-8-naphthol-4-sulfonic acid (S-acid, K and K Laboratories, Jamaica, N.Y.), 1 g KOH, 1 g ammonium sulfamate, 70% ethanol added up to 100 ml and then for 3 × 30 min at 20° in 0.1 *N* HCl. All steps were carried out in light-excluding containers.

Carbohydrates. To give well-stained bands after electrophoresis on 5-mm gels, a staining procedure using the paS reaction was applied. The gels were treated for 16 h at 4° using 2.5 g sodium-*m*-periodate, 86 ml of H_2O, 10 ml of glacial acetic acid, 2.5 ml of conc. HCl and 1 g trichloroacetic acid; for 8 h at 4° with several changes of a solution of 90 ml of water, 10 ml of glacial acetic acid and 1 g trichloroacetic acid; for 16 h at 4° with Schiff's reagent (Merck); and for 2 × 2 h at 4° with 1 g potassium disulfite

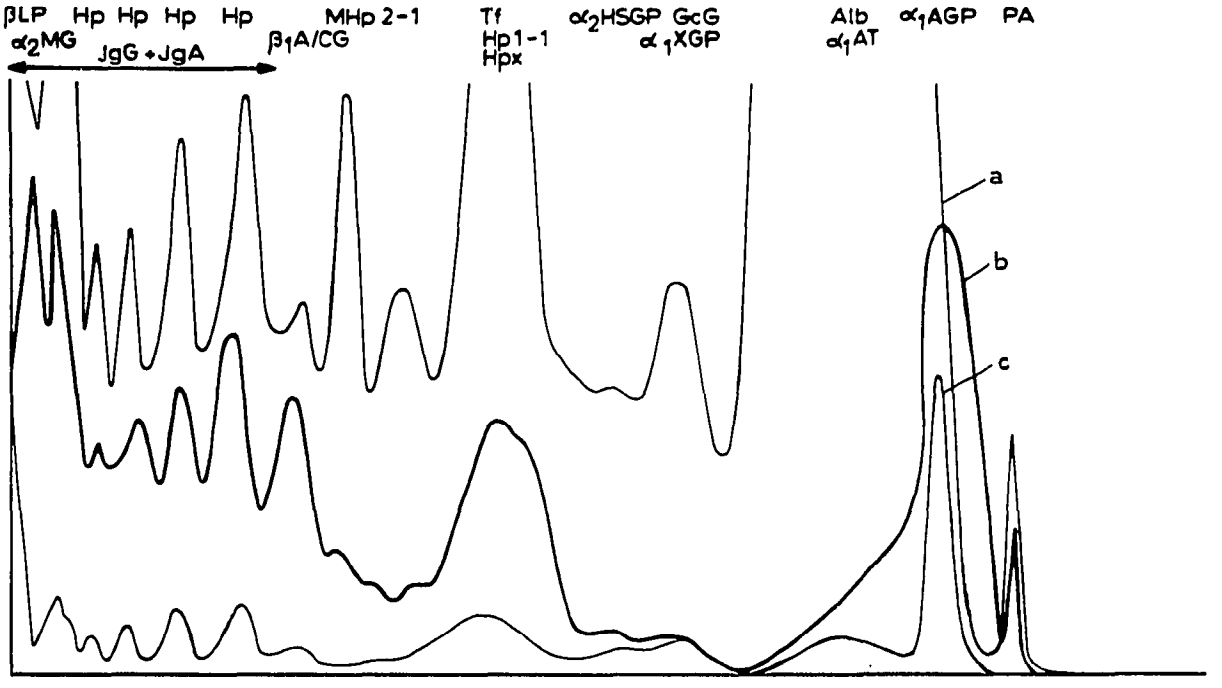

Fig. 1. Protein characterization after polyacrylamide gel electrophoresis. Technical details are described in the text; tryptophan and carbohydrate contents are according to SCHULTZE AND HEREMANS[7]. Abbreviations see p. 119. (a) Standard Amido Black stain; (b) Tryptophan detection (Try = tryptophan content); (c) Detection of glycoproteins (CH = carbohydrate content). Acid α_1 glycoprotein (CH, 41.4%; Try, 1.4%) is uncovered from the dominating albumin, which has a very low carbohydrate and tryptophan content (CH, 0.08%; Try, 0.13%). α_1 Antitrypsin (CH, 12.4%; Try, 0.55%) can be specifically demonstrated by the paS method. Note the considerable differences in the curve profiles in the post-albumin and post-transferrin region (Gc globulin CH, 4.2%; Try, 0.8%; α_2HS glycoprotein CH, 13.4%; Try, 0.82%). Increasing base line of tryptophan tracing in the last third of the electropherogram is caused by immunoglobulin G, which has a high tryptophan but low carbohydrate content (CH, 2.9%; Try, 3.83%). The position of several proteins, localized mainly by immunological methods[8], is indicated in the graph.

crystals, 20 ml of conc. HCl and 980 ml of water. Densitometric recording was made at 543 mμ. After completing the paS reaction the gels can be stained in 0.5% Amido Black–5% acetic acid for 2 h at 20°. After rinsing in running water for several minutes, they are destained in 2% acetic acid for about 16 h.

Discussion

The usefulness of specific techniques for amino acid constituents of proteins could be demonstrated in the previous study by the fact that certain constituents of serum, *e.g.* immunoglobulin G, are known to have a high tryptophan but a low carbohydrate content (Fig. 1); this is also true of the protein, amyloid, which initiated the present study. The ability to localize these proteins and to distinguish them from other proteins not having similar characteristics is extended by the present techniques. The method described for tryptophan has an absolute specificity only for indole-containing proteins[1]. In the case of the tyrosine reaction, only phenol-containing proteins will react[2]. The specificity of the paS reaction is not as great as those for the previously described methods for amino acids. The paS reaction will demonstrate not only carbohydrate constituents containing vicinal hydroxyl components but also any protein having an N-terminal serine or an α-hydroxy carboxylic acid. Despite the relative unselectivity of this method, however, the most significant reaction with proteins obtained will be those containing carbohydrates. The above methods have a much greater selectivity than methods using Sudan Black for phospholipids or lipoproteins which are capable of staining many nonlipid substances.

The use of the micro gel technique[5] in the demonstration of tyrosine (Fig. 2) was necessitated by the fact that during the chemical reaction nitrous oxide is released and the gas thus formed produced bubbles in routine gels. This was not the case when the micro method was used, since the small diameter of the gel permitted rapid diffusion of gas and eliminated, to a large extent, entrapment of gas bubbles within the gel.

Although, on a theoretical basis, methods for sulfydryl and disulfide com-

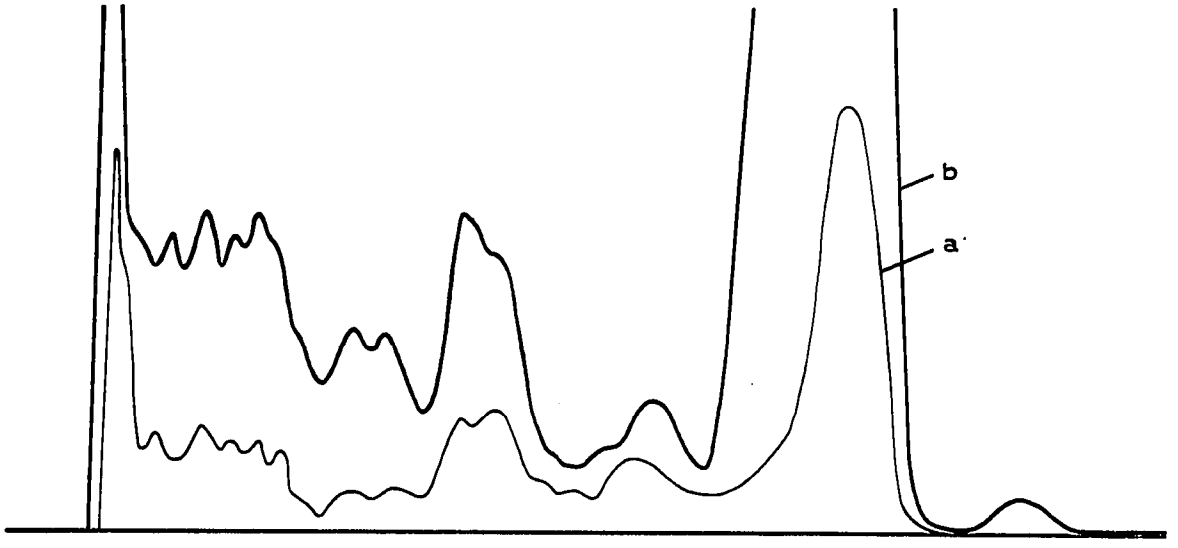

Fig. 2. Tyrosine reaction after microelectrophoresis of human serum. Technical details are as described in the text. (a) Tyrosine reaction; (b) Standard Amido Black stain.

ponents of proteins (cysteine and cystine) should be applicable to proteins demonstrated in the gel following electrophoresis, production of disulfide interchange during electrophoresis is known to occur. Usually this is prevented during electrophoresis by the incorporation in the sample of 0.1% thioglycollic acid. The incorporation of this reagent, however, would also interfere with the histochemical demonstration, since the histochemical reagent would react with unreacted thioglycollic acid still present in the gel.

Abbreviations

Alb	= Albumin
α_1AT	= α_1 Antitrypsin
α_1AGP	= α_1 Acid glycoprotein
α_1XGP	= α_1 X glycoprotein
α_2HSGP	= α_2 HS glycoprotein
α_2MG	= α_2 Macroglobulin
βLP	= β Lipoprotein
β1A/C G	= β1A/C globulin
Gc G	= Gc globulin
Hpx	= Haemopexin
Hp	= Haptoglobin
IgA	= Immunoglobulin A
IgG	= Immunoglobulin G
MHp 2-1	= Monomeric haptoglobin 2-1
Pa	= Praealbumin
Tf	= Transferrin

Nervenklinik der Universität Köln, Cologne (G.F.R.)
K. FELGENHAUER
A. WEIS

National Institutes of Health, Bethesda, Md. (U.S.A.)
G. G. GLENNER

1 G. G. GLENNER, *J. Histochem. Cytochem.*, 5 (1957) 297.
2 G. G. GLENNER AND R. D. LILLIE, *J. Histochem. Cytochem.*, 7 (1959) 416.
3 B. J. DAVIS, *Ann. N.Y. Acad. Sci.*, 121 (1964) 404.
4 L. ORNSTEIN, *Ann. N.Y. Acad. Sci.*, 121 (1964) 321.
5 K. FELGENHAUER, *Biochem. Biophys. Acta*, 133 (1967) 165.
6 K. FELGENHAUER, S. BACH AND A. STAMMLER, *Klin. Wschr.*, 45 (1967) 371.
7 H. E. SCHULTZE AND J. F. HEREMANS, *Molecular Biology of Human Proteins*, Vol. I, Elsevier, Amsterdam, London, New York, 1966.
8 K. FELGENHAUER, *Clin. Chim. Acta*, in press.

First received September 8th, 1969; revised manuscript received October 7th, 1969