ISOTACHOPHORESIS OF SERUM PROTEINS USING AMINO ACIDS AS SPACER ION

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In isotachophoresis of serum proteins, ampholine is widely used as spacer ion. The isotachopherogram obtained in this method is complicated to the process.

We used amino acids as spacer ion in our experements and obtained easy-to-decipher isotachopherograms.

The equipment was a Shimadzu Isotachopheretic Analyzer IP-2A. The first migration tube was 1.0mmI.D., 80mm long, and the second migration tube was 0.5mmI.D., 200mm long. The migration current was kept at 150μA for 12 min. and then decreased to 75μA. The leading electrolyte was 5mM-MES+10mM-ammediol+0.1% HPMC (15000), and the terminal electrolyte 5mM-EACA+10mM ammediol+$Ba(OH)_2$ (PH 10.8). The sample was human serum. The spacers were 4mg/5mℓ aqueous solutions of tricine, asparagine, glutamine, glycine, valine, leucine, and β-alanine, serum proteins were separated into eight fractions having a spacer ion between them. It is necessary to add the spacer solution in a volume more than twice that of serum sample. Our experiments showed that the mixing ration of 3 and sample volume of 1μℓ gave the best results.

The peak area repeatablity obtained under the optimum condition was 1.2%, 1.4%, 1.3%, 4.2%, 1.4%, 2.6%, 2.7%, and 4.8% for the fractions 1∿8. The repetability of percentage for total peak area was 0.7∿3.9%.

The calibration curve was linear up to 0.8μℓ under this operational condition.

A myeloma serum sample was analyzed as an example of practical sample.

Memo:

ISOTACHOPHORESIS AND ISOELECTRIC FOCUSING OF HUMAN SERUM PROTEINS

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Recently isotachophoresis (ITP) is employed to analyze human fluid proteins. Carrier ampholytes were mixed with proteins to get high-resolution. However, the separation mechanisms of proteins by ITP in the presence of carrier ampholytes were not fully investigated. In this report, we tried to examine the protein distribution during ITP.

For capillary ITP, Shimadzu IP-2A and LKB 2127 Tachophor both equipped with a UV detector were used. Six UV absorbing pK markers (2-Thiobarbituric acid, Barbital, Hypoxanthine, Uracil, Tryptophan, and Phenol) were used as standards of mobility. Capillary gel ITP and capillary gel IEF of proteins were performed in 4% polyacrylamide gel columns (1.3 mm I.D. x 3 cm). Proteins separated in capillary gels were further subjected to 4-17% lenear gradient polyacrylamide slab gel electrophoresis and stained with C.B.B. R-250.

When UV-absorbing chemicals were subjected to capillary ITP after injection of Ampholine, they were separated as six UV peaks, suggesting that Ampholine worked as spacers. Thiobarbituric acid which had the lowest pK value (4.0) showed the largest mobility. When serum proteins were subjected to capillary ITP, albumin and IgG showed a mobility close to that of barbital (pK=7.4) and Tryptophan (pK=9.4), respectively. Comparing the separational patterns of proteins on the slab gel by these two methods, in IEF serum proteins spreaded widely on the gel, and in ITP they distributed to the narrow part on the gel. The range of migration of the carrier ampholyte corresponded to that of proteins separated by each methods.

Memo:

EVIDENCE FOR TWO NEW ALLELES IN THE COAGULATION FACTOR XIII SUBUNIT B SYSTEM,FXIIIB*4 AND FXIIIB*6,REVEALED BY IMMUNOFIXATION AGAROSE GEL ISOFOCUSING

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Immunofixation agarose gel isoelectric focusing (IAGIF) with monospecific coagulation factor XIII subunit B (S) antiserum gave evidence for the existence of two new alleles in this system,designated FXIIIB*4 and FXIIIB*6.After 3h IAGIF of neuraminidase(CPN+)-treated serum samples in a 1mm isogel agarose pH 5-7 on gelbond film at a cooling temperature of 10°C at maximum settings of 1200V,20mA,10W, a 1:1 dilution of antibody was spread over the cathodal half of the gel,incubated 90 min at 37°C,pressed under filter paper,washed out in saline overnight, dried and stained.Whereas the B*4 and B*6 gene products(phenotypes B4-1,B4-2,and B6-1,confirmed by family studies) were located anodally to B2 after 4h immunofixation agarose gel electrophoresis(IAGE), IAGIF of CPN+ sera shifted the B4 and B6 band in a more cathodal position.The pI of B2 became very similar to that of B1, suggesting that a)there are three common alleles present in the major races b)FXIIIB*B' (0.013 in Japan) is identical with B*2 in Caucasoids (0.095 in Germany) c)the pI of B2 asialo-molecules is decreased by the carrier ampholytes during IAGIF.

Memo:

ISOELECTRIC FOCUSING OF IMMUNOCOMPLEXES

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Isoelectric focusing (IEF) in 150 µm thick gels of polyacrylamid and agarose was performed with bovine serum albumin (BSA) - anti BSA complexes and soluble human immunocomplexes consisting of IgM-anti IgG (rheumatoid factors) or autoantibodies developed after infection with Treponema pallidum.

Resolution was superior with acrylamid gels and results with either polyacrylamid or agarose alone were better than with composite gels. It can be shown, that immunocomplexes were dissociated during IEF. By using immunofixation for demonstration of antigen and antibody IEF can be used to analyse the immunoglobulin class and the nature of antigen in soluble immune complexes.

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Memo:

INCREASING RESOLUTION AND IMPROVING REPRODUCIBILITY OF ISOELECTRIC FOCUSING AND 2D-ELECTROPHORESIS BY PERFORMING IEF IN IMMOBILIZED PH GRADIENTS.

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The extremely fine resolution of immobilized pH gradients is successfully applied for the analysis of genetically determined heterogeneity. The data presented include the screening of legume seed proteins and enzymes of different species and varieties, the analysis of inherited heterogeneity in the human Gc system and the classification of genetic variants in the human Pi (α_1-antitrypsin) system.

The increased resolution and improved reproducibility of IEF in immobilized pH gradients are also of great advantage for the 2D-electrophoresis where the results are highly dependent on the first dimension, the IEF. 2D maps of vertical as well as horizontal electrophoresis systems are shown. By running both dimensions horizontally and by casting both gradient gels (pH gradient gel first dimension, pore gradient gel second dimension) with the same principle also the methodical efforts are reduced to a minimum.

Memo:

GENERATION OF HIGHLY-REPRODUCIBLE, EXTENDED pH INTERVALS IN IMMOBILINE GELS

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The use of immobilized pH gradients for extremely high resolution separations has been recently described (Bjellqvist *et al.* J. Biochem. Biophys. Methods 6, 1982, 317-339). Since these gels represent a medium of fully controlled environmental parameters, such as ionic strength (I) and buffering power (β), and are completely free from vexing decay problems, they appeared to be particularly attractive also for 2-D separations, where pI reproducibility along the pH axis is a must. We have therefore tried to generate extended pH intervals covering 3 to 6 pH units, for developing 2-D protein maps. In connection with this, a computer program has been developed which, given the molarities and pK's of the different Immobilines in the (two or more) chambers of the gradient mixer, can generate the theoretical pH profile, together with the I and β courses. In one approach, a five chamber gradient mixer has been built, containing five different Immobilines (pK's 4.4, 4.6, 6.2, 7.0 and 8.5), titrated in the pH 4-8 interval with non-buffering Immobilines pK 9.3 (for the two acidic species) and pK 3.6 (for the three basic components).

In a second approach, pH intervals covering 3 pH units (pH 4-7, 5-8, 6-9 and 7-10) or a wide pH 4-10 span, have been generated by simply utilizing a two chamber gradient mixer. All these pH intervals have been optimized for pH linearity, constant β power and controlled I variations along the pH gradient. Examples will be given of the application of this technique to the 2-D analysis of serum, urines, amniotic, other body fluids and other complex protein mixtures. (Supported in part by grants fr from Consiglio Nazionale delle Ricerche and Ministero Pubblica Istruzione, Roma).

Memo: