

HIGH RESOLUTION ISOELECTRIC FOCUSING: NEW APPROACHES
TO ANALYTICAL AND PREPARATIVE SEPARATIONS OF
PROTEINS AND ENZYMES

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Over the past few years high field strength is increasingly exploited for improving resolution in isoelectric focusing. The essence of ultrathin-layer isoelectric focusing is the use of gels of reduced thickness (50 - 250 μm) and field strengths of 200-1000 V/cm. Miniature ultrathin-layer systems, utilizing 1-3 cm separation distances, combine high resolution with operational simplicity and reduce focusing time from hours to minutes. High resolution in the separation of enzymes is achieved by new, fast visualization techniques designed to overcome the limitations of conventional techniques.

For improved resolution, preparative isoelectric focusing is carried out in 0.3 - 1 mm horizontal layers of granulated gels, instead of the thicker layers employed so far. Increased versatility is gained by using rehydratable layers of granulated gels (e.g. Sephadex G-200 or Bio-Gel P-60) which are sprayed before use with a solution of carrier ampholytes of any desired pH range and composition. By using field strengths of 100-500 V/cm over 10-40 cm separation distances proteins differing by as little as 0.01 pH can be resolved. High resolution preparative isoelectric focusing is a straight forward, single-step approach to the purification of proteins and enzymes which could replace multi-step procedures.

Memo:

A SERUM ENZYME ANOMALY: BINDING OF ENZYMES WITH
IMMUNOGLOBULINS

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Among the serum enzyme abnormalities detected by electrophoresis, the highest number of incidence was recorded by those resulting from complex formation of enzymes and immunoglobulins. The chance to find many such complex was provided by unreasonable rises of enzyme activity in serum and abnormality in electrophoretic pattern of the enzyme, or the enzymogram. These researches have been also under way at many places in Japan. Especially, a nation-wide research-survey was conducted by Prof. Kanno during the period from 1981 to 1982, to compile data for the number of enzyme anomaly incidences, their relationship with diseases and other factors. As a result, possible relations were found between the enzyme anomaly incidents and autoimmune disease, cancers and others, in the IgG cases linked to LDH, ALP or CK. And through improvement of the identification method, the rate of detecting complex has risen.

As a future problem, the clarification of the mechanism responsible for the complex formations between enzymes and immunoglobulins is remained. For instance, among the LDH-IgA complexes, there are some unusual formations, in which IgA binds only with LDH₂ or LDH₃ and their binding ratio is 1 : 1.

Memo: