DISCRIMINATION BETWEEN MONOCLONAL AND HETEROGENEOUS IMMUNOGLOBULIN M BY ISOELECTRIC FOCUSING AND RADIOAUTOGRAPHY

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1. Introduction

Although human heterogeneous and monoclonal IgG and IgA have been investigated by isoelectric focusing (IEF) [1–4], so far very little has been reported about IEF of immunoglobulin M. We describe a method which permits the determination of the IEF pattern of IgM after reduction to 7S-subunits. The sensitivity of the method permitted the detection of this pattern on polyacrylamide gel plates of very small amounts of IgM without prior purification, by the use of ^{125}I-labelled antibodies against this Ig-class. This can be of considerable interest for studies of IgM-producing tumours which do not secrete their product, a type of tumour that is more common than previously thought [5].

2. Materials and methods

Normal and monoclonal IgM were isolated from pooled human serum and from sera of macroglobulinemic patients [6,7]. The reduction of IgM was carried out for 1 h at 20°C in Tris buffer (0.2 M Tris; 0.2 M NaCl, pH 8) which was made 0.1 M in 2-mercaptoethanol. After cooling to 4°C the reduced material was alkylated by addition to a 20% molar excess of sodium iodoacetate [8]. After 45 min., the solution was dialyzed against 0.15 M NaCl.

IEF on polyacrylamide plates was performed as described by the manufacturer of the apparatus [9]. Ampholines (LKB, Bromma, Sweden) of the pH ranges 3.5–10 and 5–8 (v/v, 3:1) were used in a concentration of 2%. 10 μl IgM solution (0.1 to 1 mg/ml) were applied in paper pieces.

Purified sheep antibodies against IgM (μ-chain) were isolated using affinity chromatography on glutaraldehyde-insolubilized IgM [10]. The antibodies were labelled with ^{125}I by the lactoperoxidase method [11]: Three mg of antibodies were labelled in 1 ml phosphate buffer with 1 mCi ^{125}NaI (Amersham-Buchler, Braunschweig). Immediately after completion of the IEF, the gel plates were covered with a sheet of gaze which then was saturated with 3 ml of a solution containing 3 mg ^{125}I-labelled antibodies against IgM. After 2–3 h, the immunodiffusion was discontinued by removal of the gaze and flushing of the plate for approx. 15 h with a total of about 60 litres of 0.15 M NaCl. The gel was then removed from the supporting glass plate, dried on a paper covered glass plate and covered with an X-ray film [12].

3. Results and discussion

The large molecular diameter of the IgM-pentamer renders it incapable of entering the 4% polyacrylamide plate, and its insolubility in salt-free medium precludes IEF of IgM in native state. We therefore reduced its dimensions to those of the monomer and increased the solubility by the introduction of carboxymethyl groups. The possibility that the reduction and modification process introduced additional heterogeneity was tested by applying the same procedure to monoclonal IgG and IgA. Since the IEF pattern of the latter two classes remained unchanged after the treatment, apart

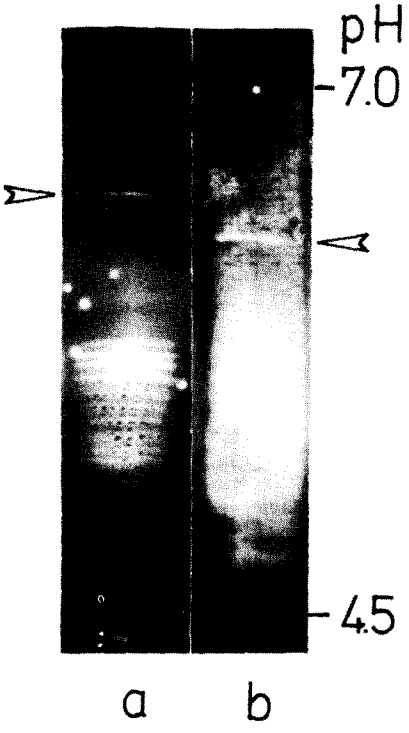

Fig.1. Radioautograph of reduced, and carboxymethylated isoelectrofocused IgM (positive print). (a) Monoclonal IgM from macroglobulinemia serum. (b) Normal IgM from pooled sera. Arrow indicates site of application.

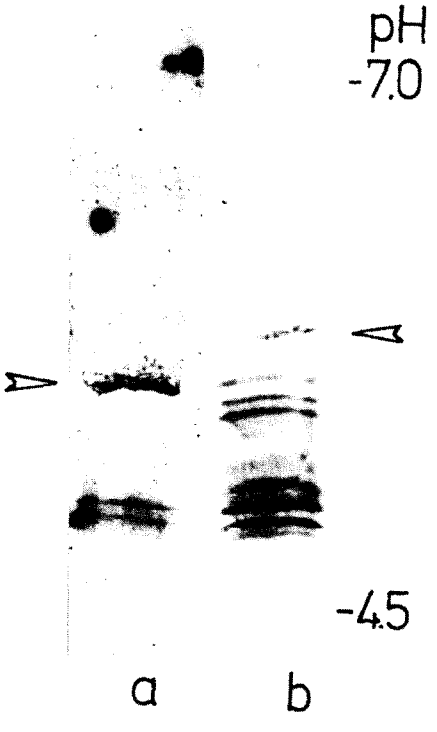

Fig.2. Radioautograph of reduced, and S-carboxymethylated isoelectrofocused IgM; both monoclonal (negative print) (a) lymphnode homogenate from chronic lymphatic leukemia patient. (b) lymphnode homogenate from immunoblastic sarcoma patient. Arrow indicates site of application.

from the displacement in isoelectric points caused by the introduction of the carboxymethyl groups, this possible source of error appears to be negligible. We recommend the use of very small amounts of IgM, since the application of higher concentrations yielded poorer results. Furthermore, it is practically impossible to isolate the IgM from tissue homogenates and serum aliquots in such amounts and purity that it can be stained properly on gels. The confinement of the labelled antibodies to a volume as small as 3 ml shortens the time required for completion of the formation of the immune complexes, thus reducing the blurring of the IEF pattern by diffusion after removal of the electrical field. The method suffers from a minor disadvantage, caused by impression marks of the gaze on the gel. This artefact, which we so far have not been able to eliminate completely, appears as a weak grid on the radioautograph. It might in unfortunate cases cover faint bands which then escape the attention of the experimentator.

Nevertheless, unequivocal differences between the IEF pattern of reduced, normal and monoclonal IgM are visible on the radioautographs (fig.1). Normal IgM gives a diffuse unsymmetrical pattern. In contrast, monoclonal IgM gives a clear line pattern consisting of 2–10 bands, which bears a certain resemblance to those of IgA and IgG group I [2,3]. The pattern of monoclonal IgM shows the same shift on neuraminidase treatment as IgA (unpublished results H. Bouman) and IgG group I [3].

The method has been successfully applied to samples of intracellular IgM from lymphnode homogenates (fig.2). Such studies proved to be of considerable value for the characterization of certain diseases (e.g. tumours) of the lymphatic system (H. Stein and H. Bouman in preparation).

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