Isolation of Monoclonal Antibodies to Phencyclidine from Ascites Fluid

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Abstract

A monoclonal antibody (Mab) to the hapten phencyclidine (PCP), or angel dust, was developed, produced in mouse ascites, and purified. The purification used only preparative-scale isoelectric focusing in the Rotofor® isoelectric focusing cell and dialysis. In 4 hr, 25% (4 mg) of the antibody from ~10 ml of ascites was purified to homogeneity while 63% of the total antibody was recovered (Egen et al. 1988). The purified antibody (ab) and its antigen binding fragments (Fab) were used for in vitro studies of drug metabolism and detoxification.

Methods

Antibody Preparation

Mice were immunized with a PCP-ovalbumin conjugate. Spleen cell suspensions were prepared, pooled, and fused with a HAT-sensitive myeloma cell line. Wells were screened for anti-PCP activity by enzyme immunoassay (EIA) and radioimmunoassay (RIA). Anti-PCP producing clones were selected and subcloned by limiting dilution techniques to establish a stable line of Ab secreting clones. The Mab was identified as IgG_{2a} by an isotype-specific EIA.

Irradiated pristane-primed mice were injected with 2×10^6 hybridoma cells. Ascites fluid was tapped every 2–3 days and tested for anti-PCP antibody activity by an ^{125}I-PCP RIA. Estimations of antibody concentration in the Rotofor fractions were determined by interpolation from a standard curve prepared from this untreated (prefractionated) ascites.

Untreated ascites was fractionated on a preliminary analytical IEF-PAGE gel stained with either silver (Merril et al. 1981) or Coomassie Blue R-250 (Blakesley et al. 1977). Mabs typically focus in the pH range 5–9, while the majority of mouse ascites proteins focus between pH 3 and 6. Stained gels were compared to an autoradiograph of an identical but unstained focused gel overlaid with radioligand (Williamson 1978). Results revealed 5 isoforms of the anti-PCP antibody focused in the pH 7.3 region. The Mabs were separated from other more acidic ascites proteins by about 0.5 pH units. This was sufficiently basic to facilitate purification of Mabs from other proteins by preparative IEF on the Rotofor cell (see Figure 1).

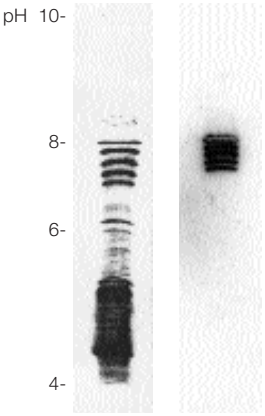

Fig. 1. Coomassie Blue-stained IEF PAGE (pH 3.5–10, 3 M urea) of ascites fluid (left) and an autoradiograph from a similar, but unstained, gel overlaid with radioligand (right). Five isoforms of the Mab are secreted by the hybridoma used in this study. The anode is at the bottom.

Treatment of Ascites Fluid for Preparative IEF on the Rotofor System

Ascites fluid (11 ml) was desalted by diafiltration in 3 M urea to increase the number of Rotofor fractions on the linear portion of the pH gradient, 2.06 ml of ampholyte solution (pH range 3.5–10; 40% w/v) was added, and the sample was diluted to 55 ml with 3 M urea (a 1:5 dilution). The mixture was clarified by centrifugation, and 51 ml was loaded into the Rotofor cell.

Without the use of urea the loss of Mab activity due to precipitation was excessive. Incubating the ascites fluid with purified 3 M urea for 4 hr at 4°C irreversibly inactivates 25% of the antibody without precipitation. Removing the urea by dialysis against PBS decreases the activity another 6%. (A higher urea concentration of 6 M did not affect results.) Because the Rotofor fractions had to be dialyzed to remove the urea and ampholytes from the Mabs, the maximum recovery of 69% was expected. At present other additives are being sought that will maintain Mab activity and prevent precipitation during focusing.

Running Conditions

Isoelectric focusing in the Rotofor cell required 4 hr at 12 W constant power at 4°C. The initial conditions were 550 V and 22 mA. At equilibrium the values were 1,000 V and 12 mA. Twenty fractions were collected, their pH values were measured, and aliquots were analyzed on silver-stained and Coomassie Blue R-250 stained gels (see Figure 3). Fractions were then dialyzed against PBS by adding 1/9 volume 10x PBS to each fraction and quantified for PCP binding capacity (activity) by RIA. The protein concentrations were determined by optical density measurement at 280 nm. Results from the antibody assay are summarized in Figure 2.

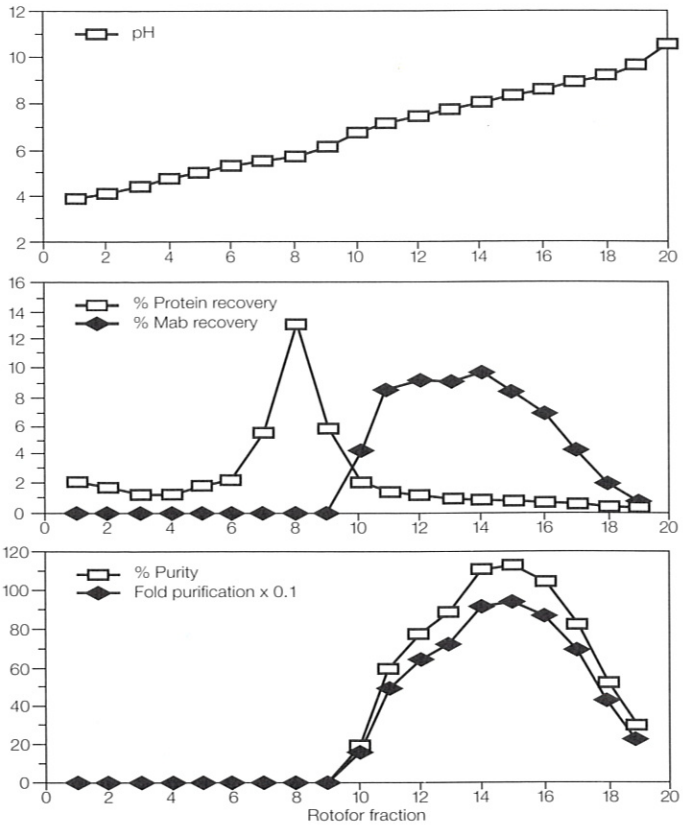

Fig. 2. Analysis of 20 Rotofor fractions by pH, protein assay, and antibody activity (RIA). The pH range in the Rotofor unit was 3.5–10. The ascites fluid contained 15 mg protein/ml and 1.7 mg PCP-Mab/ml. Specific activity is the weight ratio of Mab to total protein, while the fold purification is the ratio of the specific activity of the ascites fluid (1.7/15 = 0.11). A total of 43% of the protein and 63% of the Mab was recovered.

Results

The major protein-containing fraction (fraction 8) does not contain antibody. A summation of the anti-PCP activity recovered from fractions 10–19 is 63% of that in the original ascites fluid. Antibody recovery plateaued at approximately 9% in fractions 11–15. Fractions 14–16 contained 25% of the Mab from the original ascites fluid. A total of 11 mg was recovered in fractions 10–19. Of the 11 mg recovered, 4.3 mg in fractions 14–16 was pure IgG_{2a}.

Anti-PCP antibody concentration was estimated to be ~1.7 mg Mab/ml ascites fluid by 3 independent methods: focusing on the Rotofor cell, affinity chromatography on protein A (Hudson and Hay 1980), and RIEF (Bier 1986). 11 mg of active Mab (63% of total) was recovered in fractions 10–19. Maximum expected recovery of active anti-PCP antibody based on deactivation by urea treatment is 11.73 mg. Thus, 94% of

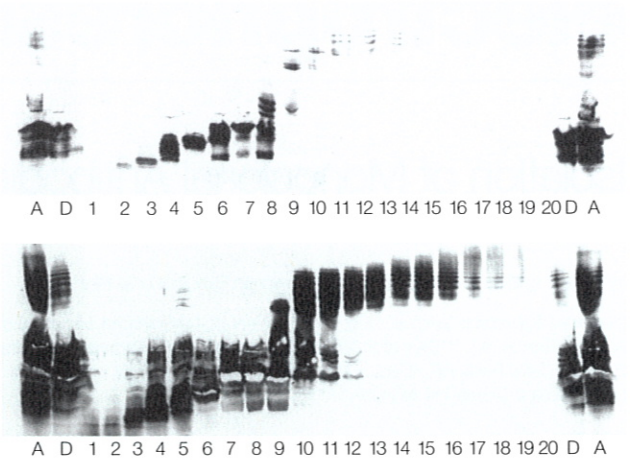

Fig. 3. Analysis of Rotofor fractions 1–20, ascites fluid (A), and desalted ascites fluid (D) in 3 M urea, by IEF (pH 3.5–10, 3 M urea) in PAGE. The top panel is a Coomassie Blue-stained gel. The bottom is a silver-stained gel. The gels were loaded with the same sample quantities. The silver-stained gel reveals trace contaminants in the Mab-containing fractions 10–20.

the active antibody was recovered in fractions 10–19. In a single run, 4.3 mg Mab was purified to homogeneity in fractions 14–16 as indicated by gel electrophoresis (Figure 3).

Conclusion

These results show that the Rotofor cell brought about significant purification of Mabs in a minimum number of steps. Using the purified antibody and its high-affinity PCP-specific antigen binding fragments (Fab), it should be possible to produce both a redistribution and an inactivation of the drug through high-affinity binding. This has been shown to be the case for digoxin-specific antibodies (Butler et al. 1979).

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