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Note

A general chromatographic procedure for the purification of murine myeloma immunoglobulins A

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Most of the work on immunoglobulins that are either homogeneous or of restricted heterogeneity involves immunoglobulins having specificity for polysaccharides[1]. Immunoadsorption[2] (affinity chromatography) is the preferred technique for the purification of these homogeneous immunoglobulins from sera or ascites; there are, however, two main difficulties associated with this technique. Firstly, the specificity of a homogeneous immunoglobulin may not be known, thus making the preparation of an immuno-adsorbent impossible. Secondly, even if a polysaccharide-specific immuno-adsorbent can be prepared, the immunoglobulin in question is, not infrequently, adsorbed with such tenacity that removal with hapten solution, or even with denaturing buffers, is not possible[3].

The latter occurred when, in our laboratory, we prepared an immuno-adsorbent for MOPC 384 and 870. Both these murine myeloma immunoglobulins precipitate with the O-antigen determinant of a lipopolysaccharide (lps) from *Salmonella tranaroa* The lypopolysaccharide was heated with 1% aqueous acetic acid, the lipid A removed, and the mixture separated on a column of Sephadex G-50. The single peak of O-antigen polysaccharide[4] precipitated with these two immunoglobulins, but the core material did not. When the O-antigen fraction was prepared, and then attached to Sepharose[3], the resulting immuno-adsorbent indeed bound the two IgA's, but with too high an affinity, and it proved impossible to remove significant amounts of immunoglobulin, even by using such strong denaturing agents as 5M guanidine hydrochloride (pH 11.0), 1% dodecyl sodium sulfate, or 8M urea. Most antipolysaccharide immunoglobulins show this effect of tenacious binding, which makes difficult their purification by affinity chromatography. As almost all binding studies on homogeneous immunoglobulins currently involve antipolysaccharide immunoglobulins, we have sought a general method for their purification.

The method described here will probably prove generally applicable to the purification of myeloma IgA's.

EXPERIMENTAL

Materials. — Plasmacytomas (MOPC 384 and 870) were of BALB/c origin[5], and ascites were generously supplied by Dr. M. Potter (NCI). The immunoglobulins belonged to the IgA class. Goat anti whole mouse serum was obtained from Cappel Laboratories (Donningtown, PA 19335) and rabbit anti IgA was prepared by immunization with SAPC-10 IgA. Lipopolysaccharide (lps) was isolated from *Salmonella tranaroa* by extraction with aqueous phenol[6]. O-Antigen could be cleaved from the lps by hydrolysis with 1% acetic acid for 1.5 h at 100°; the resulting mixture of core- and O-polysaccharide was resolved by chromatography[4] on Sephadex G-50. Both the whole lps and the O-antigen precipitate with MOPC 870 and 384 (see Fig. 1).

Methods. — Ascites fluid (either MOPC 384 or 870) was diluted with an equal volume of phosphate-buffered saline (PBS), and then made 40% in ammonium sulfate. Precipitated proteins were collected by centrifugation, dissolved in water, and the solution dialyzed against PBS, pH 7.4. This solution was applied to the top of a column of DEAE-Sephadex A-25 pre-equilibrated in the same buffer. Unadsorbed material was removed by washing with PBS (pH 7.4), and the eluant was changed to 0.1M disodium phosphate (pH 8.0)–0.15M sodium chloride to elute bound components (see Fig. 2), which made up ~50% of the load. The latter material (Fraction II) precipitates with lps, whereas the unabsorbed Fraction I does not.

The IgA-rich fraction was dialyzed against 0.1M Tris hydrochloride (pH 8.1), and converted into monomeric IgA by reduction with 0.01M 1,4-dithiothreitol for 2 h at room temperature, followed by alkylation with 22mM 2-iodoacetamide[3] for 1 h at 0°. The excess reactants were removed by dialysis *versus* PBS, pH 7.4, and the alkylated monomers were loaded onto the top of a column of Sephadex G-200 pre-equilibrated in the same buffer. Elution with that buffer yielded two components:

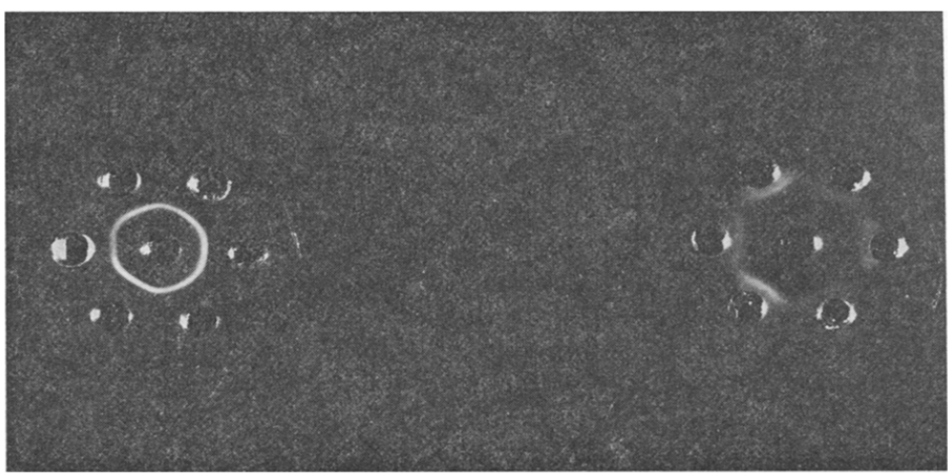

Fig 1. Double diffusion of lps (left) and O-antigen (right) of *Salmonella tranaroa* with MOPC 384 and 870. (Peripheral wells contained the two immunoglobulins alternately. Antigen was in the central well.)

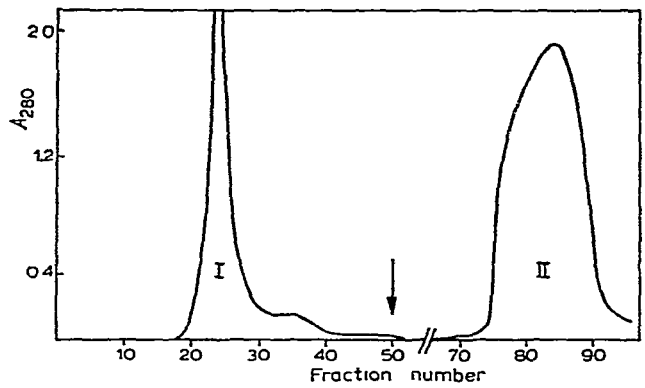

Fig. 2. DEAE-Sephadex A-25 chromatography of MOPC 384 precipitated with ammonium sulfate. [The material (~100 mg) was loaded on a column (2.5 × 30 cm), and 2.5-ml fractions were collected. Initial buffer (PBS, pH 7.4) was changed to 0.1M disodium phosphate (pH 8.0) containing 0.15M sodium chloride at tube no. 50, the position indicated by the arrow. Fractions I and II respectively weighed 36 and 50 mg.]

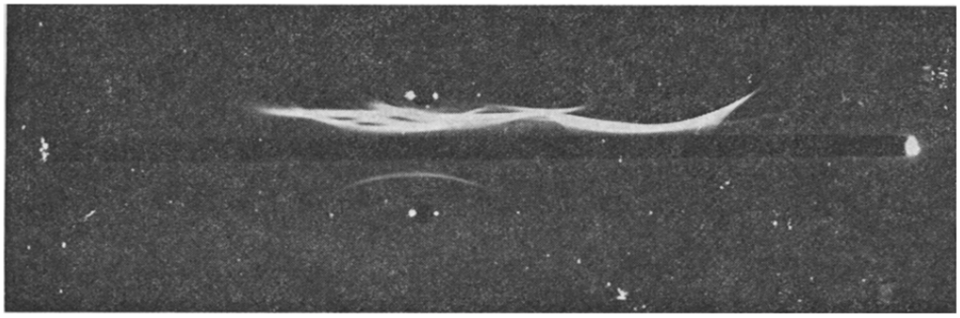

Fig. 3. Immunoelectrophoresis of MOPC 384 ascites (top) and chromatographically purified MOPC 384 (bottom). (The trough contained goat anti-whole mouse serum.)

one, eluted near the void volume, and the second, just before the position where rabbit IgG would be obtained. Eluates containing the latter peak were pooled, and concentrated. On immunoelectrophoresis, a faint line adjacent to the anode side of the IgA band appeared; this contaminant could be removed by recycling once more on a column of Sephadex G-200. The immunoglobulin thus obtained showed a single band against goat anti-whole mouse serum (see Fig. 3). Typically, ascites fluid yielded ~2.0 mg of pure immunoglobulin A per ml. Sequential, amino acid analysis showed the purified material to be homogeneous. No advantage could be found in using ultro-gel AcA 34 (LKB, Rockville, MD) instead of Sephadex G-200 in the gel-filtration chromatography of the immunoglobulin-rich fraction. It was also found that, if chromatographed on Sephadex G-200 without reduction and alkylation, Fraction II from the column of DEAE-Sephadex A-25 was eluted as a single peak near the void volume. When reduced and alkylated, this material showed three bands in immuno-electrophoresis; thus, the last purification step is only possible

if the monomeric mixture is used. The results of the second fractionation on Sephadex G-200 can also be achieved by re-adsorption of the sample on DEAE-Sephadex A-25. Elution with PBS, pH 7.8, now *removes* the monomeric IgA's, whereas removal of the contaminant requires a higher salt concentration and pH 8.0.

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