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Isolation of a 70000 Molecular Weight Antigen of the Novikoff Hepatoma†

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ABSTRACT: Previous reports from this laboratory have indicated that a number of cytosol and nuclear proteins of Novikoff hepatoma cells were immunologically related [Yeoman, L. C., Jordan, J. J., Busch, R. K., Taylor, C. W., Savage, H., & Busch, H. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 3258; Busch, R. K., & Busch, H. (1977) *Tumori 63*, 347]. In preparation for analysis of their structure and function, studies were undertaken to purify nuclear antigen 2 from the cytosol of Novikoff hepatoma cells in high yield and purity. It was shown on Ouchterlony gels that cytosol nuclear antigen 2 formed a single immunoprecipitin band of identity with one of the bands extracted from Novikoff nuclear chromatin. In this study, a 70000 molecular weight antigen was isolated from the cytosol of Novikoff hepatoma cells by ammonium sulfate fractionation, ion-exchange chromatography, and isoelectric focusing in a granulated gel bed. This protein which focused at a p*I* of 6.3 was labeled with ^{125}I-labeled Bolton–Hunter reagent and purified on an Ultrogel AcA-44 column. As shown by electrophoresis on NaDodSO$_4$–polyacrylamide gels, the antigen in the excluded volume migrated as a single protein with a molecular weight of 70000. The overall purification over the starting material was 2890-fold.

A number of "tumor-associated" nuclear antigens have been described in the chromatin of Novikoff hepatoma cells by using antibodies prepared to specific nuclear and nucleolar protein fractions (Yeoman & Busch, 1978; Busch et al., 1978). Several of the nuclear antigens showed immunological identity with cytosol antigens of the Novikoff hepatoma as well as nuclear fractions of the Walker 256 carcinosarcoma (Yeoman et al., 1976). Three of these antigens were found to be oncofetal proteins and were designated NAg-1, NAg-2,[1] and NAg-3 with molecular weights of 27000, 72000 and 110000, respectively (Yeoman, 1978; Yeoman & Busch, 1978a; Yeoman et al., 1978). Additional biochemical properties of these antigens have been reported (Yeoman, 1978).

Purification of these antigens from the cell nucleus was limited by the amount of nuclear starting material (0.6 M NaCl extract of chromatin). Inasmuch as the antigen NAg-2 was found in higher concentrations in the cytosol, it seemed desirable to isolate it from this fraction. The present studies were designed to use classical nondenaturing methods of isolation since the yields obtained with affinity columns were low and the nativeness of the isolated products were uncertain (Marashi et al., 1979).

An antigen with a molecular weight of 70000 and a p*I* of 6.3 was purified 2890-fold by ammonium sulfate fractionation, DEAE-Sephacel chromatography, CM-Bio-Gel A chromatography, isoelectric focusing in a granulated gel bed, and gel filtration on an Ultrogel AcA-44 column. The isolated product migrated as a single band on a NaDodSO$_4$-containing polyacrylamide gel. An immunoprecipitation line of identity was demonstrated for cytosol NAg-2 with the 0.6 M NaCl nuclear chromatin extract of Novikoff hepatoma cells. The isolated antigen had the same molecular weight and p*I* as nuclear antigen 2 (NAg-2), which was found in earlier studies (Yeoman, 1978; Yeoman & Busch, 1978; Yeoman et al., 1978b).

Materials and Methods

Preparation of Novikoff Hepatoma Cytosol. Novikoff hepatoma cells were transplanted intraperitoneally in male Holtzman rats (Holtzman Co., Madison, WI) and removed by abdominal puncture 6 days later. The cells were washed with NKM (0.13 M NaCl–0.005 M KCl–0.008 M $MgCl_2$). The washed tumor cells were disrupted with a Tissumizer (Tekmar Co., Cincinnati, OH) in 10 volumes of TKM buffer (0.05 M Tris–0.005 M $MgCl_2$–0.025 M KCl–0.001 M PMSF, pH 7.4). The crude nuclei were sedimented by centrifugation at 1000*g* for 20 min. The cytosol fraction used was the

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[1] Abbreviations used: IE, immunoelectrophoresis; M_r/pI, molecular weight × 10^{-3}/isoelectric point; NAg-2, nuclear antigen 2; NKM, 0.13 M NaCl–0.005 M KCl–0.008 M $MgCl_2$; PBS, phosphate-buffered saline; PMSF, phenylmethanesulfonyl fluoride; NaDodSO$_4$, sodium dodecyl sulfate; TKM, 0.05 M Tris–0.005 M $MgCl_2$–0.025 M KCl; Tris, tris(hydroxyethyl)aminomethane.

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postribosomal supernatant after centrifugation at 100000*g* for 16 h.

Ammonium Sulfate Fractionation of Cytosol Proteins. The clear postribosomal supernatant was concentrated to 20 mg/mL over an Amicon UM-10 membrane (Amicon Corp., Lexington, MA). Solid ammonium sulfate was added to the concentrated protein solution to a final concentration of 30%. This solution was kept at 4 °C for 8 h and was then centrifuged at 48000*g* for 20 min; similarly, 30–60, 60–80, and 80–100% ammonium sulfate fractions were obtained.

Antiserum. The antiserum used in these experiments was obtained from New Zealand white rabbits immunized with the 0.14 M NaCl soluble nuclear proteins of Novikoff hepatoma cell chromatin as previously reported by Yeoman et al. (1976). Rabbits were immunized on days 1, 7, 14, and 21 with chromatin proteins in complete Freund's adjuvant. Rabbits were bled on day 30. Subsequently, rabbits were boosted on day 21 and bled on day 30. The immunoglobulin fraction was prepared by adding an equal volume of saturated ammonium sulfate to the antiserum, collecting the precipitate at 21000*g*, and resuspending the pellet in 0.15 M NaCl (Kendall, 1938). Immunoglobulin fractions were dialyzed against 0.15 M NaCl prior to use.

Immunoprecipitation Methods. Ouchterlony plates were prepared with 0.8% agarose (Bio-Rad, Richmond, CA), 0.9% saline, and 0.1% thimerosal. The wells (20 μL) were filled with ammonium sulfate fractions or other protein fractions at a concentration of 10 or 20 mg/mL. For Ouchterlony analysis, each ammonium sulfate fraction was dissolved in 0.6 M NaCl–0.01 M Tris–1 mM PMSF, pH 8.0, dialyzed against several changes of the same buffer, and adjusted to a final concentration of 10 or 20 mg/mL. Immunoelectrophoresis was performed on 5-μL aliquots of antigen containing sample for 35 min at 75 V on precast 1% agarose IE gels (Immunoagaroslides; Worthington Diagnostics, Elk Grove Village, IL). Troughs were loaded with 50 μL of immunoglobulin fraction, and precipitin arcs were allowed to develop for 18 h. The plates were deproteinized for 18 h in PBS and stained with 0.013% Coomassie brilliant blue R-250 (Cawley, 1969).

DEAE-Sephacel Separation. The 30–60% ammonium sulfate cytosol fraction was dialyzed to 25 mM Tris–25 mM NaCl–1 mM PMSF, pH 8.3, and was concentrated to 30 mg/mL. A DEAE-Sephacel column (1.5 × 35 cm) (Pharmacia, Piscataway, NJ) which had been equilibrated with the loading buffer was loaded with 150 mg of the 30–60% ammonium sulfate fraction. The column was eluted with a stepwise sodium chloride gradient to 0.5 M NaCl, pH 8.3. All operations were carried out at 4 °C.

CM-Bio-Gel A Chromatography. The DEAE-Sephacel flow-through fraction was dialyzed to 0.02 M sodium phosphate–0.025 M NaCl–1 mM PMSF, pH 8, concentrated to 10 mg/mL, and loaded onto a CM-Bio-Gel A column (2.0 × 30 cm) (Bio-Rad, Richmond, CA) which had been equilibrated with the same buffer. The column was developed with a gradient from 0.025 to 0.5 M NaCl.

Electrofocusing on a Modified LKB Granulated Gel Bed. A 4-mL slot was made in an LKB glass tray by the addition of two strips of glass (0.3 × 2 × 24.4 cm) attached to the glass tray with vacuum grease. Protein fractions (0.4 mg) that had been dialyzed against 1% glycine, pH 7.2, were mixed with 0.16 g of Ultrodex (LKB Instruments, Inc., Rockville, MD) and pH 3.5–10 ampholines to a final concentration of 2%. Isoelectric focusing was performed for 18 h at 4 °C with the LKB constant power supply set for 4 W and a maximum voltage of 1500 V (Radola, 1973). At 18 h, a paper map was made by placing Whatman 1 paper in contact with the granulated gel bed, washing several times in 10% Cl_3AcOH, and staining with Coomassie brilliant blue R-250. Antigens were eluted from the Ultrodex with 0.6 M NaCl–0.01 M Tris, pH 8.3. The pH was measured on a separate gradient formed on each side of the tray.

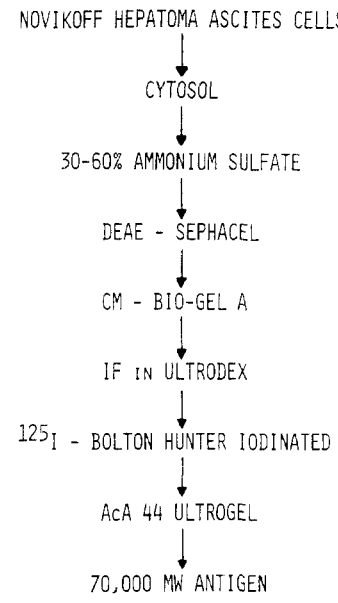

FIGURE 1: Flow diagram of steps used in the isolation of a 70000 molecular weight cytosol antigen.

^{125}I Labeling. Protein recovered from the Ultrodex gel was dialyzed to 0.1 M sodium borate and 1.0 M NaCl, pH 8.5. Protein was labeled for 15 min at 4 °C with 1 mCi of ^{125}I-labeled Bolton–Hunter reagent (Amersham, Arlington Heights, IL). The reaction was terminated by the addition of 0.5 mL of sample buffer containing 0.2 M glycine. The labeled product was recovered in the void fraction from a Sephadex G-25 column.

Separation of ^{125}I-Labeled Material on AcA-44. The ^{125}I-labeled flow-through fraction from Sephadex G-25 was concentrated and dialyzed to 0.6 M NaCl–0.01 M Tris–1 mM PMSF, pH 8.3, and purified on an Ultrogel AcA-44 column, 1.5 × 60 cm (LKB Instruments, Inc., Rockville, MD).

Acrylamide Gel Electrophoresis. Antigen from the AcA-44 column was analyzed on 6% $NaDodSO_4$ gels. The gels were sliced into 2-mm sections, placed in counting vials, and dissolved with 0.4 mL of 60% $HClO_4$ and 0.8 mL of 30% H_2O_2 at 60 °C for 16 h. The dissolved gel slices were counted in Aquasol (Beckman Instruments, Inc., Irvine, CA) in a Beckman LS-230 liquid scintillation counter. Acid–urea gels (6%) were run for 4.5 h at 120 V as previously described (Busch et al., 1974) and stained with 1% Buffalo Black in 7% acetic acid.

Results

Isolation of TKM–Cytosol. In order to isolate protein NAg-2 from the cytosol of Novikoff hepatoma cells, the cells were homogenized with TKM buffer, and the clear 100000*g* supernatant was collected for ammonium sulfate fractionation.

Ammonium Sulfate Fractionation of Cytosol Antigens of Novikoff Hepatoma Cells. The TKM–cytosol obtained was fractionated as outlined in Figure 1. The 100000*g* supernatant of Novikoff hepatoma cytoplasm was separated into 0–30, 30–60, 60–80, and 80–100% ammonium sulfate fractions. The yields of protein in each fraction are shown in Table I. The largest number of antigenic components was in the 30–60% ammonium sulfate fraction; one was a dense precipitin band

Table I: Ammonium Sulfate Fractionation of 2.5 g of Novikoff Hepatoma Cytosol

% of saturation	protein[a] (mg)	% yield
0–30	710	29
30–60	840	35
60–80	800	33
80–100	80	3

[a] Protein was determined by the Bio-Rad protein assay.

Table II: Purification Table

fractions	protein (mg)	total units[b]	sp act. (units/mg)	% yield	purifn (x-fold)
cytosol	2500	325	0.13		
$(NH_2)_4SO_4$ (30–60% fraction)	840	109	0.13	33.5	0
DEAE-Sephacel (peak 1)	68	18	0.26	5.5	2
CM-Bio-Gel A (peak b)	0.65	21.6	33	6.7	254
IF-Ultrodex (fraction 8)	0.020[a]	7.5	376	2.3	2890

[a] Protein yield is a maximum estimate since aliquots of the sample were at the minimum level of detection. The specific activity, therefore, is a minimum value. [b] One unit of immunoreactivity was achieved at the last serial onefold dilution at which a precipitin band could be detected in an Ouchterlony gel for a given sample.

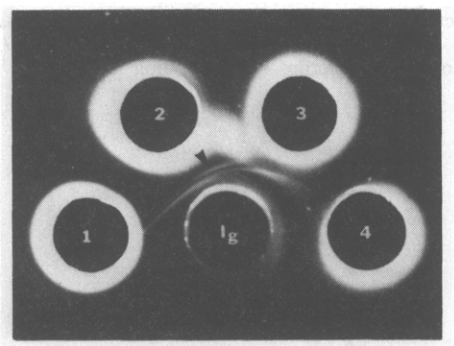

FIGURE 2: Ouchterlony double-diffusion analysis of ammonium sulfate fractions from cytosol of Novikoff hepatoma cells. Aliquots (20 mg/mL) of each ammonium sulfate fraction were placed in wells. (1) 0–30; (2) 30–60; (3) 60–80; (4) 80–100%. The center well (Ig) contained immunoglobulin fraction at 40 mg/mL. Precipitin band formation was detected after 18 h of immunodiffusion. The arrowhead indicates the antigen selected for purification.

selected for further purification (Figure 2, arrowhead). This antigen was also detected with antiserum preabsorbed with normal rat liver chromatin proteins, indicating that it was quantitatively enriched in Novikoff hepatoma cell nuclei (Davis et al., 1978). Inasmuch as the Ouchterlony assay used for antigen units does not take into account the number of antigens in a fraction, the purification in this step (Table II) was not apparent.

The 30–60% ammonium sulfate fraction was loaded on a DEAE-Sephacel column and eluted with a 25–500 mM NaCl stepwise gradient at pH 8.3 (Figure 3). Peaks 1–6 were analyzed on 6% acid–urea–polyacrylamide gels as shown in Figure 4. Ouchterlony analysis showed that the antigen designated by the arrowhead (Figure 2) was present in fraction 1.

Isolation of the 70 000 Molecular Weight Antigen. The antigens of fraction 1 of the DEAE-Sephacel column were applied to a CM-Bio-Gel A column and eluted with a

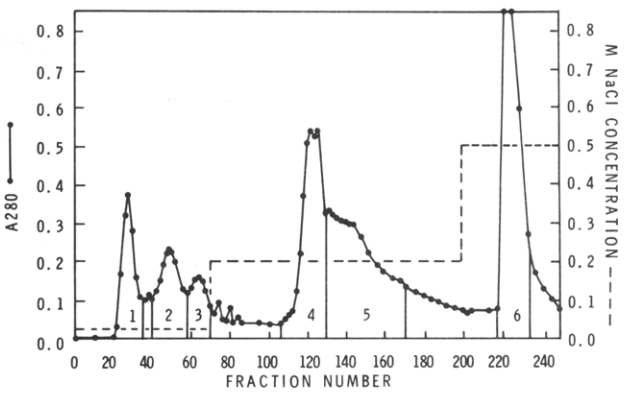

FIGURE 3: DEAE-Sephacel elution profile for the 30–60% ammonium sulfate fraction of Novikoff hepatoma cytosol. Protein (150 mg) soluble in 25 mM Tris–25 mM NaCl–1 mM PMSF, pH 8.3, at 30 mg/mL was loaded on a 1.5 × 35 cm DEAE-Sephacel column. The column was eluted stepwise with increasing sodium chloride concentrations up to 0.5 M, pH 8.3. Absorbance was read at 280 nm. The eluate was divided into six fractions as indicated, and tubes were pooled.

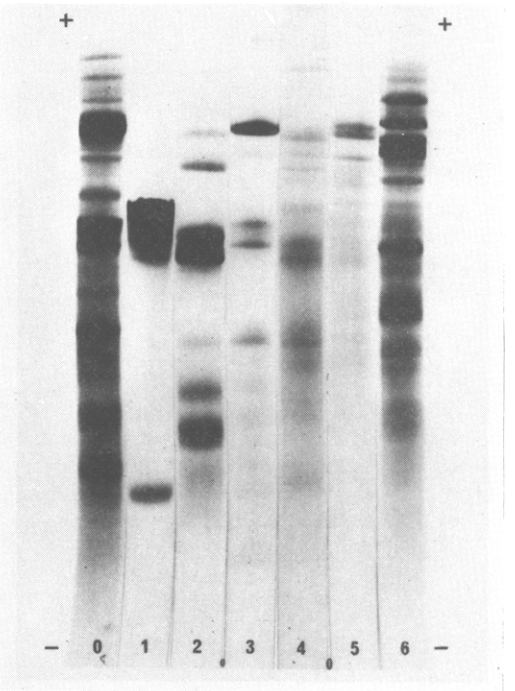

FIGURE 4: Acid–urea–6% polyacrylamide gel electrophoresis of DEAE-Sephacel column fractions. Slot 0 is the 30–60% ammonium sulfate fraction. Slots 1–6 correspond to fractions 1–6. Fraction 1 (slot 1) was selected for further purification. Gels were run at 120 V for 4.5 h and stained with 1% Buffalo Black in 7% acetic acid.

0.025–0.5 M NaCl gradient in the presence of 0.02 M sodium phosphate, pH 8 (Figure 5). The small peak (b) which eluted at an ionic strength of 0.225 contained the immunoreactivity which corresponded to the outer band of Figure 2. Peak a contained minimal immunoreactivity, and its visualization in an Ouchterlony gel required over 48 h of diffusion.

The highly antigenic peak b (400 μg) was electrofocused (Figure 6) on a modified LKB granulated gel bed (see Materials and Methods). The antigens located by Ouchterlony assay (Figure 7A) of 0.5-cm gel fractions (Figure 6, arrows 5 and 8) had p*I* values of 7.0 and 6.3. The antigen bands with p*I* values of 6.3 and 7.0 were well resolved from the major stained bands with p*I* values of 7.6, 7.2, and 6.5 (Figure 7B).

Figure 8 shows an immunoelectrophoretic analysis of the highly antigenic fraction 8 from the isoelectric focusing

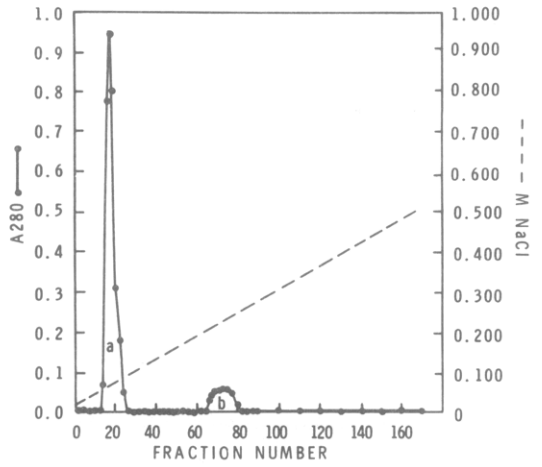

FIGURE 5: CM-Bio-Gel A elution profile of the DEAE-Sephacel flow-through fraction. Protein fraction 1 (50 mg) was dialyzed to 0.02 M sodium phosphate–0.025 M NaCl–1 mM PMSF, pH 8, and was loaded on a 2 × 30 cm CM-Bio-Gel A column. The column was eluted with a gradient from 0.025 to 0.5 M NaCl. The eluate was divided into two fractions as indicated, and the tubes were pooled.

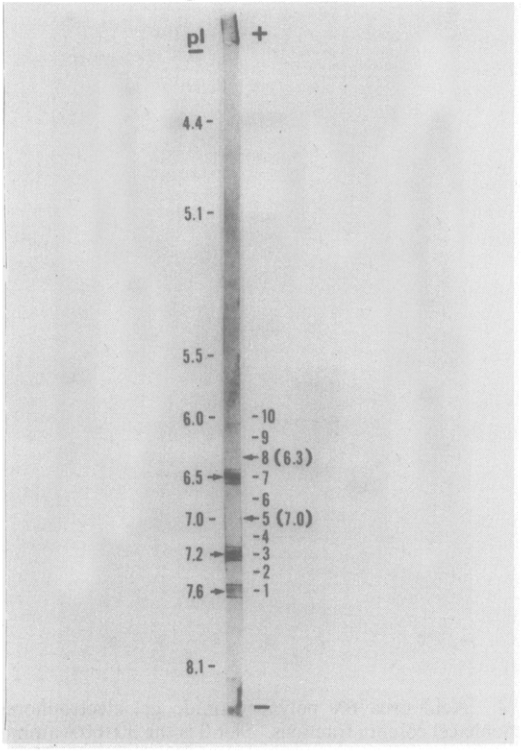

FIGURE 6: Preparative isoelectric focusing of CM-Bio-Gel A column fraction b on a pH 3.5–10 granulated gel bed. The sample was focused at 4 W and a maximum voltage of 1500 V for 18 h. The protein bands were detected by a paper map detection method which was described under Materials and Methods. The pH gradient was measured in side strips which did not contain the sample. The bands cut from the gel bed are numbered 1–10.

granulated gel bed. Slot 1 is the antigen in band 8 (Figure 6), and slot 2 is the CM-Bio-Gel A peak b (Figure 5). The highly purified antigen had the same immunoelectrophoretic migration as the antigen in peak b.

The p*I* 6.3 antigen was purified 2890-fold over the starting material (Table II). The yield was 2.3% overall and 6.9% of the 30–60% ammonium sulfate fraction. The p*I* 6.3 fraction from the preparative isoelectric focusing experiment was labeled (see Materials and Methods) with ^{125}I using the Bolton–Hunter reagent; it was further purified by gel filtration on an AcA-44 column (Figure 9).

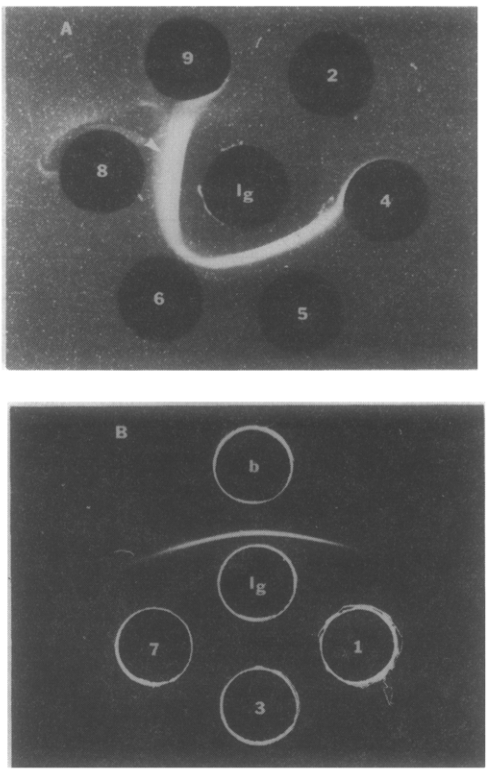

FIGURE 7: Ouchterlony double-diffusion analysis of the 10 fractions cut from the granulated gel bed of Figure 6. Panel A: 2 is fraction 2; 4 is fraction 4; 5 is fraction 5; 6 is fraction 6; 8 is fraction 8; 9 is fraction 9. Panel B: b is peak b from the CM-Bio-Gel A column; 1 is fraction 1; 3 is fraction 3; 7 is fraction 7.

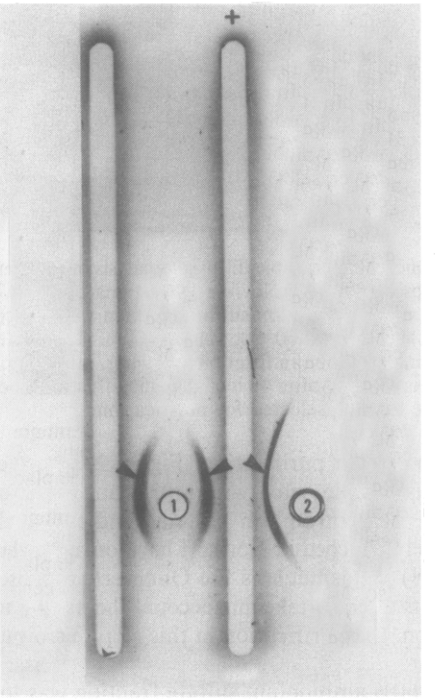

FIGURE 8: Immunoelectrophoresis of fraction 8 cut from the granulated gel bed of Figure 6. Slot 1 is fraction 8 from the granulated gel bed (p*I* 6.3). Slot 2 is CM-Bio-Gel A column peak b.

Figure 10 shows the analysis of the flow-through fraction of the AcA-44 column on a $NaDodSO_4$–polyacrylamide gel. The only major peak migrated relative to British Drug House (BDH) molecular weight standards with a molecular weight of 70 000. Isolated antigen was analyzed in an Ouchterlony

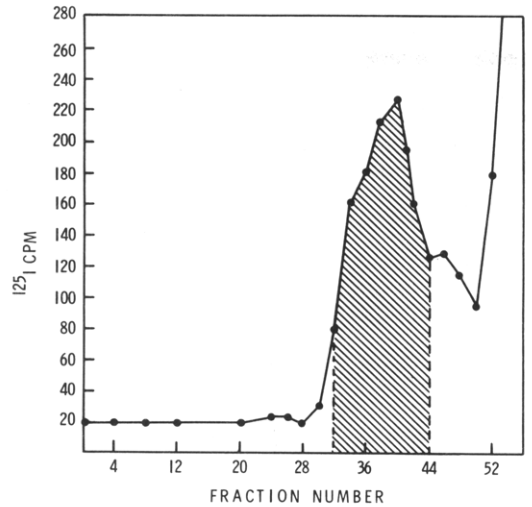

FIGURE 9: Separation of ^{125}I-labeled antigen on a 1.5 × 60 cm AcA-44 Ultrogel column. The ^{125}I-labeled flow-through fraction from Sephadex G-25 was concentrated to 1 mL, dialyzed to 0.6 M NaCl–0.01 M Tris–1 mM PMSF, pH 8.3, loaded onto the AcA-44 column, and eluted with the same buffer. The flow-through fraction was pooled as indicated.

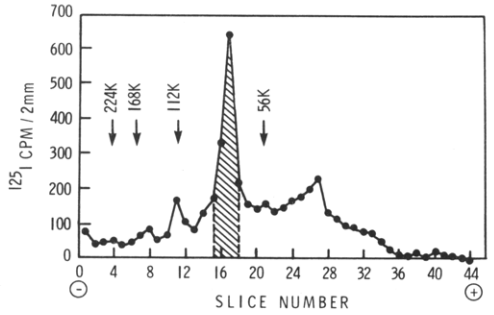

FIGURE 10: NaDodSO$_4$–5% polyacrylamide gel electrophoresis of the flow-through fraction from the AcA-44 column. See Materials and Methods for the gel run conditions. The molecular weight markers shown by the arrows at 56 000, 112 000, 168 000, and 224 000 were BDH's high molecular weight standards run on identical gels.

gel with the 0.6 M NaCl extract of Novikoff hepatoma chromatin.

Figure 11 shows an immunoprecipitation line of identity for the isolated antigen (well B) with the nuclear antigens in well A. The line of precipitation in the 0.6 M NaCl extract of nuclear chromatin corresponds in position to that of nuclear NAg-2 (Yeoman et al., 1976).

Discussion

By use of antibodies to nuclear nonhistone proteins, it was shown in this study (Figure 2) and in previous studies (Yeoman et al., 1976) that a number of antigens could be immunoprecipitated from the Novikoff hepatoma cytosol. The antigen selected for purification was detectable with immunoglobulin preabsorbed with the 0.6 M NaCl extract of normal rat liver chromatin. Successive ammonium sulfate precipitations, ion-exchange chromatography, preparative isoelectric focusing, and gel filtration effected a 2890-fold purification of the antigen. The final purified antigen was homogeneous with respect to p*I* (6.3) and to gel electrophoretic molecular weight (70 000).

Immunoprecipitation analysis of the isolated antigen showed a line of identity with the nuclear antigen NAg-2. The molecular weight, p*I*, and line of confluency indicated that the isolated antigen was nuclear antigen 2 (Yeoman, 1978; Yeoman & Busch, 1978; Yeoman et al., 1978). As an aid to analysis and as a preliminary to the development of a radioimmunoassay, the isolated antigen was labeled with ^{125}I after elution from the Ultradox granulated bed. Labeled antigen was used for further purification and for analytical polyacrylamide gel studies.

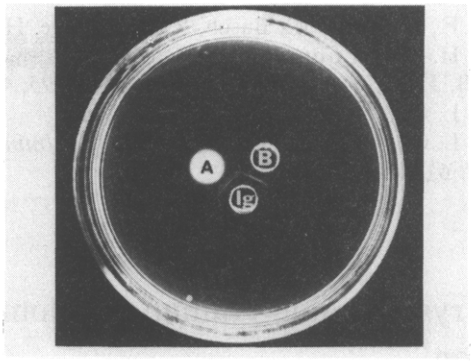

FIGURE 11: Ouchterlony gel analysis of isolated cytosol antigen and nuclear antigens. The 0.6 M NaCl extract of Novikoff hepatoma chromatin (5 mg/mL) was put in well A. Cytosol-purified antigen was put in well B (<1 μg). Immunoglobulin was put in the Ig well at 80 mg/mL. Immunoprecipitation was observed after 18 h of diffusion.

The technology employed in this purification utilized standard methods of protein chemistry. This approach was selected to maintain nativeness and to minimize the losses associated with affinity isolation techniques (Marashi et al., 1979). The antigenicity was demonstrated by immunoelectrophoresis and Ouchterlony immunoprecipitation techniques.

The isolated antigen exhibited high immunoreactivity. Its association with the Novikoff hepatoma was shown by immunoprecipitation with antiserum preabsorbed with normal liver chromatin protein. Such highly purified antigens are useful for isolation of more specific antibodies and for the increased specificity required for meaningful quantitative studies on a broad range of tumors and normal tissues. In addition, approaches similar to those employed here are directly applicable to the isolation of similar antigens from human neoplastic cells.

The detection of a cytoplasmic antigen with antibodies to nuclear proteins may result from an equilibrium that exists for some proteins which are distributed between the cell nucleus and the cytoplasm as has been described for nonhistone and cytosol proteins by Bonner (1978). Inasmuch as it is generally accepted that nuclear proteins are synthesized on cytoplasmic polysomes, this may in fact account for amounts of nuclear proteins detected in the cytoplasm.

Acknowledgments

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The Crystallizable Human Myeloma Protein Dob Has a Hinge-Region Deletion†

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ABSTRACT: During experiments to prepare heavy-metal derivatives of the crystallizable human IgG1 (κ) immunoglobulin Dob, it became apparent that this protein has several unusual features. (1) Instead of the four labile interchain disulfide bridges ordinarily found in IgG1, the Dob protein has only a single interchain disulfide bridge, which connects its two light chains. (2) The Dob heavy chain appears to be slightly smaller than a control γ1 chain, as judged by polyacrylamide gel electrophoresis in sodium dodecyl sulfate and by gel filtration in guanidine. (3) The Dob heavy chain has three fewer residues of half-cystine than expected in γ1 chains. (4) The Dob IgG is relatively resistant to digestion with papain and trypsin; however, it is readily digested with pepsin, although at an unusual site. These findings suggest that some or all of the γ1 hinge region is missing in Dob. To localize the deletion, we prepared an $F(ab')_2$ fragment consisting of two heavy-chain pieces (Fd') noncovalently associated with the light-chain dimer. The Fd' piece was isolated and digested with trypsin. The sequence of the C-terminal tryptic peptide was Val-Ala-Pro-Glu-Leu-Leu-Gly-Gly-Pro-Ser-Val. Positions 2–11 of this peptide are identical with residue positions 231–240 of the γ1 chain. The N-terminal valine could be either Val-211 or Val-215 of the γ1 sequence. A tryptic peptide, Val-Asp-Lys-Lys, was also isolated from Dob Fd'; this sequence is not found in the variable region of the Dob heavy chain [Steiner, L. A., Garcia Pardo, A., & Margolies, M. N. (1979) *Biochemistry* (following paper in this issue)] but corresponds to positions 211–214 of the γ1 constant region. Therefore, the deletion cannot include these residues and must begin after Val-215; normal γ1 sequence resumes at Ala-231. The same 15-residue deletion has been found in two other IgG1 proteins, Mcg [Fett, J. W., Deutsch, H. F., & Smithies, O. (1973) *Immunochemistry 10*, 115] and Lec [Rivat, C., Schiff, C., Rivat, L., Ropartz, C., & Fougereau, M. (1976) *Eur. J. Immunol. 6*, 545]. Possible explanations for the occurrence of identical hinge-region deletions in three different immunoglobulins are suggested by recent experiments demonstrating that the three constant domains and the hinge region of mouse γ1 chains are each encoded by separate segments of DNA [Sakano, H., Rogers, J. H., Hüppi, K., Brack, C., Traunecker, A., Maki, R., Wall, R., & Tonegawa, S. (1979) *Nature (London) 277*, 627].

Most information on the three-dimensional structure of immunoglobulins has been derived from X-ray diffraction studies of fragments obtained by proteolytic digestion [reviewed by Davies et al. (1975) and Poljak et al. (1976)]. However, to obtain information on the structural relations of the different immunoglobulin domains to each other and to gain insight into the functioning of the whole molecule, it is necessary to analyze the intact protein. Unfortunately, crystals suitable for X-ray studies have been obtained from only a few undigested immunoglobulins (Terry et al., 1968; Edmundson et al., 1970; Colman et al., 1976; Edmundson et al., 1978; Ely et al., 1978). The first of these to be investigated by crystallographic techniques was the human myeloma protein Dob, an IgG1 (κ) cryoglobulin (Terry et al., 1968). An electron density map at 6-Å resolution was consistent with a model in which the Fc fragment[1] forms the stem and the two Fab fragments form the arms of a T (Sarma et al., 1971). This interpretation was supported initially by electron micrographs of the protein crystals (Labaw & Davies, 1971) and, more recently, by a study in which domain coordinates derived from higher resolution studies of immunoglobulin fragments were fitted to the original diffraction data (Silverton et al., 1977).

Experiments were initiated in our laboratory to prepare heavy-metal derivatives of the Dob protein that might be useful for the X-ray diffraction studies. Our approach was based on earlier studies showing that divalent mercuric ions could be specifically inserted into the interchain disulfide bridges of immunoglobulins (Steiner & Blumberg, 1971). During the course of these experiments, it became clear that the covalent linkage between heavy and light chains was modified in the Dob protein. Instead of the four labile interchain disulfide

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[1] The nomenclature used for immunoglobulins is the following: γ1 is the heavy chain of the class IgG1; C_H1, C_H2, and C_H3 are domains or homology regions of the constant portions of the heavy chain; V_H is the variable domain of the heavy chain; the hinge region is the segment of heavy chain between the C_H1 and C_H2 domains; $F(ab')_2$ is a fragment, obtained by digestion with pepsin, that is composed of the two light chains and two fragments, called Fd', each comprising the N-terminal half of the heavy chain; Fab' is identical with $F(ab')_2$ but contains only one light chain and one Fd' fragment; Fab is a similar but slightly smaller fragment produced by digestion with papain; Fc is the fragment, obtained by digestion with pepsin, that is composed of the carboxy-terminal halves of the heavy chains; pFc' is a fragment, obtained by digestion with pepsin, that corresponds to the C-terminal half of Fc (the two C_H3 domains); details concerning the definition and preparation of these fragments are provided by Nisonoff et al. (1975).

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