New Biol. 230, 69.
Li, J. C. M., and Chang, P. (1955), *J. Chem. Phys. 23*, 518.
Metcalfe, J. C., Birdsall, N. J. M., Feeney, J., Lee, A. G., Levine, Y. K., and Partington, P. (1971), *Nature (London) 233*, 199.
Metcalfe, J. C., Birdsall, N. J. M., and Lee, A. G. (1972a), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 21*, 335.
Metcalfe, J. C., Birdsall, N. J. M., and Lee, A. G. (1972b), *FEBS Symp. Biomembranes* (in press).
Price, H. I., and Lewis, W. C. M. (1929), *Biochem. J. 23*, 1030.
Reiss-Husson, F. (1967), *J. Mol. Biol. 25*, 363.
Resing, H. A., and Torrey, H. C. (1963), *Phys. Rev. 131*, 1102.
Robinson, J. D., Birdsall, N. J. M., Lee, A. G., and Metcalfe, J. C. (1972), *Biochemistry 11*, 2903.
Robles, E. C., and Van den Berg, D. (1969), *Biochim. Biophys. Acta 187*, 520.
Scandella, C. J., Devaux, P., and McConnell, H. M. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 2056.
Sheard, B. (1969), *Nature (London) 223*, 1057.
Singer, S. J., and Nicolson, G. L. (1972), *Science 175*, 720.
Small, D. M. (1967), *J. Lipid Res. 8*, 551.
Taylor, R. B., Duffus, W. P. H., Raff, M. C., and de Petris, S. (1971), *Nature (London), New Biol. 233*, 225.
Tiddy, G. J. T. (1972), *J. Chem. Soc., Faraday Trans. 1, 68*, 670.
Torrey, H. C. (1953), *Phys. Rev. 22*, 962.
Trauble, H., and Sackmann, E. (1972), *J. Amer. Chem. Soc. 94*, 4499.
Van Deenen, L. L. M. (1971), *Pure Appl. Chem. 25*, 25.
Vilallonga, F. (1968), *Biochim. Biophys. Acta 163*, 290.
Vold, R. L. (1972), *J. Chem. Phys. 56*, 3210.
Watkins, C. L., and Johnson, C. S. (1971), *J. Phys. Chem. 75*, 2452.

The Binding of a Naphthalene Dye Associated with J-Chain Attachment in an Immunoglobulin A Mouse Immunoglobulin†

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ABSTRACT: Native multimeric protein 460, a γ A mouse myeloma protein, binds 8-anilinonaphthalene-1-sulfonate (ANS) with a $K_0 = 3 \times 10^5$ l./mol. Neither the 7S monomers nor the isolated J chain derived from this protein bind ANS. When 7S monomers and J chains are recombined, ANS binding is partially restored. The evidence is consistent with ANS binding to the site of the attachment of the J chain rather than to the J chain itself. Dimeric and trimeric forms of protein 460 bind 1 mol of ANS/mol of 7S monomer. ANS shows a blue shift in its fluorescence emission maximum and a 200-fold increase in fluorescence quantum yield on binding to the protein. This suggests that the binding site for ANS is relatively hydrophobic.

A new immunoglobulin polypeptide chain, designated the J or joining chain, has recently been described (Halpern and Koshland, 1970; O'Daly and Cebra, 1971; Morrison and Koshland, 1972; Parkhouse, 1972). Its role appears to be to join the subunits of IgA and IgM immunoglobulins together, to form the naturally occurring IgA dimers and trimers as well as the IgM pentamers (Morrison and Koshland, 1972). This polypeptide component has been characterized by its mobility in alkaline–urea polyacrylamide gels (Halpern and Koshland, 1970; O'Daly and Cebra, 1971; Parkhouse, 1972), and its size (O'Daly and Cebra, 1971; Morrison and Koshland, 1972). It has a different amino acid composition when isolated from human or rabbit immunoglobulins (O'Daly and Cebra, 1971; Morrison and Koshland, 1972).

We have identified a similar polypeptide chain in native protein 460 synthesized and excreted by a mouse plasmacyte tumor, MOPC 460. We have employed the technique of fluorescence enhancement (Stryer, 1965, 1968; Parker *et al.*, 1967; Green *et al.*, 1972) using the naphthalene dye 8-anilinonaphthalene-1-sulfonate as the fluorescent probe, to study the nature of the site of attachment of the J chain to protein 460.

Materials and Methods

Reagents. Reagent grade ANS[1] as the magnesium salt (Eastman) was used without further treatment. Tritium-labeled ANS was synthesized by the method of Parker and Osterland (1970) and had a specific radioactivity of 0.70 Ci/mol. ANS concentration was calculated assuming $\epsilon_M = 4.99 \times 10^3$ at 350 nm (Parker and Osterland, 1970). Dithiothreitol (Sigma Chemical Co., St. Louis) was used without further treatment. Iodoacetic acid (Aldrich Chemical Co.) was recrystallized twice from water. [^{3}H]Iodoacetic acid was supplied by New England Nuclear. The specific radioactivity was adjusted by the addition of unlabeled iodoacetic acid to 7.8 Ci/mol.

Preparation of Proteins. MOPC 460 and 315 tumor lines were generous gifts of Drs. Herman Eisen and Ernest Simms and were maintained in BALB/c mice as previously described (Rosenstein *et al.*, 1972).

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[1] Abbreviation used is: ANS, 8-anilinonaphthalene-1-sulfonate.

Protein 460, the IgA myeloma protein produced by plasmacyte tumor MOPC 460, was isolated from both the serum and ascites of tumor-bearing mice by an affinity column chromatographic method (Goetzl and Metzger, 1970) and, except where indicated in the text, the reduction and alkylation step was omitted. Protein concentration was determined by measuring the optical density, assuming $E_{1\%}^{280} = 15.0$. Protein 315 was isolated by the same procedure.

N-Terminal analysis by the dansyl chloride procedure (Bruton and Hartley, 1970) yielded the following results. Protein 460 preparations were found to have only aspartic acid (light chains) and glutamic acid (heavy chains) on the N termini. Protein 315 was found to have aspartic acid (H chains); the light chains are blocked by pyrrolidonecarboxylic acid (Schulenburg *et al.*, 1971). The analysis method is sensitive enough to pick up a 1–3% contamination by other unblocked polypeptide chains had these been present.

Protein 460 isolated from either serum or ascites yielded only two optical density peaks when reduced and alkylated and subjected to Sephadex G-100 chromatography, using 1 M propionic acid–6 M urea buffer. The first peak gave a single protein staining band when analyzed by sodium dodecyl sulfate gel electrophoresis. This band ran in the same position as a marker protein of mol wt 53,000, and therefore corresponds to protein 460 H chains. The second G-100 peak also yielded a single band when analyzed by sodium dodecyl sulfate gel electrophoresis. This band ran in the same position as an L-chain marker protein.

Purified proteins Fis and Bil, two IgM myeloma macroglobulins, were the generous gifts of Dr. William Konigsberg. These proteins were chromatographed on Sephadex G-200 columns, and only the large molecular weight peak (18 S) was collected and used. Both proteins have been used for sequence analysis studies and are known to contain a single amino acid sequence of more than 96% purity.

For preparation of Fab and Fc fragments, protein 460 was digested by papain in 0.1 M phosphate, pH 7.4, containing 0.01 M cysteine for 2 hr at 37° and the digest was chromatographed on DEAE-cellulose (Whatman DE-52) as previously described (Eisen *et al.*, 1968). Amino acid analyses were performed on a Jeol 5AH amino acid analyzer. Samples were hydrolyzed for 24 hr at 110° in evacuated sealed tubes, containing 1 ml of glass-distilled 6 N HCl and 0.01 M dithiothreitol. Equilibrium dialysis of protein 460 was performed in 100-μl Lucite chambers (Gateway Immunoserum Co., Cahokia, Ill.) (Jaffe *et al.*, 1971).

Flourescence enhancement was measured on a Farrand Mark I spectrofluorometer, at an excitation wavelength of 375 nm and an emission wavelength of 475 nm. These measurements were always performed in a 4.0-ml quartz cell of 10-mm path length in either 0.2 M Tris (pH 8.0) containing 0.075 M NaCl, or in 0.01 M phosphate (pH 7.4) containing 0.155 M NaCl. Emission spectra for quantum yield determination were corrected for instrument factors (Calvert and Pitts, 1967) in the wavelength range 400–600 nm by comparing the observed spectrum obtained with quinine sulfate in 0.1 N H_2SO_4 with its absolute spectrum (Eastman, 1967). Lamp intensities were measured by the ferrioxalate actinometry method of Parker (1968). Quantum yields were determined by the method of Parker and Rees (1960). An appropriate correction for the index of refraction of the solvents was made.

Reduction and Alkylation. Proteins 460 and 315 were incubated with 0.01 M dithiothreitol in 0.2 M Tris buffer (pH 8.0), containing 0.075 M NaCl for 2 hr at room temperature. Reduction was terminated by alkylation with the addition of neutralized iodoacetic acid to a final concentration of 0.025 M. Under these conditions the polymeric forms of the proteins are converted to the monomeric, 7S L_2H_2 unit. It has been found that these conditions do not alter the hapten binding properties of these immunoglobulins (Jaffe *et al.*, 1969, 1971). In certain experiments [^{3}H]iodoacetic acid was used for the alkylation step.

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis was performed in 8 M urea by the method of Ornstein (1964) and Davis (1964). The gels were run at a constant current of 2.5 mA/gel until the tracking dye (Bromophenol Blue) moved to within 1 cm of the bottom of the gel. The gels were stained in a solution of 0.25% Amido Black in 5:1:5 v/v methanol–acetic acid–water medium. For the detection of the J chain, the gels were overloaded with respect to other protein components (100–200-μg total protein) and stained for 12 hr at room temperature. Analytical sodium dodecyl sulfate polyacryamide gel electrophoresis was also performed in 5% acrylamide containing gels run with 0.1 M phosphate (pH 7.1), containing 0.1% sodium dodecyl sulfate (Shapiro *et al.*, 1967).

Ultracentrifugation. Analytical ultracentrifugation was performed on a Beckman Model E ultracentrifuge, equipped with schlieren optics. Centrifugations were carried out at 59,780 rpm. The sedimentation coefficients were corrected to 20° in water.

Isolation of J Chain. Either serum derived or ascites derived polymeric protein 460 was reduced and radioalkylated as described and then chromatographed on DEAE-cellulose. In a typical experiment, 25 mg of protein was chromatographed on a 1 × 10 cm DEAE-cellulose column. The 7S monomers are not retained by the column and are eluted at the buffer front. J chain is eluted by means of a linear salt gradient consisting of 300 ml of 0.2 M Tris (pH 8.0), containing 0.075 M NaCl in a mixing chamber, and 300 ml of 0.2 M Tris (pH 8.0), containing 1.0 M NaCl in the second buffer reservoir. J chain is eluted at about 0.13 M NaCl. The molecular weight of J chain, as judged by sodium dodecyl sulfate polyacrylamide gel electrophoresis, was 15,000–20,000.

Reoxidation Conditions. Protein 460 (3×10^{-6} M) was reduced for 2 hr with 0.01 M dithiothreitol. These reducing conditions, when followed by alkylation, have been shown to liberate J chains from intact immunoglobulin molecules. Protein disulfide bonds, which were reduced in the presence of 0.01 M dithiothreitol, were allowed to re-form by dialyzing the reaction mixture against three changes of buffer, pH 8.0 (0.2 M Tris containing 0.075 M NaCl) at room temperature.

G-200 Chromatography of Proteins. To determine the size distribution of protein 460, samples were chromatographed on a 4 × 50 cm column of Sephadex G-200, eluted with either 0.2 M Tris (pH 8) containing 0.075 M NaCl or phosphate-buffered saline, 0.01 M in phosphate (pH 7.4). Optical density, monitored at 280 nm, was used to locate protein fractions.

Temperature. All experiments were performed at room temperature (25°) unless otherwise stated.

Results

Isolation and Characterization of J Chain from Protein 460. When protein 460 is reduced with dithiothreitol and alkylated with iodoacetic acid and then subjected to electrophoresis on alkaline–urea polyacrylamide gels, a fast-moving protein band is observed. This band is not present when native protein 460 undergoes electrophoresis, or after the reduced and alkylated protein is subjected to chromatography on DEAE-cel-

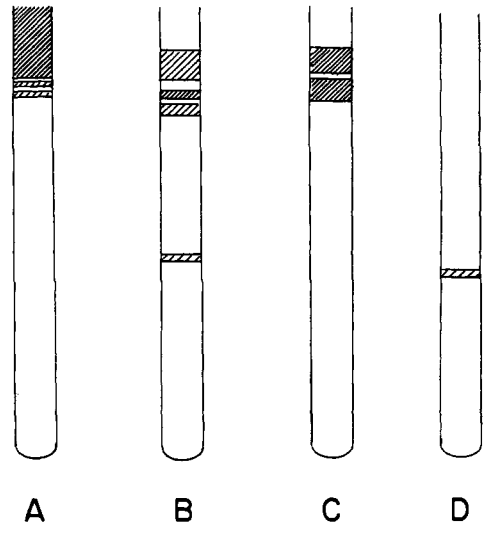

FIGURE 1: Polyacrylamide alkaline–urea gel electrophoresis of protein 460: (A) protein 460; (B) protein 460 reduced with dithiothreitol and alkylated with iodoacetic acid; (C) reduced and alkylated protein 460 chromatographed on a DEAE-cellulose column; (D) isolated "J" chain obtained by DEAE-cellulose chromatography of reduced and alkylated protein 460. The hatched areas represent protein bands stained with Amido Black. The relative intensities of the various bands are indicated by the number of lines. Only gels B and D show a "fast"-moving protein band, a characteristic property of the "J" chain.

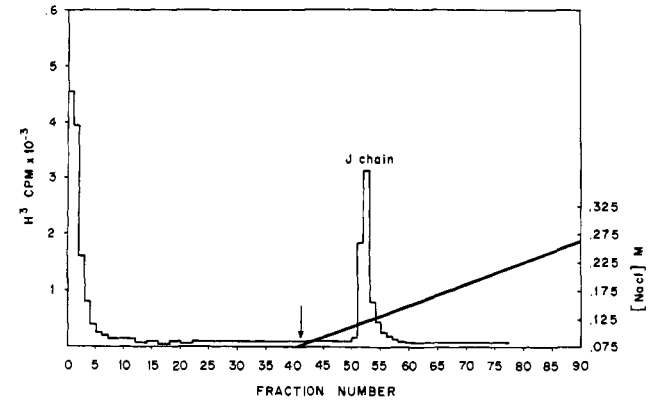

FIGURE 2: Protein 460 (5.0 mg) reduced with 0.01 M dithiothreitol and alkylated with [^{3}H]iodoacetic acid. Eluted from 1 × 10 cm DE-52 column with 0.2 M Tris buffer (pH 8.0.) 0.075 M with respect to NaCl. Vertical arrow: start of 0.075–1.0 M NaCl gradient (300 ml each compartment). The indicated gradient is calculated.

lulose, or after the protein is purified with a reduction and alkylation step included in the purification procedure (Figure 1). The electrophoretic mobility is identical with that obtained by other workers for the J, or joining chain (O'Daly and Cebra, 1971; Morrison and Koshland, 1972; Parkhouse, 1972). DEAE-cellulose chromatography was used to purify J chain from a reduced and alkylated preparation of protein 460. Figure 2 shows a typical isolation profile.

When the isolated J-chain fraction from DEAE-cellulose chromatography is subjected to electrophoresis on alkaline–urea polyacrylamide gels, it moves as a single band in the expected position (see Figure 1).

The amino acid composition of the isolated J chain is given in Table I. For comparison the amino acid compositions of 460 light and heavy chains are also included. The amino acid composition of the J chain is distinct from that of either protein 460 light or heavy chains. It is also different from the composition of J chain for man (Morrison and Koshland, 1972) and rabbit (O'Daly and Cebra, 1971). It appears, therefore, that amino acid composition of J chain is different in each of the three species so far studied.

N-Terminal analysis of isolated J chain by the dansyl method (Bruton and Hartley, 1970) yielded no detectable dansyl-amino acid. The quantity of J chain used (1.5×10^{-8} mol) was over three orders of magnitude above the detection limits of the method (1×10^{-11} mol). This is consistent with J chain possessing a blocked N terminus.

Flourescence Enhancement Studies of ANS Binding. Free ANS in aqueous solvents has a fluorescence emission maximum of 520 nm and a fluorescence quantum yield of 0.004 (Stryer, 1965). Upon addition of protein 460 there is a blue shift in the emission maximum of 45 nm and the quantum yield increases to 0.82 ± 0.02; a 200-fold increase. Figure 3 shows that the fluorescence enhancement titration of ANS in the presence of protein 460 is consistent with ANS binding to a uniform site having a K_0 of 3.0×10^5 l./mol. The value of this constant is close to the value found when ANS interacts with albumin (Weber and Young, 1964) and apomyoglobin (Stryer, 1965).

ANS binding to protein 460 that had been subjected to a reduction and alkylated step during purification, or to native 7S monomers, was less than 4% of that obtained with the multimeric protein. The inability of 7S monomers to bind ANS would suggest that ANS binding does not occur within the antibody combining sites, which are left intact by the reduc-

TABLE I: Amino Acid Composition (Residues per Molecule) of J, Heavy, and Light Chains of Protein 460.

Amino Acid	J Chain[a]	H Chain[b]	Light Chain[b]
Lys	13 ± 0.9	20	13
His	5 ± 0.4	6	5
Arg	6 ± 1.3	14	8
Asp	15 ± 0.5	37	23
Thr	10 ± 0.5	42	18
Ser	14 ± 2.0	48	27
Glu	24 ± 1.7	46	19
Pro	9 ± 0.6	40	11
Gly	14 ± 1.4	36	14
Ala	15 ± 1.2	22	8
Val	9 ± 0.04	34	13
Met	2	6	2
Ileu	5 ± 0.4	17	7
Leu	15 ± 1.9	44	17
Tyr	6 ± 0.3	21	7
Phe	7 ± 0.4	11	10
Molecular weight	18,500		

[a] Values are an average of two separate determinations. Values were calculated by dividing amino acid residues found by the number of methionine residues. The molecular weight was calculated without including cysteine and tryptophan, which were not determined. This molecular weight is in the range previously found for J chain (15,000–24,000) (O'Daly and Cebra, 1971; Morrison and Koshland, 1972). [b] Taken from Jaffe *et al.* (1971).

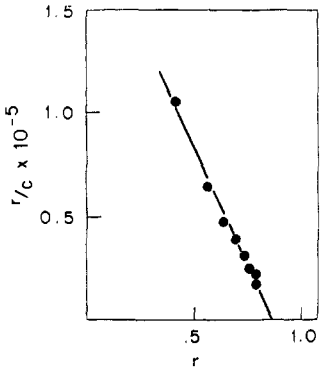

FIGURE 3: Scatchard plot of ANS binding to protein 460 (preparation S-4); r = number of molecules of ANS per calculated 7S monomer, obtained from fluorescence enhancement data; c = concentration of ANS, mol/l. The number of sites at saturation was determined by equilibrium dialysis.

TABLE II: Correlation of ANS Fluorescence and J-Chain Quantity in Preparations of Protein 460 Purified from Ascites and Serum.

Protein 460 Sample	ANS[a] Fluorescence	ANS Fluorescence Ratio Ascites/Serum	J Chain[b] (cpm of ^{3}H)	J Chain cpm Ratio Ascites/Serum
S-4				
Ascites	15.4		39,387	
		1.40		1.58
Serum	11.0		24,833	
S-8				
Ascites	135		42,192	
		1.73		1.80
Serum	78		23,513	

[a] ANS fluorescence at saturation was measured in arbitrary units. [b] J chain was isolated from reduced and radioalkylated protein 460 by DEAE-cellulose chromatography.

tion and alkylation step used to produce monomers (Jaffe *et al.*, 1971; Rosenstein *et al.*, 1972). This was confirmed by preparing Fab and Fc fragments by papain digestion. Equilibrium dialysis with radioactive ANS showed that it was bound to neither fragment. The Fab fragments have previously been demonstrated to bind the haptens ε-dinitrophenyllysine and menadione (Jaffe *et al.*, 1971; Rosenstein *et al.*, 1972).

Correlation of ANS Binding with J-Chain Attachment. Approximately 20% of mice inoculated with MOPC 460 plasmacytoma cells develop an ascitic form of the tumor.

Protein 460 isolated from the ascites of these mice was compared to the protein isolated from the serum for ANS binding. It was found that the ascites derived protein 460 has a greater fluorescence enhancement than serum 460, indicating a greater number of ANS binding sites. The ratio of ANS fluorescence of ascites derived protein over serum derived protein closely correlated with the ratio of J chain isolatable from the ascites protein over the serum protein. This can be seen in Table II. The amount of J chain was quantitated by measuring the amount of radiolabeled alkylating agent incorporated into J chain during the reduction and alkylation step of the purification. We assume that the specific radioactivity of pure J chain is uniform when it is derived from protein isolated from serum or ascites under identical conditions. Isolated J chains were found to bind ANS at a level of less than 5% of that of native 460 molecules.

ANS binding could, however, be partially reconstituted by allowing a reduced protein 460 preparation to reoxidize. This can be seen in Figure 4. In this experiment reducing agent was removed and the reaction mixture exposed to air. About 30% of the original ANS binding was restored. Reduced 7S monomers, isolated by DEAE-cellulose chromatography run in the presence of 0.005 M dithiothreitol, showed no ANS binding when reoxidized in the absence of J chain.

Number of ANS Binding Sites. The number of ANS binding sites was determined by equilibrium dialysis employing [^{3}H]ANS. Three protein 460 samples were examined: S-4 serum (0.80), S-8 serum (0.72), and S-8 ascites (0.95). The numbers in parentheses are the number of ANS binding sites per mole of 7S monomer. The number of ANS binding sites for the serum samples is significantly lower than 1.0.

When one of these serum samples (S-4) was subjected to chromatography on Sephadex G-200, two fractions were obtained. The first fraction, representing 80% of the applied protein 460, when subjected to analytical ultracentrifugation, was found to be composed of two sedimenting species, one of 10.4 S and the other of 13.6 S. These sedimentation values are consistent with this fraction being composed of both dimers and trimers.

The second fraction from the G-200 chromatography, representing 20% of the applied protein 460, was found to contain monomers only.

The monomer fraction bound no ANS and was found to have no J chain as judged by analytical polyacrylamide gel electrophoresis. The multimer fraction bound ANS. The number of ANS binding sites per monomeric unit calculated for this fraction was 1.06. The observed number of sites per monomeric unit for the S-4 serum preparation, 0.8, can therefore be accounted for by correcting the observed value for the amount of monomer present.

These data indicate that the multimeric forms of protein 460 appear to have only one ANS binding site per monomeric unit, irrespective of whether the multimer is a dimer or a trimer. Neither monomers derived from native, unreduced populations nor monomers prepared by reduction of polymers contain J chain or bind ANS.

ANS Binding to Other Immunoglobulins. Three additional myeloma immunoglobulins were tested for their ability to bind ANS. Protein 315, an IgA mouse myeloma protein, bound ANS in a similar manner to protein 460. Preliminary evidence substantiates the observation that ANS binding is dependent on the presence of a bound J chain. This immunoglobulin appears to bind 1 mol of ANS/calculated monomeric unit.

Two human IgM macroglobulins, Bil and Fis, both of which were found to have J chains when tested by polyacrylamide gel electrophoresis were also examined for ANS binding. Bil did not bind ANS, but Fis showed fluorescence enhancement. The ANS binding by protein Fis, quantitated by equilibrium dialysis employing [^{3}H]ANS, showed 2 mol of ANS bound per calculated mol of monomeric unit.

Discussion

We have presented evidence showing that the fluorescence

of ANS is greatly enhanced when it is bound to native protein 460. Protein 460 was found to have a J chain, presumably attached by disulfide bonds. If the J chain is removed from protein 460 by reduction and alkylation, followed by chromatography on DEAE-cellulose, ANS no longer binds. Isolated J chains do not bind ANS. Monomeric 7S units of protein 460, and Fab or Fc fragments derived from them, also do not bind ANS. When protein 460 is reduced under conditions which separate the J chain from the rest of the molecule, and it is then reoxidized by removal of the reducing agent and exposure to air, it regains about 30% of its ability to bind ANS.

There are two possible explanations for these data. One possibility is that ANS binds either to J chain or to the H_2L_2 monomer. Dissociation of the J chain could produce a conformational change in either fragment which abolishes ANS binding. A second explanation is that ANS binds to the site where J chain attaches to the 7S monomer, and that this attachment site is destroyed by the separation of the chains. There is no easy way to determine whether ANS binds to one or more than one chain.

The binding of ANS to protein 460 is accompanied by a blue shift of 45 nm and an increase in fluorescence quantum yield from 0.004 to 0.82. The blue shift and the quantum yield indicate that the ANS binding site is probably hydrophobic. The dependence of the quantum yield and emission maximum of ANS on the polarity of the environment was shown by Stryer (1965). It must be pointed out, however, that restriction of solvent relaxation in a polar environment may also cause similar fluorescence changes (Ainsworth and Flanagan, 1969).

In addition to protein 460, several other multimeric myeloma proteins were tested for ANS binding. Our preliminary evidence shows that all three (protein 315, an IgA protein; Fis, an IgM protein; Bil, an IgM protein) have an attached J chain. However, only two of these (protein 315 and protein Fis) bind ANS. Clearly, therefore, not all J-chain-containing immunoglobulins bind ANS.

We do not know, however, whether the J-chain attachment site is in the same location on all γA and γM immunoglobulins. A difference of this type or perhaps some more subtle alteration in conformation could be responsible for absent ANS binding in some immunoglobulins. However, ANS was the only fluorescent probe tested, and it is possible that other fluorescent probes (McClure and Edelman, 1967; Parker *et al.*, 1967) might bind.

It has been shown (O'Daly and Cebra, 1971; Morrison and Koshland, 1972) that multimeric immunoglobulins possess only one J chain, irrespective of the size of the polymer. There appears to be only one ANS binding site per monomeric unit in protein 460, and two ANS sites per monomeric unit in protein Fis. This could also reflect different modes of attachment of the J chain to the momomeric units. In IgM molecules (Fis), J chain is probably attached via both μ chains (Morrison and Koshland, 1972). Each μ chain–J chain interaction could form one ANS binding site. For protein 460 only one α chain may be involved on the J-chain attachment. We expect that a cysteine disulfide bridge connects the α and J chains. An analogous link has been described in IgM molecules (Morrison and Koshland, 1972). If only one of the two α chains were involved in J-chain attachment, then one sulfhydryl group (on the unattached α chain) would be expected to be unreacted. There is evidence in the case of protein 460 and several other mouse IgA myeloma proteins that there are unreacted sulfhydryl groups associated with the α chains (Rosenstein *et al.*, 1972).

The binding of ANS associated with J-chain attachment in protein 460 may yield information about the J chain–heavy chain interaction. The technique of resonance energy transfer (Stryer and Haugland, 1967; Steinberg, 1971; Werner *et al.*, 1972) could be used to measure the distance between the ANS binding site (presumably the site of attachment of the J chain) and the combining sites of the immunoglobulin. Using ANS as the energy donor, and attaching an appropriate fluorescent acceptor to a hapten capable of binding at the active site, the transfer of energy could be measured and hence, the distance between acceptor and donor could be calculated.

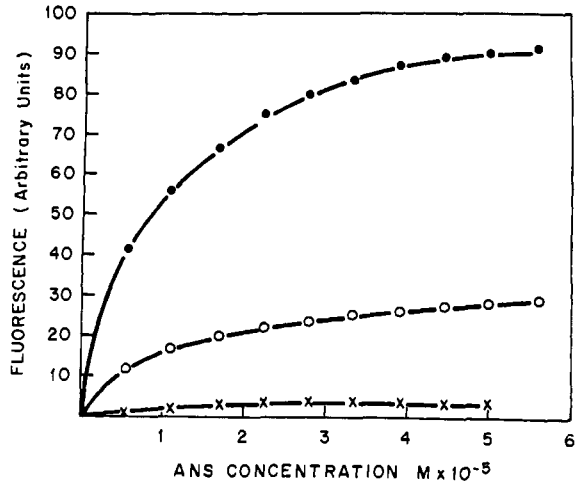

FIGURE 4: Plot of fluorescence as a function of ANS concentration for native polymeric protein 360, preparation S-8 derived from ascites (●); reduced 7S monomers allowed to reoxidize by dialysis for 24 hr against three changes of 0.2 M Tris (pH 8.0) buffer containing 0.075 M NaCl at 4° (×); and reduced 460 allowed to reoxidize under the same conditions in the presence of reduced J chain. J-chain concentration was not measured directly, but is estimated to be between 1 and 2×10^{-6} M (○). Protein concentration was 3×10^{-6} M based on the 7S monomers.

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References

Ainsworth, S., and Flanagan, M. T. (1969), *Biochim. Biophys. Acta 194*, 213.

Bruton, C. J., and Hartley, B. S. (1970), *J. Mol. Biol. 52*, 165.

Calvert, J. G., and Pitts, J. M. (1967), Photochemistry, New York, N. Y., Wiley, p 798.

Davis, D. J. (1964), *Ann. N. Y. Acad. Sci. 121*, 404.

Eastman, J. W. (1967), *Photochem. Photobiol. 6*, 55.

Eisen, H. N., Simms, E. S., and Potter, M. (1968), *Biochemistry 7*, 4126.

Goetzl, E. J., and Metzger, H. (1970), *Biochemistry 9*, 1267.

Green, A. M., Marshall, J. S., Pensky, J., and Stanbury, J. B. (1972), *Science 175*, 1378.

Halpern, M. S., and Koshland, M. E. (1970), *Nature (London) 228*, 1276.

Jaffe, B. M., Eisen, H. N., Simms, A., and Potter, M. (1969), *J. Immunol. 103*, 872.

Jaffe, B. M., Simms, E. S., and Eisen, H. N. (1971), *Biochemistry 10*, 1693.
McClure, W. O., and Edelman, G. M. (1967), *Biochemistry 6*, 559.
Morrison, S. L., and Koshland, M. E. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 124.
O'Daly, J. A., and Cebra, J. J. (1971), *Biochemistry 10*, 3843.
Ornstein, L. (1964), *Ann. N. Y. Acad. Sci. 121*, 321.
Parker, C. A. (1968), Photoluminescence of Solutions, Amsterdam, Elsevier, p 208.
Parker, C. A., and Rees, W. T. (1960), *Analyst 85*, 587.
Parker, C. W., and Osterland, C. K. (1970), *Biochemistry 9*, 1074.
Parker, C. W., Yoo, T. J., Johnson, M. C., and Godt, S. M. (1967), *Biochemistry 6*, 3408.
Parkhouse, R. M. E. (1972), *Nature (London) 236*, 9.
Rosenstein, R. W., Musson, R. A., Armstrong, M. Y. K., Konigsberg, W. H., and Richards, F. F. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 877.
Schulenburg, E. P., Simms, E. S., Lynch, R. G., Bradshaw, R. A., and Eisen, H. N. (1971), *Proc. Not. Acad. Sci. U. S. 68*, 2623.
Shapiro, A. L., Viñuela, E., and Maziel, J. (1967), *Biochem. Biophys. Res. Commun. 28*, 815.
Steinberg, I. Z. (1971), *Ann. Rev. Biochem. 40*, 83.
Stryer, L. (1965), *J. Mol. Biol. 13*, 482.
Stryer, L. (1968), *Science 162*, 526.
Stryer, L., and Haugland, R. P. (1967), *Proc. Nat Acad. Sci. U. S. 58*, 719.
Weber, G., and Young, L. B. (1964), *J. Biol. Chem. 239*, 1415.
Werner, T. C., Bunting, J. R., and Cathou, R. E. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 795.