Purification and Binding Properties of Nurse Shark Antibody*

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ABSTRACT: Antibodies to the 2,4-dinitrophenyl haptenic group were elicited in nurse sharks immunized with 2,4-dinitrophenylhemocyanin. 18S and 7S immunoglobulin antibodies were obtained by antibody purification with a 2,4-dinitrophenyl immunoadsorbent. Equilibrium dialysis binding studies with [ε-^{3}H]2,4-dinitrophenyl-L-lysine resulted in valences of five for the 18S molecule and one for the 7S molecule. Both molecular forms possessed similar association constants and gave the same degree of fluorescence quenching with the homologous ligand. Ligand bound to both the 18S and 7S molecules exhibited a spectral shift, similar but qualitatively different from the shift obtained with mammalian anti-2,4-dinitrophenyl antibodies. On the basis of binding data it is suggested that the 7S molecule is either a synthetic precursor or a breakdown product of the 18S antibody molecule.

Antigen binding studies with various IgM[1] immunoglobulins have raised questions regarding the number of combining sites per antibody molecule (valence). Pentavalency of human IgM had been inferred from studies by Franklin *et al.* (1957) working with sera of patients afflicted with rheumatoid arthritis. Onoue *et al.* (1965) measured a valence of five (based on a molecular weight of 900,000) with rabbit IgM specifically purified to the azobenzene arsonate heptenic group. Following this report several groups verified five antigen binding sites per IgM molecule (Lindqvist and Bauer, 1966; Voss and Eisen, 1968; Clem and Small, 1968; Frank and Humphrey, 1969; Schrohenloher and Barry, 1968).

Merler *et al.* (1968) measured ten combining sites with a human IgM antibody to the *Salmonella typhi* O 12 antigen. The ten active sites were measured by equilibrium dialysis with a tetrasaccharide ligand derived from the somatic antigen of *S. typhi*. An average intrinsic association constant of 2.7×10^4 l./mole was measured for a relatively homogeneous set of sites. Onoue *et al.* (1968) in a subsequent report measured ten binding sites per molecule of IgM antibody (rabbit) specific for the 1-azonaphthalene-4-sulfonate haptenic group. Binding data suggested that the affinity for hapten in five of the sites was 100 times greater than a second set of five weaker sites. Stone and Metzger (1968) measured the binding properties of a Waldenstrom IgM antibody to the IgG antigen and found the subunits to be potentially divalent but functionally univalent. Ashman and Metzger (1969) recently reported a valence of ten for a human myeloma IgM with demonstrable antibody activity to the 2,4-DNP group.

Because of these diverse results it has become imperative to study the IgM molecule in depth. The shark provides a model system for this approach because of the existence of 18S and 7S IgM antibody protein (Clem *et al.*, 1967) and the apparent lack of 7S IgG. In addition, adequate amounts of antibody can be obtained thereby alleviating the quantitative problems associated with antigen-induced IgM antibody studies.

Materials and Methods

Preparation of Nurse Shark Antisera. Nurse sharks, weighing 15–20 kg, were maintained in tidal sea water in Bimini Bay. Immunizations and bleedings were accomplished as previously described (Clem and Small, 1967). Sharks were anesthetized with ethyl *m*-aminobenzoate methanesulfonic acid (Sigma Chemical Co.) for both immunization and bleeding.

Dinitrophenylated antigen was prepared as described by Eisen (1964). Keyhole limpet hemocyanin or *Limulus polyphemus* hemocyanin (Pacific Bio-Marine Supply Co.) was dissolved in water (10–20 mg/ml) and an equal weight of K_2CO_3 and 2,4-dinitrobenzenesulfonic acid, sodium salt (Eastman Organic Chemicals), was added. Reactions proceeded for 24 hr at 37° in the dark. Reaction mixtures were passed through Dowex 1-X8 (J. T. Baker Chemical Co.) equilibrated in water to remove dinitrophenolate and dinitrobenzenesulfonate anions. Based on an assumed molecular weight of 1×10^6, about 200 DNP groups were substituted per mole of hemocyanin. Sharks received 1–1.5 mg of antigen in complete Freunds adjuvant per pound of weight.

Preparation of Immunoadsorbent. DNP immunoadsorbent was prepared as described by Robbins *et al.* (1967) and modified according to the method of Gallagher and Voss (1969).

Radioimmunoelectrophoresis. Glass slides were coated with 1% Ionagar in sodium barbital buffer (pH 8.6, $\mu = 0.1$). Each slide contained samples of normal shark and the antibody fraction to be analyzed. Samples were electrophoresed for 1 hr at 10 V/cm (5–8 mA). After electrophoresis rabbit

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[1] The nomenclature used for immunoglobulins is that adopted by the World Health Organization and summarized in *Bull. World Health Organ.*, *30*, 447 (1964).

antishark whole serum was used for development of precipitin lines. [^{125}I]DNP_2-RNase was added to the trough after diffusion of the antiserum. Barbital buffer was subsequently added to maintain solubility of all components.

Slides were developed in a moist atmosphere for 20–24 hr and washed in 0.05 M PO_4 (pH 8.0) to remove nonspecific protein, followed by distilled water to remove salts. Slides were dried under a hot stream of air. X-Ray film (Eastman Kodak Royal Blue) was placed in contact with the slides for 24–48 hr before development.

Preparation of [^{125}*I*]*DNP-RNase.* DNP_2-RNase was labeled by the ICl method of McFarlane (1958). Carrier-free [^{125}I]Na (Iso/Serve Division of Cambridge Nuclear Corp.) was diluted with ICl in proportions designed for 10 iodine groups to be substituted per mole of protein. Unreacted ^{125}I was removed by extensive dialysis. The labeled product was 95% precipitable by 5% trichloroacetic acid with a specific activity of 19.5×10^6 cpm/mg.

Polyacrylamide Gel Disc Electrophoresis. Disc gel electrophoresis was performed at pH 9.5 according to the method of Ornstein (1964) and Davis (1964). Gels (7.5%) were electrophoresed at 4 mA/tube for 2 hr at room temperature and 100–200-μg samples of protein were applied per tube.

Equilibrium Dialysis. Equilibrium dialysis chambers were prepared with 0.05 ml of purified shark anti-DNP antibody and 0.05 ml of [ϵ-^{3}H]DNP-L-lysine (ligand) separated by dialysis membrane. All experiments included two control chambers containing ligand and buffer for equilibration measurements. Chambers were equilibrated for 22–24 hr at 5–6°. After equilibration 0.025-ml samples of protein and ligand were placed in 8.0 ml of Bray's (1960) scintillation fluid. Samples were counted in a Nuclear-Chicago liquid scintillation spectrometer.

Preparation of [ϵ-^{3}*H*]*DNP-L-lysine.* [^{3}H]2,4-Dinitrofluorobenzene (New England Nuclear, 1.55 Ci/mmole in benzene) was reacted with a 50-fold weight excess of α-*t*-Boc-L-lysine (Pierce Chemical Co.) in 0.05 M potassium phosphate (pH 8.0) as described by Eisen *et al.* (1968). After reacting for 24 hr at 37° the contents were dried and the acid-labile *t*-Boc group was removed with 1 N HCl in glacial acetic acid. The dried mixture was dissolved in water and extracted with ether. Further purification and identification was accomplished by thin-layer chromatography on silica gel in water-saturated methyl ethyl ketone. The R_F (1.8) of the radioactive ϵ-DNP-L-lysine was identical with that of a reference compound obtained from Pierce Chemical Co. The labeled product, diluted properly with unlabeled ϵ-DNP-L-lysine, was used as a ligand in equilibrium dialysis studies.

Fluorescence Quenching. Fluorescence quenching measurements were performed with an Aminco-Bowman spectrophotofluorometer, according to the method of Eisen (1964).

Passive Hemagglutination Test. Antibodies were assayed by the passive hemagglutination reaction with DNP-bovine γ-globulin and DNP-hemocyanin-coated, tanned sheep erythrocytes (Stavitsky, 1954). Naturally occurring shark hemagglutinins for sheep erythrocytes were removed by adsorption with sheep cells.

Ultracentrifugation. Analytical ultracentrifugation was performed with a Spinco Model E ultracentrifuge as previously described (Gallagher and Voss, 1969).

Rabbit Anti-Nurse Shark Sera. Albino rabbits were immunized with 2 ml of normal nurse shark sera in complete Freunds adjuvant. Rabbits were hyperimmunized with 2.0 ml in adjuvant after 60 days. Bleedings were obtained 10–14 days after the second immunization.

Spectral Shift Measurements. Difference spectra were obtained as described by Little and Eisen (1967).

Results

Purified anti-DNP antibodies were obtained from nurse shark sera by immunoadsorption as described in Materials and Methods. In general, sharks responded poorly to the immunogen but shark 4336, in particular, produced up to 200 μg of purified anti-DNP antibody/ml of sera. One adsorption yielded close to 90% of the total purifiable antibody. Readsorption of the serum supernatant yielded 10% and a third adsorption gave less than 1% of the total antibody. These percentages were characteristic if an excess of immunoadsorbent was used based on the measured capacity of the adsorbent (Gallagher and Voss, 1969). Purified antibody from the first and second adsorptions was pooled and represented the total antibody obtained in micrograms per milliliter of serum adsorbed. Serum supernatants after adsorption with DNP immunoadsorbent agglutinated hemocyanin-coated sheep erythrocytes.

Control adsorptions of normal shark serum resulted in less than 10 μg/ml of adsorbable protein (presumable nonspecific).

Shark sera generally contain natural agglutination and hemolysin activity against sheep erythrocytes. Normal nurse shark serum possessing an agglutination titer of 1500/ml was adsorbed with the DNP immunoadsorbent and the eluted protein was assayed for agglutinating activity. No titer was detectable suggesting that the adsorbent was selecting for only DNP-specific antibody.

Purity of the shark antibody was verified by radioimmunoelectrophoresis. Figure 1 shows that the eluted antibody from the Dowex 1-X8 column gave a diffuse band with electrophoretic mobility characteristic of IgM. Precipitin patterns with whole shark serum indicated a heavy line developed with rabbit anti-whole shark serum in the IgM region and a component migrating toward the cathode similar to mammalian IgG. Purified shark antibody always showed IgM mobility and never cathodic migration. The serum component exhibiting a mobility similar to mammalian IgG has been tentatively identified as transferrin by Clem *et al.* (1967) and is not considered an immunoglobulin. Figure 1 shows that the IgM antibody reacted specifically with [^{125}I]DNP_2-RNase antigen. Controls with normal shark serum or purified normal shark IgM developed distinct precipitin lines but did not bind radioactive antigen.

Unlike immunoelectrophoresis, disc gel electrophoresis of purified shark antibody revealed two components. One component diffused into the gel with a mobility comparable with rabbit 7S IgG (purified anti-DNP) as shown in Figure 2. The second component showed minimal diffusion into the gel suggesting a large molecular weight (18 S). These proteins were analyzed on gels containing 6 M urea and dentical results were obtained. This suggested that the heavy component was not aggregated 7S material. To verify the distribution of components by molecular size the purified antibody was applied to a Bio-Gel P-200 column (110 × 2 cm) equilibrated in 0.05 M PO_4 (pH 8.0). Figure 3 shows the elution

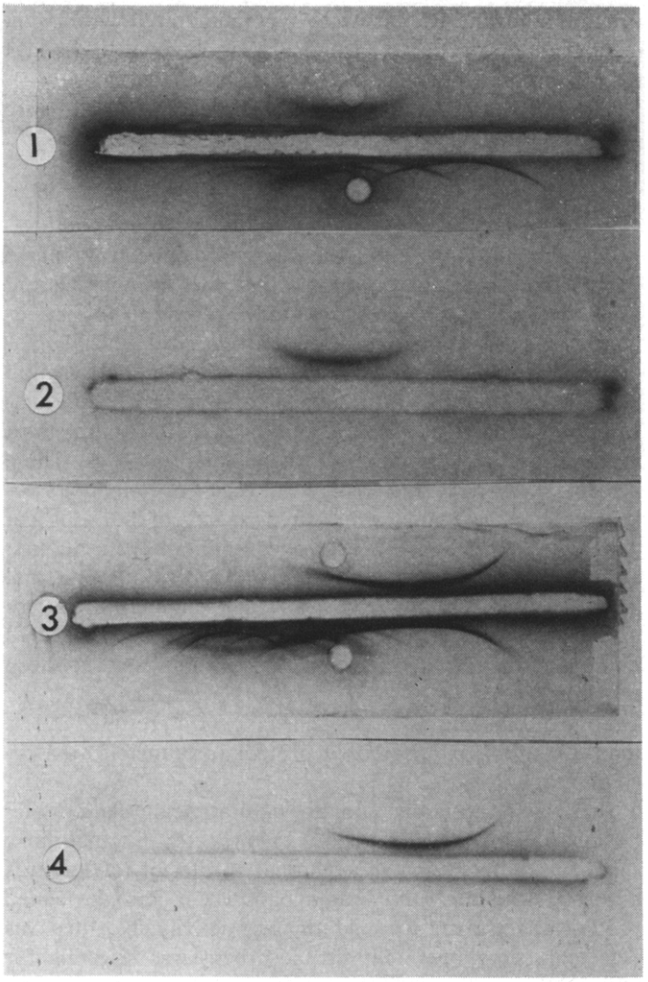

FIGURE 1: Radioimmunoelectrophoresis of purified nurse shark and rabbit anti-DNP antibodies after Dowex 1-X8 chromatography. Slide 1, precipitin patterns of purified shark anti-DNP (top, A_{278} 6.0) and normal shark serum (bottom). Rabbit anti-nurse shark serum and [^{125}I]DNP_2-RNase was applied to the central trough after electrophoresis (230 V, 5 mA, 50 min). Slide 2, 48-hr X-ray developed from slide 1 showing binding of radioactive antigen. Slide 3, precipitin patterns of purified rabbit anti-DNP (top, A_{278} 5.0) and normal rabbit serum (bottom). Goat anti-rabbit serum and [^{125}I]-DNP_2-RNase were applied as described above. Slide 4, 48-hr X-ray of slide 3.

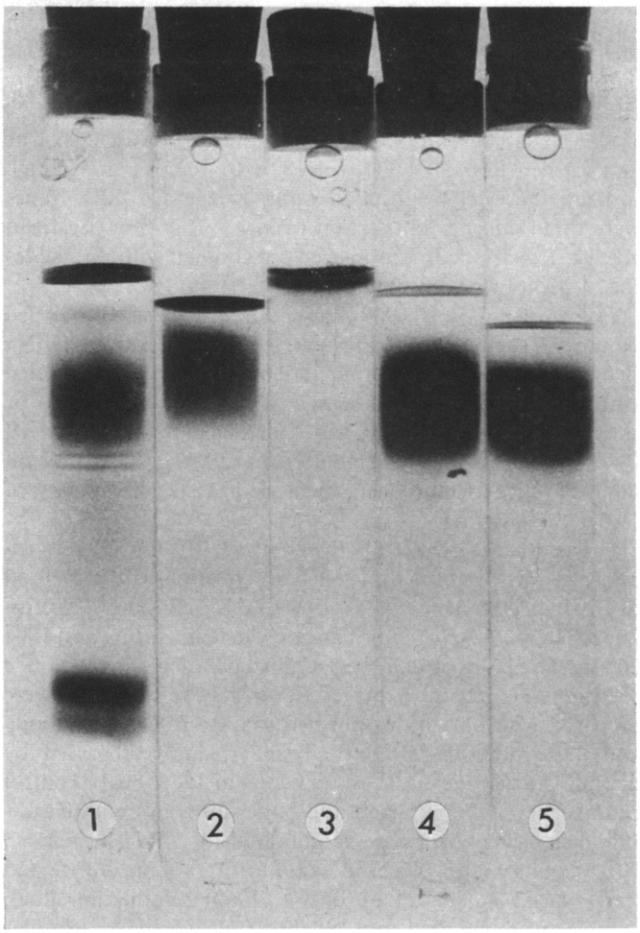

FIGURE 2: Disc gel electrophoresis patterns of whole nurse shark serum (tube 1), purified shark anti-DNP antibodies after Dowex 1-X8 chromatography (tube 2), 18S purified shark anti-DNP antibody from a Bio-Gel P-200 column as described in Figure 3 (tube 3), 7S shark anti-DNP antibody from P-200 column (tube 4), and purified rabbit anti-DNP IgG antibody (tube 5). Gels were electrophoresed at 4 mA/tube for 2 hr at room temperature.

TABLE 1: Comparative Q_{max} Determinations[a] for Purified 18S and 7S Nurse Shark Anti-DNP with Purified Antibody from Other Species.

Species	n[b]	Q_{max}	K_0[c]
Shark 18 S	5	35	2×10^5
Shark 7 S	1	35	2×10^5
Chicken 7 S	2	85	2×10^6
Rabbit 7 S	2	72	1×10^6

[a] Based on fluorometric titrations with ε-DNP-L-lysine (A_{360} 5.00) and purified antibody (A_{287} 0.100). [b] Valence or the limiting value for r at infinite c. [c] Average intrinsic association constant expressed as liters per mole.

pattern. It should be noted that all bleedings did not yield the distribution of the two molecular forms (18 and 7 S) indicated in Figure 3.

Both components isolated from Bio-Gel P-200 were analyzed in the ultracentrifuge for purity and estimation of molecular size. Figure 4 shows schlieren pat erns obtained from the first and second peaks of the column. The first peak indicated a sedimentation coefficient of 18 S and the second peak 7 S. Both components appear pure as shown in Figure 4.

Binding Properties. Valence and the average intrinsic association constant, K_0, were measured by equilibrium dialysis. Figure 5 shows the results of equilibrium dialysis binding studies for the purified 18S and 7S components. A valence of five was measured for 18 S and a valence of one for 7 S. Both proteins were at least 90% active when tested by readsorption to the DNP immunoadsorbent. It is important to note that the 18S and 7S species showed similar binding constants (2×10^5 l./mole). Sips' plot of these data indicated a heterogeneity index, a, of 0.5.

Equilibrium dialysis binding studies were verified by fluorescence-quenching studies. Figure 6 shows that the

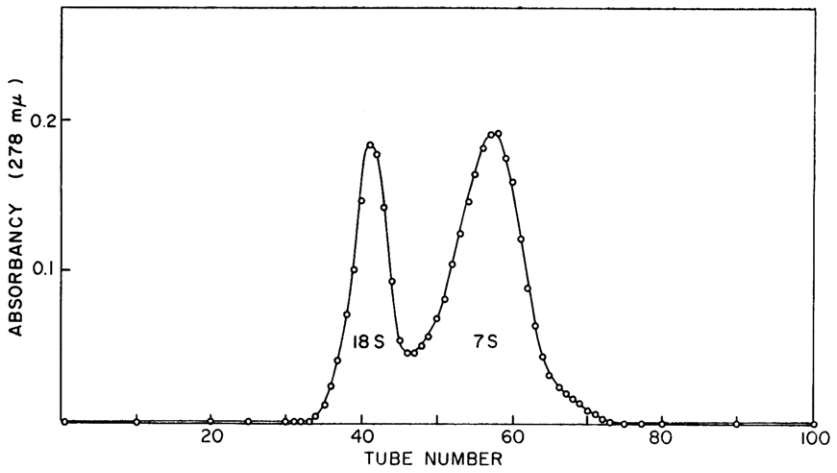

FIGURE 3: Molecular seive chromatography of purified nurse shark anti-DNP antibodies. Bio-Gel P-200 was equilibrated in 0.05 M potassium phosphate buffer (pH 8.0) and packed to a bed volume of 110 × 2 cm. Fractions (1 ml) were collected at a drop rate of 1 drop/10 sec. Column was calibrated with Blue Dextran (average molecular weight 2 × 10^6) and the void volume corresponded to tube 32 shown above. The total recovered protein was greater than 95%. As a control, iodinated ^{125}I shark antibody was applied to the same column and greater than 98% of the radioactivity was recovered.

18S and 7S components gave identical titration curves. Since the degree of quenching is directly related to K_0 this supports the previous binding data which indicated closely related association constants.

Q_{max} studies are presented in Table I. Again, it is interesting to note that both 18 and 7 S gave Q_{max} values of 35% with a high ligand concentration. Control experiments were performed with purified rabbit and chicken anti-DNP antibodies. Q_{max} values for rabbit and chicken were about two times higher than shark antibody.

Passive Hemagglutination and Precipitation Studies. The multivalent 18S molecule and the univalent 7S molecules were assayed for their agglutinating activities in the passive hemagglutination assay. The 18S molecule was capable of agglutinating DNP-coated erythrocytes at a concentration as low as 1 μg/ml. The 7S molecule did not agglutinate at concentrations up to 1.0 mg/ml. The agglutination of DNP-coated erythrocytes by the 18S antibody molecule was inhibited by concentrations of ε-DNP-L-lysine in the range of 10^{-4} and 10^{-5} M. Partial inhibition of 18S agglutination was observed by the addition of univalent 7 S.

The 7S molecules did not precipitate with DNP proteins in either gel diffusion or capillary precipitin tests. Precipitin tests were run in various salt (NaCl) concentrations up to 2.0 M to see if the immune precipitation of shark antibody was salt dependent as described for fowl antibody (Hektoen, 1918). Precipitation with 7 S was not observed as shown in Figure 7. In Figure 7, shark 7 S appeared to inhibit rabbit anti-DNP precipitation with DNP-antigen as evidenced by the clear zone around the center well. 18 S appeared to precipitate very weakly at physiological conditions and high concentrations of antibody protein.

Thus, the lack of hemagglutination and precipitation by 7 S further supports the univalence determined by equilibrium dialysis.

Spectral Shift. Little and Eisen (1967) showed previously that bound DNP ligands exhibit spectral changes with induced anti-DNP antibodies. Since these spectral changes were

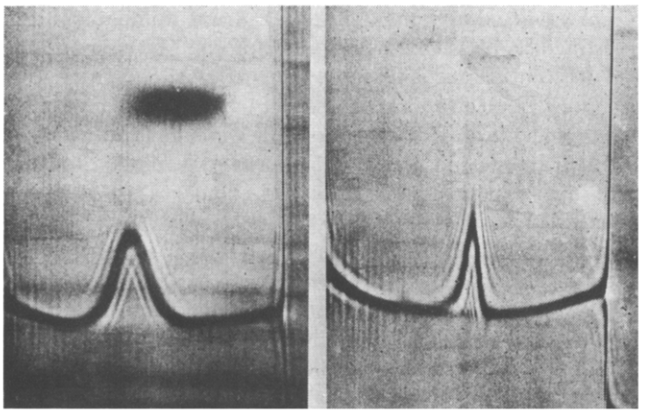

FIGURE 4: Schlieren patterns of purified shark anti-DNP antibodies after chromatography on Bio-Gel P-200 column. Left, 7S antibody (A_{278} 6.0) and right, 18S antibody (A_{278} 5.0). Proteins were dissolved in 0.05 M phosphate buffer (pH 8.0) Rotor speed 59,780 rpm.

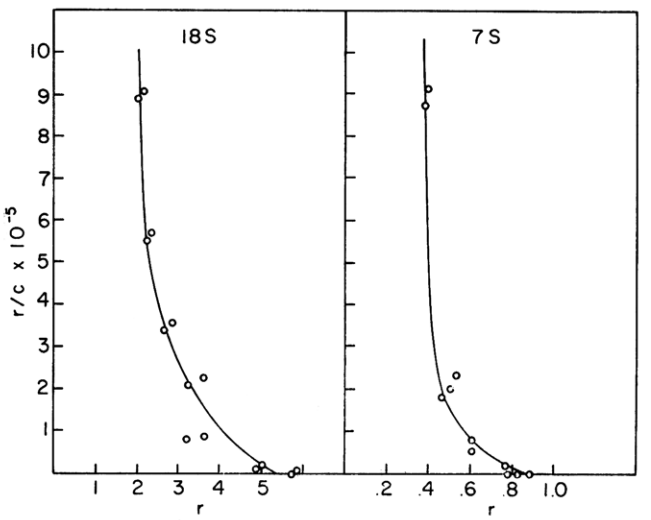

FIGURE 5: Equilibrium dialysis. Fifty microliters of purified 18 S (A_{278} 2.0/ml) and 7 S (A_{278} 1.9/ml) shark anti-DNP antibodies was equilibrated in chambers for 22 hr at 5° with various concentrations of [ε-^{3}H]DNP-L-lysine. Samples (25 μl) from each side of the chambers were obtained and assayed after equilibration had been established with the buffer controls. In all cases r is moles of ligand bound per mole of antibody (molecular weight assumed to be 150,000 for 7 S and 900,000 for 18 S, $\epsilon_{A_{278}}^{1\%}$ assumed to be 13), c is free-ligand concentration expressed as moles per liter.

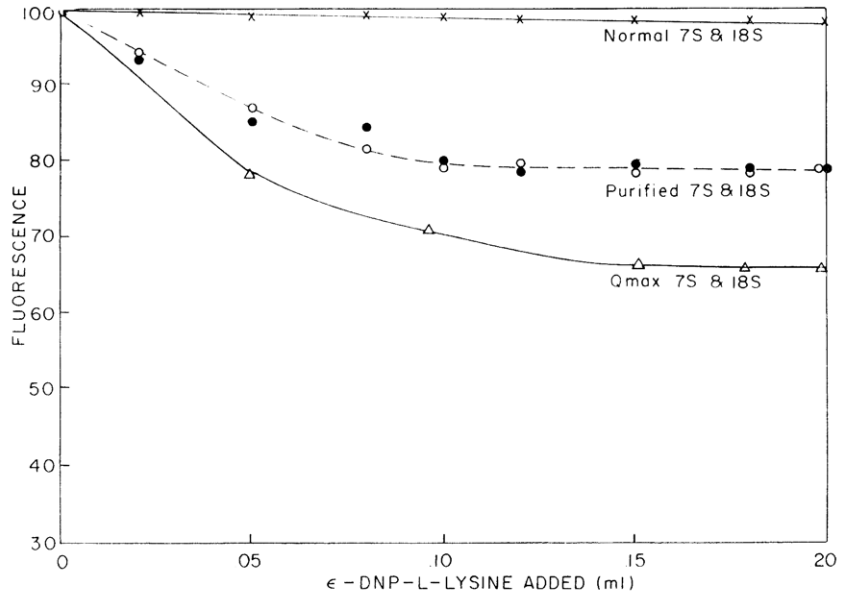

FIGURE 6: Fluorescence-quenching titrations. Both normal (*—*) and purified shark antibodies (– – –) were titrated at A_{278} 0.100 with 20 μl of ligand ϵ-DNP-L-lysine. A_{360} 0.100 Q_{max} titrations (—△—) were done with high concentrations of ligand (A_{360} 5.00) and the nonspecific attenuation of fluorescence was corrected by control titrations with tryptophan and normal shark 7 or 18 S.

obtained from a variety of species (rabbit, guinea pig, goat, and horse) it was suggested that this may be a universal property of all anti-DNP sites. Data indicated that the spectral change was consistent with the presence of a tryptophan residue in the anti-DNP active site. To extend this hypothesis to the primitive shark system spectral shift studies were performed with both 18S and 7S species. Figure 8 displays the difference spectra obtained between free and antibody-bound ϵ-DNP-L-lysine.

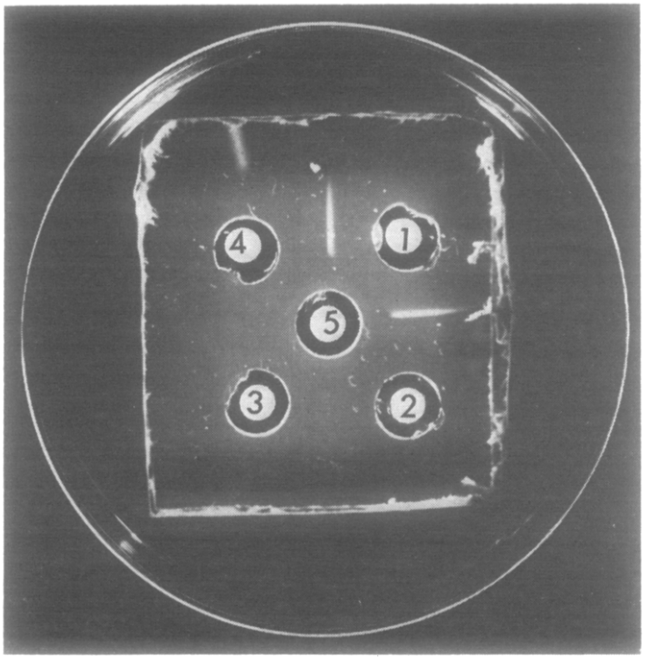

FIGURE 7: Comparative double-diffusion precipitin reactions of purified nurse shark 7S anti-DNP antibody and purified rabbit IgG anti-DNP in agar gel. Well 1 contained purified rabbit anti-DNP (A_{278} 2.00). Wells 2, 3, and 4 contained DNP_{24} human serum albumin at 1.96, 0.98, and 0.49 mg per ml, respectively. Well 5 contained purified shark 7 S anti-DNP (A_{278} 1.55).

Ligand bound to shark antibody shows two peaks at 378 and 425 mμ. Control studies with rabbit antibody show peaks at 380 and 470 mμ as previously reported by Little and Eisen (1967). Thus, the bound ligand with shark antibody exhibits a spectral shift but the shift is not qualitatively identical with that of the rabbit anti-DNP system.

Discussion

These studies have described the induction and purification of antibodies specific for the 2,4-dinitrophenyl group from nurse shark sera. A large proportion of the data reported were obtained from antibody purified from shark 4336. However, low levels of antibody showing relatively weak binding properties have been purified by immunoadsorption from several nurse sharks.

Of primary interest was the finding of 18S and 7S IgM-purified antibodies coexisting in sera in quantities sufficient to measure their binding properties. Data presented in this paper give indirect evidence for the assumption that the 7 S is the natural subunit. First, it has been previously shown (Clem *et al.*, 1967) that 18 and 7 S are immunologically indistinguishable. Antisera specific for the H chains of the 18S molecule react with the H chains of the 7S molecule. Second, the electrophoretic mobility of the two molecular forms is identical. If the 7S molecule was similar to mammalian IgG one would have expected a more cathodic mobility than that experimentally observed (Figure 1). Third, the valence of five for the 18S (900,000 molecular weight) component and a valence of one for 7 S is further evidence for a naturally occurring subunit. One would expect the subunits derived from thiol reduction of the 18S component to yield a monovalent 7S subunit as reported by Onoue *et al.* (1965). Preliminary studies have indicated that the 7S subunits derived from the 18S molecule give similar binding measurements as the naturally occurring 7S molecule. The valence of five for the shark 18S molecule is consistent with both studies by Onoue *et al.* (1965, 1968). They first reported a valence of five for a rabbit IgM molecule with an association

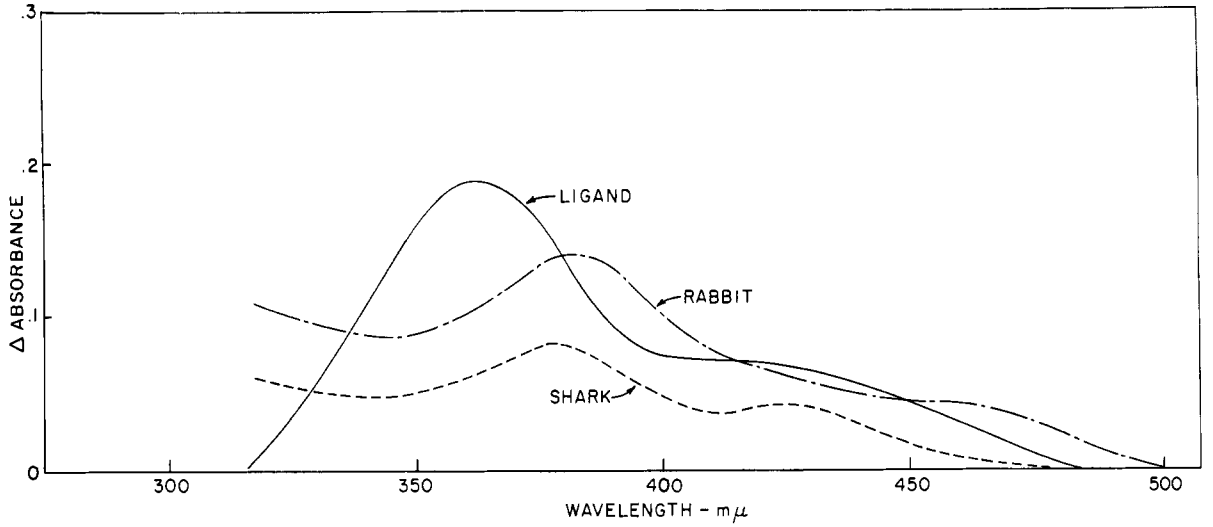

FIGURE 8: Difference spectra of free and antibody-bound ϵ-DNP-L-lysine. The experimental cell contained purified shark anti-DNP (A_{278} 1.94). The reference cell contained the ligand (A_{360} 1.25). The solid line is the spectrum of the free ligand against buffer with maxima at 260 and 430 mμ. The spectra depicting bound ligand are differences between ligand plus antibody (rabbit -----, shark -----) *vs.* ligand alone.

constant approximately equivalent to the constant measured for the shark antibody in this report. When Onoue *et al.* (1968) produced a relatively high-affinity rabbit IgM, they measured two sets of active sites with a 10^2 differential in binding constants (*i.e.* 10^7 *vs.* 10^5 l./mole). Since the primary set of sites in the purified shark antibody has an average intrinsic association constant, K_0, of 2×10^5 l./mole, the second set of sites (2×10^3 l./mole) would be too weak to assay in equilibrium dialysis. This relationship is further exemplified in the Q_{max} studies. The Q_{max} values of 35% are consistent with only five quenchable sites (Table I). Ligand concentrations high enough to quench sites with association constants of 2×10^3 l./mole are not possible in fluorometric titrations. These same arguments are extrapolatable to the 7S IgM shark molecule. The fact that the 18S and 7S molecules yielded similar binding constants is extremely important in the hypothesis for naturally occurring 7S subunits. If the two binding constants between 18 and 7 S had been grossly different, then the concept of a precursor subunit would be invalid. It should be pointed out that excessively high concentrations of ligand at high concentrations of purified antibody were used in equilibrium dialysis studies to attempt to uncover additional sites with both the 18S and 7S molecules. These experiments were not successful and did not alter results reported in Figure 5.

Both the 18S and 7S molecules gave identical spectral shifts with bound ligand. Although the shift is qualitatively different from that reported with mammalian anti-DNP antibodies (Little and Eisen, 1967), a shift was measured. Studies are currently in progress to determine if the difference between the spectra for shark and rabbit antibody is due to the contribution of amino acids other than tryptophan.

The finding of a univalent 7S molecule raises some interesting questions. Suran *et al.* (1967) reported both a 17S- and a 7S-precipitating antibody (anti-keyhole limpet hemocyanin) in leopard sharks. Clem *et al.* (1967) demonstrated mercaptoethanol-resistant hemagglutinating 7S anti-bovine serum albumin antibodies in nurse sharks. Clem and Small (1967) showed similar results with anti-bovine serum albumin antibodies from lemon sharks. These data would suggest a bivalent 7S IgM antibody capable of precipitation and hemagglutination. There are two possible explanations for the discrepancies between a univalent 7S class and bivalent 7S class. First, there are in fact, two distinct classes of 7 S, one of which is bivalent and the other univalent; second, that these are all the same class of molecules with different K_0 values which have been elicited by the protein antigen *vs.* the hapten. If protein antigens, such as bovine serum albumin, elicit a 7S molecule with sites possessing association constants similar to those reported by Onoue *et al.* (1968) for antiazonaphthalene the effective valence is two. Both sites are strong enough to function as bivalent molecules in precipitation and hemagglutination reactions. On the other hand, if the anti-DNP shark antibodies possess relatively weak binding sites they may exhibit an effective valence of one (10^5 *vs.* 10^3 l./mole). Results from other sharks in this study have indicated weakly binding anti-DNP antibodies. If sharks show an increase in association constants with time as described for rabbits (Eisen and Siskind, 1964), then the 7 S may evolve from a functionally univalent molecule to two functional sites. These studies are now in progress.

It would appear on the basis of binding data that the 7S IgM molecule is "potentially" either a synthetic precursor or a breakdown subunit of the 18S IgM antibody molecule. It is not clear why the 7S molecule has not polymerized (disulfide-bond formation) to 18 S if the precursor concept applies or under what control mechanism a breakdown has occurred if the latter is the process explaining its presence in shark serum.

Acknowledgments

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Chloroquine and Synthesis of Aminoacyl Transfer Ribonucleic Acids. Tryptophanyl Transfer Ribonucleic Acid Synthetase of *Escherichia coli* and Tryptophanyladenosine Triphosphate Formation*

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ABSTRACT: The antimalarial drug, chloroquine, enhances both the rate and extent of enzymatic charging of tryptophan to transfer ribonucleic acid of *Escherichia coli*. The effect occurs throughout a 230-fold purification of tryptophanyl transfer ribonucleic acid synthetase. Chloroquine does not affect the rate of tryptophan-dependent adenosine triphosphate–[^{32}P]inorganic pyrophosphate exchange. Although the synthetase forms tryptophanyladenosine triphosphate ester in addition to tryptophanyl transfer ribonucleic acid, chloroquine does not significantly affect formation of the tryptophanyladenosine triphosphate. Chloroquine changes neither the K_m nor the V_{max} for the active form of tryptophan transfer ribonucleic acid. The effect of chloroquine on reaction rate can be attributed entirely to its conversion of the inactive form of tryptophan transfer ribonucleic acid into the active form when the latter is at suboptimal concentration. The inactive form of tryptophan transfer ribonucleic acid is not an inhibitor of the enzyme.

The antimalarial drug, chloroquine, 7-chloro-4-(4-diethylamino-1-methylbutylamino)quinoline (CQN[1]), binds to RNA, probably by a combination of ionic and other forces, with a consequent change in the absorbance spectrum of the drug (Irvin *et al.*, 1949; Parker and Irvin, 1952; Muench, 1966). The drug also combines with DNA (Parker and Irvin, 1952; Allison *et al.*, 1965; Cohen and Yielding, 1965a; Stollar and Levine, 1963; O'Brien *et al.*, 1966a; Blodgett and Yielding, 1968), the interaction with double-stranded DNA being characterized by a higher association constant than that of the interaction with single-stranded DNA (Parker and Irvin,

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[1]Nonstandard abbreviations used are: MAK, methylated albumin kieselguhr; CQN, chloroquine; Trp-ATP, ATP esterified to tryptophan on the 2′- or 3′-hydroxyl group; $tRNA_i^{Trp}$, the inactive form of $tRNA^{Trp}$; $tRNA_a^{Trp}$, the active form of $tRNA^{Trp}$; $tRNA_T^{Trp}$, the sum of both forms of $tRNA^{Trp}$; 1 A_{260} unit of tRNA has an A_{260} of 1.0 in a 1.0-cm optical path when dissolved in 1.0 ml of 5 mM KH_2PO_4–5 mM K_2HPO_4 buffer (pH 6.9).