Sims, J. L., Juarez-Salinas, H., & Jacobson, M. K. (1980) *Anal. Biochem. 106*, 296–306.
Skidmore, C. J., Davies, M. I., Goodwin, P. M., Halldorsson, H., Lewis, P. J., Shall, S., & Zia'ee, A. (1979) *Eur. J. Biochem. 101*, 135–142.
Smulson, M. E., Stark, P., Gazzoli, M., & Roberts, J. (1975) *Exp. Cell Res. 90*, 175–182.
Sudhakar, S., Tew, K. D., Schien, P. D., Wooley, P. J., & Smulson, M. E. (1979) *Cancer Res. 39*, 1411–1417.
Tegtmeyer, P., & Macasset, T. (1972) *J. Virol. 10*, 599–604.
Whish, W. J. D., Davies, M. I., & Shall, S. (1975) *Biochem. Biophys. Res. Commun. 65*, 722–730.
Yoshihara, K., Hashida, T., Tanaka, Y., Ohgushi, H., Yoshihara, H., & Kamiya, T. (1978) *J. Biol. Chem. 253*, 6459–6466.
Yoshihara, K., Hashida, T., Tanaka, Y., Matsunami, N., Yamaguchi, A., & Kamiya, T. (1981) *J. Biol. Chem. 256*, 3471–3478.

Specificity of Autoimmune Monoclonal Fab Fragments Binding to Single-Stranded Deoxyribonucleic Acid†

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ABSTRACT: Fab fragments from hybridoma HEd 10 [Lee, J. S., Lewis, J. R., Morgan, A. R., Mosmann, T. R., & Singh, B. (1981) *Nucleic Acids Res. 9*, 1707–1721] were prepared in large amounts by papain digestion of the immunoglobulin G (IgG) fraction from ascites fluid. Binding data were generated by a fluorescence quenching technique, and binding constants [$K(0)$] were estimated from Scatchard plots. The Fab fragments bound tightly to poly(dT) [$K(0) = 12.7 \times 10^6$ M^{-1}], and analysis of binding constants for the series $p(dT)_2$ to $p(dT)_{17}$ showed that the recognition sequence consisted of four consecutive residues. The effect of ionic strength on the interaction suggested that only two phosphates were involved. Binding constants for poly(dU), poly[d(brU)], poly[d(brC)], and poly(rU) were 1.0×10^6 M^{-1}, 18.8×10^6 M^{-1}, 0.5×10^6 M^{-1}, and $<0.5 \times 10^6$ M^{-1}, respectively, implicating the involvment of the 3, 4, and 5 positions of the pyrimidine ring as well as the deoxyribose sugar in the recognition process. At high ionic strength (0.5 M) $K(0)$ for whole IgG binding to poly(dT) was 75×10^6 M^{-1} compared to a value of 1.1×10^6 M^{-1} for the Fab fragment. These results may have implications for the tissue damage caused by DNA-containing immune complexes in systemic lupus erythematosus.

From autoimmune NZB/NZW mice we recently prepared six hybridoma cell lines that secrete antibodies to single-stranded DNAs (Lee et al., 1981). Two of these cell lines (HEd 8 and HEd 10) were of paticular interest since, although they were derived from different mice, they produce antibodies that are indistinguishable by isoelectric focusing gels as well as on the basis of their DNA-binding specificity patterns (Lee et al., 1981). Thus they may represent a particularly common type of murine autoimmune antibody. The mechanism by which these antibodies cause tissue damage, particularly to the kidneys, in the disease systemic lupus erythematosus (SLE) is not well understood, but complexes between antibodies and nucleic acids are clearly implicated (Koffler et al., 1971; Hahn, 1981). Thus a detailed understanding of antibody–DNA interactions at the molecular level may give a better insight to this rather puzzling disease. Recently several other nucleic acid binding autoimmune antibodies have been prepared and some of their properties described (Lafer et al., 1981a; Tron et al., 1980; Eilat et al., 1980; Jacob & Tron, 1982), but as yet no unique features of autoimmune antibodies have become apparent.

Proteins that bind to nucleic acids are also of considerable interest to molecular biology, and several systems, for example, the lac repressor (Ogata & Gilbert, 1977; Lin & Riggs, 1972; Goeddel et al., 1978), the cro repressor (Ptashne et al., 1980; Anderson et al., 1981), and the gene 32 protein of phage T4 (Kowalczykowski et al., 1981; Newport et al., 1981), have been studied in detail. However, these systems are complex because of the multimeric nature of the proteins and/or the possession of several different modes of binding. In this paper we shall describe the properties of Fab fragment HEd 10 binding to single-stranded DNAs of defined sequence and length and show that this is a comparatively simple process. It may thus serve as a good model for protein recognition of nucleic acids in general.

Materials and Methods

Nucleic Acids. Poly(dT), poly(dU), poly(dC), poly(dA), poly(dA)·poly(dT), and the $p(dT)_x$ oligomers ($x = 2$–17) were purchased from P-L Biochemicals. The repeating-sequence pyrimidine DNAs were prepared by depurination, and poly[d(TG)] was prepared by alkaline ceasium chloride buoyant density centrifugation from the appropriate duplex DNAs as described previously (Harwood & Wells, 1970; Morgan et al., 1974, 1979; Lee et al., 1979). Concentrations were calculated from absorbance measurements with extinction coefficients listed previously (Morgan et al., 1979; Lee et al., 1979) or with those supplied by the manufacturer. Cardiolipin and glucose 1,6-diphosphate were purchased from Sigma, and solutions of known concentration were prepared by direct weighing.

Preparation of Immunoglobulin G (IgG) and Fab Fragment HEd 10. The hybridoma cell line HEd 10 was derived from autoimmune NZB/NZW mice (Lee et al., 1981), and injection into three C_3H pristane-primed mice produced large quantities of ascites fluid (10–15 mL/mouse). Clots after formation were removed by centrifugation, and the supernatant

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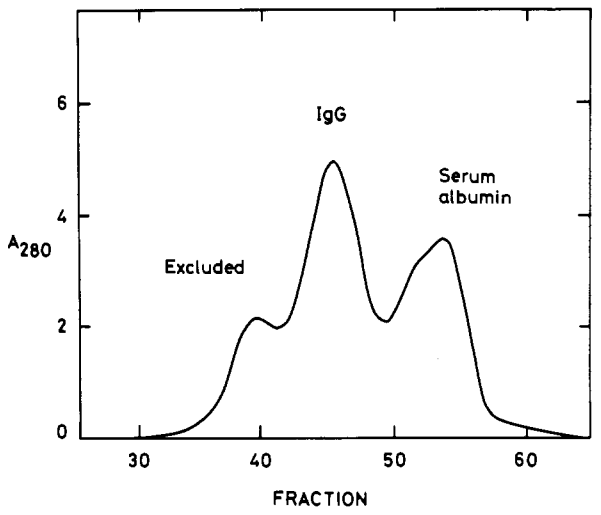

FIGURE 1: Gel exclusion chromatography of ascites fluid from HEd 10 on Sephacryl S-200. The IgG fraction is well resolved and is the major protein in the fluid.

was filtered through a Millipore 0.45-μm filter before being stored frozen until ready for use. Aliquots (10 mL) of the cleared ascites fluid were chromatographed on a 500-mL Sephacryl S-200 gel exclusion column in a buffer of 0.5 M NaCl, 1 mM EDTA,[1] and 50 mM Tris-HCl, pH 8.0. Figure 1 is a typical elution profile showing that the IgG fraction was the major protein component in the ascites fluid. The total yield was approximately 0.5 g. For preparation of Fab fragments the IgG fraction was treated with 1% papain for 5 h at 37 °C in a buffer of 0.25 M NaCl, 1 mM EDTA, 25 mM 2-mercaptoethanol, and 25 mM Tris-HCl, pH 7.5, according to the procedure of Mage (1980). After precipitation with 80% ammonium sulfate the fragments were dialyzed into 10 mM Tris-HCl, pH 8.0–0.1 mM EDTA before applying to a 100-mL DEAE-Sephacel column and eluting with a 200-mL 0–1 M NaCl gradient. The Fab fragments appeared in the flow through whereas the Fc portion eluted at the end of the gradient (Mage, 1980). The Fab fragments were again precipitated with 80% ammonium sulfate and dialyzed into 10 mM potassium phosphate, pH 6.5, before applying to a 60-mL phosphocellulose column and eluting with a 100-mL 0–2 M NaCl gradient. Approximately 5% of the applied protein appeared in the flow through whereas the purified Fab fragments eluted at approximately 0.5 M NaCl. The final yield of Fab fragments was approximately 50% by weight from the original IgG compared to a theoretical maximum of approximately 60%.

Purified IgG was prepared by rechromatographing an aliquot of the original IgG fraction on Sephacryl S-200. The IgG peak was precipitated with 80% ammonium sulfate and dialyzed into 10 mM Tris-HCl, pH 8.0–0.1 mM EDTA before applying to a 100-mL DEAE-Sephacel column and eluting with a 100-mL 0–1 M NaCl gradient. Approximately 80% of the protein eluted at 0.6 M NaCl, and this was identified as IgG HEd 10 on the basis of a positive solid-phase radioimmmuno assay (SPRIA) (Lee et al., 1981). Several other minor peaks apeared at higher NaCl concentrations, but these did not bind to DNA as judged from the SPRIA.

Isoelectric focusing gels (Awdeh et al., 1968) and NaDodSO$_4$–polyacrylamide gels (Laemmli, 1970) were used to demonstrate that the light chain of the Fab fragments was intact and that both the Fab and IgG preparations were essentially free from contaminating proteins (>90% pure). In both cases the protein concentration was estimated by assuming A_{280} (1 mg/mL) = 1.5 (Guiges & Leng, 1976), and the molecular weights were taken to be 45 000 and 150 000 for the Fab fragment and IgG, respectively.

Binding Studies. Both the Fab fragments and whole IgG gave a strong tryptophan fluorescence that was quenched in the presence of all nucleic acids tested. Binding experiments were performed at 20 °C in 1-cm^2 quartz cuvettes in a Turner Model 430 spectrofluorometer with excitation at 295 nm and emission at 355 nm. Fab fragments (1.39×10^{-7} M) or IgG (2.92×10^{-8}) in 2 mL of either PBS buffer (2.7 mM KCl, 137 mM NaCl, 10 mM Na_2HPO_4, 1.4 mM KH_2PO_4, final pH of 7.2) or 10 mM Tris-HCl, pH 8.0–0.1 mM EDTA with various concentrations of NaCl gave a good response on the ×100 sensitivity scale of the fluorometer. The initial fluorescence (F_s) of the sample was arbitrarily set to 100 before the sequential addition of small aliquots of antigen (1–10 μL) up to a maximum of 200 μL. The fluorescence (F) was read after each addition, and in all cases equilibrium appeared to be established within 30 s. The fluorescence was corrected for the volume change upon addition of antigen. No correcton was applied for internal quenching due to the small absorbtion of the excitation light at 295 nm by the nucleic acids since this was found to be insignificant. The fluorescence of the bound form of either Fab fragments or IgG (F_B) was estimated by extrapolation to infinite antigen concentration of a plot of F against [antigen]$^{-1}$. Thus

$$F = f_s F_s + f_B F_B$$

where F is measured fluorescence, F_s is initial fluorescence (=100), F_B is fluorescence of bound protein, f_s is fraction of protein in solution, and f_B is fraction of protein bound to antigen; moreover

$$f_s + f_B = 1$$

These equations were solved simultaneously for each value of F to yield f_s and f_B. Since the protein and antigen concentrations were known, f_B was converted to r, the number of protein molecules bound per phosphate (or nucleotide) residue, and f_s gives c, the free protein concentration. The data are conveniently analyzed in the form of a Scatchard plot (r/c vs. r) since the intercept on the r/c axis gives the binding constant [$K(0)$] directly (McGhee & von Hippel, 1974). For the Fab fragments this intercept was estimated by eye whereas for IgG binding the binding parameters were calculated from a computer program as described previously (Lee & Waring, 1978).

Results

Number of Consecutive Bases in the Recognition Sequence. Previous binding studies with HEd 10 IgG using a solid-phase radioimmuno assay (SPRIA) had suggested that polypyrimidine DNAs other than poly(dC) were the preferred antigens (Lee et al., 1981; see also Table I). Thus oligomers of poly(dT) provide ideal antigens for studying the effect of polymer length on the binding of Fab fragment HEd 10. Binding data were generated by the fluorescence quenching technique described in detail under Materials and Methods. The value of F_B (the fluorescence of Fab bound to antigen) varied between 37 and 39 for all the oligomers and poly(dT) except for p(dT) (or TMP), where F_B was measured to be 41. (The initial fluorescence of the unbound Fab was always set to 100.) This suggests that the mechanism of binding was very similar in all cases. The binding data were converted into a Scatchard plot (Figure 2) in which the intercept on the or-

[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; DEAE, diethylaminoethyl; NaDodSO$_4$, sodium dodecyl sulfate.

Table I: Binding Constants of Fab Fragment HEd 10 to Various Nucleic Acids[a]

polymer	binding constant $[K(0)] \times 10^{-6}$ (M^{-1})	polymer	binding constant $[K(0)] \times 10^{-6}$ (M^{-1})
poly(dT)	12.7	poly(dA)	<0.5
poly[d(TTC)]	6.0	poly(dA)·poly(dT)	<0.5
poly[d(TC)]	9.6	heat-denatured calf thymus DNA	<0.5
poly[d(TCC)]	1.1	native calf thymus DNA	<0.5
poly[d(TG)]	6.8	cardiolipin	<0.5
poly(dU)	1.0	glucose 1,6-diphosphate	<0.5
poly[d(brC)]	0.5		
poly[d(brU)]	18.8		
poly(dC)	<0.5		
poly(rU)	<0.5		

[a] $K(0)$ was measured from the intercept on the Scatchard plot as described in the legend to Figure 1. A binding constant of $<0.5 \times 10^6$ M^{-1} is considered too small to be measured accurately by this technique.

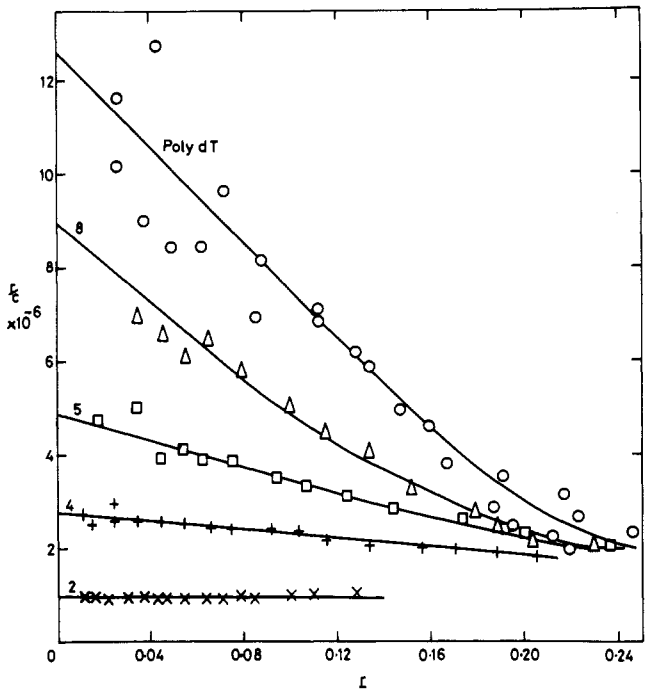

FIGURE 2: Scatchard plots for binding of Fab fragment HEd 10 to oligo(dT) and poly(dT): (○) poly(dT), (△) $p(dT)_8$, (□) $p(dT)_5$, (+) $p(dT)_4$, and (×) $p(dT)_2$. r is the number of protein molecules bound per phosphate residue, c is the free (unbound) protein concentration, and the intercept on the r/c axis gives $K(0)$, the binding constant, directly. The curves were fitted to the data points by eye.

dinate (r/c axis) gives the binding constant directly, and it should be noted that this is independent of protein concentration (McGhee & von Hippel, 1974). Although the binding constant increases from $p(dT)_2$ to $p(dT)_8$ with a further small increase for poly(dT), this *does not* imply that the Fab fragment is binding to as many as eight or more consecutive residues. The reason is that the longer the oligomer the larger the number of potential binding sites. For example, for a protein that recognizes two consecutive residues the binding constant for $p(dT)_3$ would be twice that for $p(dT)_2$ because $p(dT)_3$ has two potential binding sites. Theoretically

$$K_N = K(0)(N - m + 1)/N$$

where $K(0)$ is the intrinsic association constant for an infinite polymer, K_N is the association constant for an oligomer of length N, and m is the length of the sequence recognized for the binding of one Fab fragment (McGhee & von Hippel, 1974; Kowalczykowski et al., 1981).

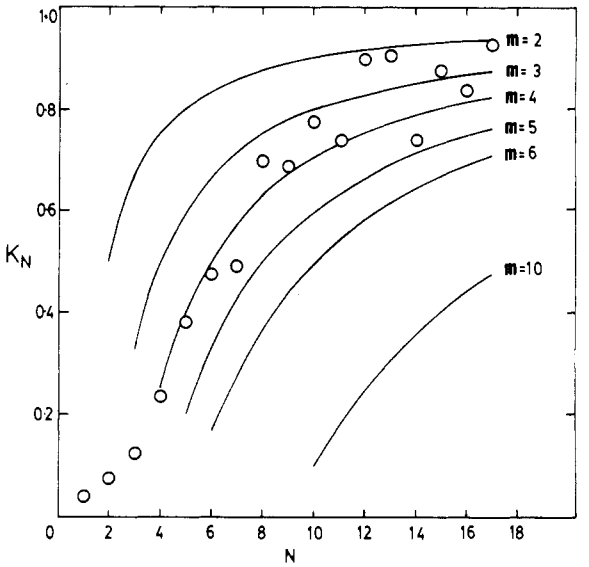

FIGURE 3: Binding constant K_N as a function of number of residues (N) in oligo(dT). All values have been normalized such that $K(0)$ for poly(dT) equals unity. K_N was measured from the intercept on the ordinate of a Scatchard plot as described in the legend to Figure 2. The error is estimated to increase from less than 5% for the smaller oligomers to as much as 15% for poly(dT). The curves shown for m = 2, 3, 4, 5, and 10 (where m = the length of the sequence recognized) were calculated from $K_N = [(N - m + 1)/N]K(0)$ as described in the text.

In Figure 3, K_N is plotted as a function of N for 17 oligomers with $K(0)$ for poly(dT) itself being normalized to unity; superimposed on these data are theoretical curves calculated from the above equation for different values of m. (For $N < m$, K_N must be less than $K(0)/m$, but the value cannot be calculated theoretically since the contribution from each residue is unknown.) It can be seen that there is a good fit between the experimental and theoretical curves for $m = 4$ especially for values of N of 4, 5, and 6, suggesting that four consecutive residues constitute the binding site. The number of bases occluded by the binding of one Fab fragment (i.e., the number of bases made inaccessible to another Fab) may be larger than four (see Discussion). It should be noted that this estimated binding site size of 4 is for poly(dT), and it is possible that bases other than thymine outside this sequence might make positive interactions with the Fab fragments.

Number of Ion Pairs Involved in the Binding Process. The phosphodiester backbone is known to be a major antigenic determinant for many antibodies to nucleic acids (Stollar, 1975), and therefore, the number of phosphates involved in the interaction is of considerable interest. Theoretically the number of ion pairs can be deduced from plots of log $K(0)$ against log (ionic strength), and this is shown in Figure 4 for poly(dT). For a single-stranded DNA the slope of this curve = 0.7(number of ion pairs) (Record et al., 1976). Therefore the number of ion pairs = $1.44/0.7 \simeq 2$; since only phosphate moieties are charged under the pH conditions of these experiments, we infer that only two phosphates out of a possible four are directly involved in the interaction. From the point of view of specificity this is encouraging because if all four phosphates were being recognized, then the antibodies might be expected to bind tightly to all nucleic acids regardless of sequence or structure.

Antigenic Determinants of Single-Stranded DNA. The other features of the nucleic acid that are being recognized can be deduced from binding constants for different but related polymers as shown in Table I. Comparison of poly(dT) and

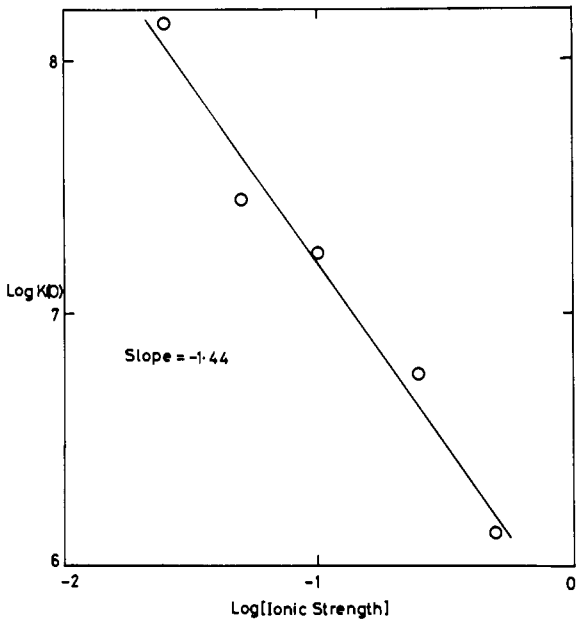

FIGURE 4: Effect of ionic strength on binding constant: log $K(0)$ vs. log (ionic strength). $K(0)$ was measured from the intercept on the ordinate of Scatchard plots. Experiments were performed in 10 mM Tris-HCl, pH 8.0–0.1 mM EDTA supplemented with NaCl to give the required ionic strength.

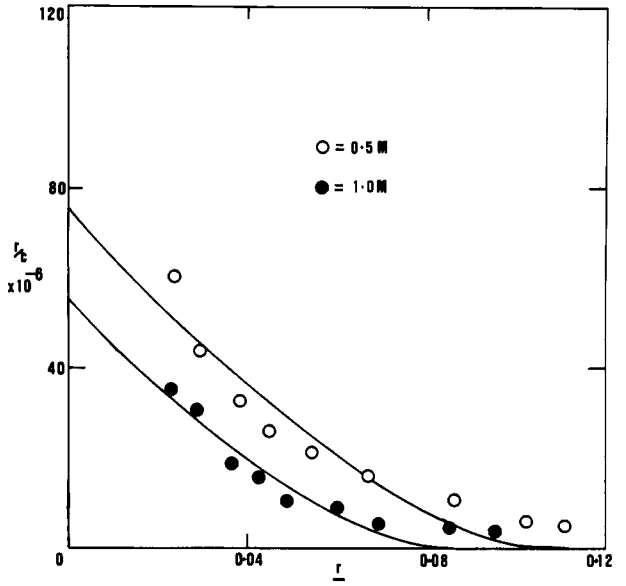

FIGURE 5: Scatchard plots for binding of IgG HEd 10 to poly(dT): (○) 0.5 M NaCl and (●) 1.0 M NaCl. r is the number of IgG molecules bound per phosphate, and c is the free (unbound) IgG concentrations. The curves were fitted to the data points with a computer program according to eq 10 of McGhee & von Hippel (1974).

poly[d(brU)] with poly(dU) suggests an important role for the 5-methyl group of pyrimidines in the recognition process. The fact that the binding constant to poly[d(brU)] is actually higher than that to poly(dT) is probably due to the higher polarizability of bromine compared to a that of methyl group and has also been noted in the interaction between the lac repressor and synthetic operator DNAs (Lin & Riggs, 1972). Comparison of poly[d(brC)] with poly[d(brU)] shows that the 4-carbonyl and/or 3-imino of thymine is bound by the antibody. Finally comparison of poly(dU) with poly(rU) suggests that some aspect of the deoxyribose backbone is also being recognized. Thus, perhaps not surprisingly, every feature of a single-stranded nucleic acid is being utilized in the interaction so that antibodies should have the potential for distinguishing between any two nucleic acids however similar.

Some further points can be made from the binding constants listed in Table I. The polymers poly(dT), poly[d(TTC)], poly[d(TC)], poly[d(TCC)], and poly(dC) form a series with decreasing thymine content. However, the binding constants do not strictly follow this pattern, and in particular that for poly[d(TC)] is greater than that for poly[d(TTC)]. This suggests that the preferred sequence is of the type TXTX or XTXT (where X is an unknown or unnecessary base) rather than one containing four consecutive thymines. However the binding constant for poly[d(TG)] is significantly less than that for poly[d(TC)] so presumably positive or negative interactions are also occurring with bases other than thymine. These points serve to emphasize the fact that the preferred sequence for HEd 10 is actually unknown although it is undoubtedly thymine rich.

Many of the binding constants in Table I are listed as $<0.5 \times 10^6$ M^{-1}, which is considered to be the limiting value that can be measured accurately by this technique. The major problem is that sufficiently high concentrations of antigen cannot be attained to estimate the limiting fluorescence, F_B. However, even these weak antigens gave rise to a significant drop in the fluorescence of the Fab fragment so that the estimated low binding constant is not an artifact due to a lack of fluorescence quenching. The small binding constant for cardiolipin is of some interest since many autoimmune DNA binding antibodies from MRL/1 mice have this compound as the preferred antigen (Lafer et al., 1981a).

Binding Constants for IgG HEd 10 to Poly(dT). For a perfectly rigid IgG molecule binding to a rigid polymer the binding constant (K_{IgG}) would be expected to be the square of the binding constant of the Fab fragment (K_{Fab}), i.e., $K_{IgG} = K_{Fab}^2$. Similarly for an infinitely flexible IgG molecule binding to a flexible polymer $K_{IgG} = 2K_{Fab}$. [A more detailed theoretical treatment has been proposed by Crothers & Metzger (1972).] Thus comparison of K_{IgG} with K_{Fab} measured under identical conditions will give some indication of the flexibility inherent in the system. Figure 5 shows Scatchard plots for the binding of IgG HEd 10 to poly(dT) in 1.0 and 0.5 M NaCl for which the binding constants are estimated to be 54×10^6 M^{-1} and 75×10^6 M^{-1}, respectively. Measurements were not performed at lower ionic strengths because, for higher binding constants, c, the unbound IgG concentration, cannot be measured accurately. These values are to be compared with 1.1×10^6 M^{-1} for the binding of Fab fragment HEd 10 to poly(dT) (Figure 4) in 0.5 M NaCl. Thus $K_{IgG} \simeq 75K_{Fab}$, suggesting an extremely flexible system. The binding constant of IgG to $p(dT)_5$ in 0.5 M NaCl was measured to be 1.0×10^6 M^{-1} (data not shown) whereas theoretically under the same conditions the Fab fragment binding constant is calculated to be 0.44×10^6 M^{-1} ($=1.1 \times 10^6$ $[(5 - 4 + 1)/5]$). Thus for $p(dT)_5$, $K_{IgG} \simeq 2K_{Fab}$ as expected for a situation where a single antigen molecule cannot bind to both arms of the IgG simultaneously. This argues that the properties of the antigen combining site have not been altered in conversion of the IgG to Fab fragments.

The computer-fitted curves of Figure 5 also yield estimates of n, the number of bases occluded by the binding of one IgG molecule. In 0.5 and 1.0 M NaCl n was found to be 7.6 and 9.5, respectively, which is approximately twice the value of m, the number of bases recognized by one Fab fragment.

Discussion

The fluorescence quenching technique for measuring binding parameters, described herein, is extremely powerful, and it has

been used extensively in a wide variety of systems (Fox et al., 1980; Guiges & Leng, 1976; Kowalczykowski et al., 1981). However, it suffers from the disadvantage that r and c are calculated by difference, and therefore, accurate estimates of these parameters can only be made simultaneously over a 10–90% saturaton range. This limitation has little effect on the estimate of $K(0)$ because the extrapolaton to the r/c axis is reasonably linear over a wide range (McGhee & von Hippel, 1974). However, the extrapolation to the r axis in order to find the number of bases occluded (n) by the binding of one ligand becomes very shallow, leading to poor estimates. Moreover, it would appear [see Figure 1 for poly(dT) for example] that many Scatchard plots generated in this study would not intercept the r axis. One explanation for this artifact is that at low r/c values the Fab fragment is binding to less than four residues, enabling artificially large r values to be reached. For this reason estimates of n have not been made for the Fab fragment.

For the whole IgG the upward curvature of the Scatchard plots (Figure 5) at large r values is less pronounced, and the data points are reasonably well fitted to eq 10 of McGhee & von Hippel (1974). In this case n was found to be approximately 8.5 or twice the value of m, the number of bases recognized for the binding of one Fab fragment. In other words there is probably no "neighbor exclusion" effect (Crothers, 1968) for these proteins. It is encouraging that the plot of K_N vs. N (Figure 3) fits the theoretical treatment adequately, and as far as we are aware, this is the first reported protein to demonstrate this effect. The gene 32 protein from T4, for example, has a binding constant to oligonucleotides that is essentially independent of length, suggesting a very different and more complex mechanism of binding to single-stranded nucleic acids (Kowalczykowski et al., 1981).

The measured binding constants to synthetic polymers of related but different sequences (Table I) have enabled the elucidation of most of the antigenic determinants of a single-stranded nucleic acid. Crystals of Fab fragment HEd 10 have been grown, and if the structure can be solved by X-ray diffraction techniques, it will hopefully confirm the findings of this work (W. Anderson, unpublished results). The only potential recognition point on the pyrimidine ring that has not been investigated is the 2 position for which no analogues were available. However, the general conclusion is that antibodies have the potential to distinguish between two single-stranded nucleic acids however similar. We are in the process of preparing Fab fragments that show specificity for duplex DNA, and comparison with the properties of these proteins will be most instructive.

The specificty pattern of Fab fragment HEd 10 can also be compared to the pattern for the whole IgG deduced previously from SPRIA (Lee et al., 1981). Two major differences are apparent. Firstly, the preferred antigen for IgG HEd 10 was poly[d(brC)] whereas the Fab fragment binds only weakly [$K(0) = 0.5 \times 10^6$ M^{-1}]. Other halogenated single-stranded pyrimidines also gave anomalous results (Lee et al., 1981). One possible explanation is that halogenated polymers bind considerably better to the PVC plates used in the SPRIA compared to other DNAs. This may have important consequences for the study of antibodies binding to "Z" DNA where brominated poly[d(GC)] is an important antigen (although admittedly there is no evidence that bromine atoms are being recognized in brominated poly[d(GC)]) (Lafer et al., 1981b; Nordheim et al., 1981). Secondly, as judged from the SPRIA, heat-denatured calf thymus DNA bound HEd 10 well whereas for the Fab fagment the binding constant was too low to be measured accurately [$K(0) < 0.5 \times 10^6$ M^{-1}]. Again the explanation may be that heat-denatured calf thymus DNA binds well to the PVC plates possibly because of its extensive secondary structure and high molecular weight. Thus these antibodies are actually more specific for particular antigens than the SPRIA would suggest.

Comparison of binding constants to poly(dT) for the Fab fragment and whole IgG has shown that $K_{IgG} \simeq 75K_{Fab}$, which is a considerably smaller difference than the theoretical maximum. It should also be noted that this enhancement factor is several orders of magnitude smaller than that found in previous studies on the role of multivalency (Hornick & Karush, 1972; Gopalakrishnan & Karush, 1974). Morevoer, since the preferred thymine-rich sequence will only be found rarely in naturally occurring DNAs such as heat-denatured calf thymus, then it would be expected that $K_{IgG} \simeq 2K_{Fab}$. Thus both arms of an IgG molecule are unlikely to be bound to the same molecule of DNA, giving rise to the tendency to form large complexes. This conclusion is in opposition to that of Aarden et al. (1976), who, using antibodies to duplex DNA, showed that cross-linking was a rather rare event. However their studies were performed in concentrated CsCl solutions, which may have profound effects on the flexibility of IgG molecules, and also antibodies to duplex DNA tend to show less sequence specificity than the single strand specific antibodies described here (Stollar, 1975).

It is known that antibody–DNA complexes as opposed to other immune complexes are primarily responsible for mediating the tissue damage in SLE, but the reason for this is unclear (Koffler et al., 1971). We propose that a sequence-specific antibody binding to a random polymer (where the preferred sequence occurs rarely) will tend to form large complexes of the type that may be responsible for the tissue damage (Wilson & Dixon, 1971). We have recently prepared another 30 monoclonal antibodies from autoimmune NZB mice, and these too show considerable specificity in their interaction with various nucleic acids (unpublished results). Moreover, the majority of them have poly(dT) as the preferred antigen. It should also be noted that oligo(dT) sequences were the first antigenic sites described for human SLE serum antibodies (Stollar et al., 1962). This suggests that it may be possible to administer oligomers of poly(dT) that will inhibit the formation of large DNA-containing immune complexes in SLE, thereby suppressing the tissue damage. Experiments along these lines are now in progress.

Acknowledgments

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References

Aarden, L. A., De Groot, E. R., & Lakmaker, F. (1976) *J. Immunol. Methods 13*, 241–252.

Anderson, W. F., Ohlendorf, D. H., Takeda, Y., & Matthews, B. W. (1981) *Nature (London) 290*, 754–758.

Awdeh, Z. L., Williamson, A. R., & Askonas, B. A. (1968) *Nature (London) 219*, 66–67.

Crothers, D. M. (1968) *Biopolymers 6*, 575–584.

Crothers, D. M., & Metzger, H. (1972) *Immunochemistry 9*, 341–357.

Eilat, D., Schmuel, A., Sasson, B., & Laskov, R. (1980) *Eur. J. Immunol. 10*, 841–845.

Fox, K. R., Gauvreau, D., Goodwin, D. C., & Waring, M. J. (1980) *Biochem. J. 191*, 729–742.

Goeddel, D. U., Yansura, D. G., Winston, C., & Caruthers, M. H. (1978) *J. Mol. Biol. 123*, 661–687.

Gopalakrishnan, P. V., & Karush, F. (1974) *J. Immunol. 113*, 764–778.
Guiges, M., & Leng, M. (1976) *Eur. J. Biochem. 69*, 615–624.
Hahn, B. H. (1981) *Clin. Immunol. Allergy 1*, 583–631.
Harwood, S. J., & Wells, R. D. (1970) *J. Biol. Chem. 245*, 5625–5634.
Hornick, C. L., & Karush, F. (1972) *Immunochemistry 9*, 325–340.
Jacob, L., & Tron, F. (1982) *J. Immunol. 128*, 895–898.
Koffler, D., Carr, R., Agnello, V., Thoburn, R., & Kunkel, H. G. (1971) *J. Exp. Med. 134*, 294–312.
Kowalczykowski, C. S., Lonberg, N., Newport, J. W., & von Hippel, P. H. (1981) *J. Mol. Biol. 145*, 75–104.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.
Lafer, E., Rauch, J., Andrzejewski, C., Mudd, D., Furie, B., Furie, B., Schwartz, R., & Stollar, B. (1981a) *J. Exp. Med. 153*, 897–904.
Lafer, E. M., Moller, A., Nordheim, A., Stollar, B. D., & Rich, A. (1981b) *Proc. Natl. Acad. Sci. U.S.A. 78*, 3546–3550.
Lee, J. S., & Waring, M. J. (1978) *Biochem. J. 173*, 115–128.
Lee, J. S., Johnson, D. A., & Morgan, A. R. (1979) *Nucleic Acids Res. 6*, 3073–3091.
Lee, J. S., Lewis, J. R., Morgan, A. R., Mosmann, T. R., & Singh, B. (1981) *Nucleic Acids Res. 9*, 1707–1721.
Lin, S. Y., & Riggs, A. D. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 2574–2578.
Mage, M. G. (1980) *Methods Enzymol. 70*, 142–150.
McGhee, J. D., & von Hippel, P. H. (1974) *J. Mol. Biol. 86*, 469–489.
Morgan, A. R., Coulter, H. B., Flintoff, W. F., & Paetkau, V. H. (1974) *Biochemistry 13*, 1596–1603.
Morgan, A. R., Lee, J. S., Pulleyblank, D. E., Murray, N. L., & Evans, D. H. (1979) *Nucleic Acids. Res. 7*, 547–569.
Newport, J. W., Lonberg, N., Kowalczykowski, S. C., & von Hippel, P. H. (1981) *J. Mol. Biol. 145*, 105–121.
Nordheim, A., Pardue, M. L., Lafer, E. M., Moller, A., Stollar, B. D., & Rich, A. (1981) *Nature (London) 294*, 417–421.
Ogata, R., & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4973–4976.
Ptashne, M., Jeffrey, A., Johnson, A. D., Maurer, R., Meyer, B. J., Pabo, C. O., Roberts, T. M., & Sauer, R. T. (1980) *Cell (Cambridge, Mass.) 19*, 1–11.
Record, M. T., Lohman, T. M., & de Haseth, P. (1976) *J. Mol. Biol. 107*, 145–158.
Stollar, B. D. (1975) *CRC Crit. Rev. Biochem. 3*, 45–67.
Stollar, D., Levine, L., Lehrer, H. I., & Van Vunakis, H. (1962) *Proc. Natl. Acad. Sci. U.S.A. 48*, 874–880.
Tron, F., Charron, D., Bach, J., & Talal, N. (1980) *J. Immunol. 125*, 2805–2809.
Wilson, C. B., & Dixon, F. J. (1971) *J. Exp. Med. 134*, 7s–17s.

Ribonucleic Acid Precursors Are Associated with the Chick Oviduct Nuclear Matrix†

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ABSTRACT: Nuclear matrix was prepared by sequential treatment of oviduct nuclei with Triton X-100, DNase I, and 2 M NaCl. Published procedures were modified such that as many steps as possible were performed at −20 °C to minimize endogenous ribonuclease activity. Examination of electron micrographs confirmed the isolation of intact nuclear matrix structures. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the proteins in these structures showed an absence of histones and an enrichment of certain nonhistone proteins. RNA was isolated from the nuclear matrix preparations and subjected to denaturing gel electrophoresis. Gels were analyzed by ethidium bromide staining and by hybridization of Northern blots to cloned DNA probes for ovalbumin, ovomucoid, 5.8S ribosomal RNA, and U1 RNA. All of the precursors to ovalbumin and ovomucoid mRNAs (including various splicing intermediates) and all of the precursors to ribosomal RNA were associated exclusively with the nuclear matrix fraction. By contrast, mature ovalbumin and ovomucoid mRNAs were distributed between matrix and nonmatrix fractions. These observations were further supported by quantitative hybridization analysis of the RNA in nuclear and matrix fractions. It was found that less than 50% of the mature message of intact nuclei was recovered in the matrix, while most significantly, over 95% of the mRNA precursors remained associated with the matrix. Finally, mature ribosomal RNAs and virtually all of the small nuclear RNAs (including U1 RNA) were also distributed between matrix and nonmatrix fractions. Our results suggest that all precursor RNAs (be they precursors to mRNA or rRNA) are exclusively associated with the nuclear matrix and support the notion that the nuclear matrix may be the structural site for RNA processing within the nuclei of eucaryotic cells.

Intervening sequences have been observed in nuclear RNA transcripts of animals, plants, and lower eucaryotes and in RNA transcripts encoded by eucaryotic viruses (Abelson, 1979; Roop et al., 1978). Although not all eucaryotic RNA transcripts are interrupted by intervening sequences [such as those for U1 RNA, histones, or interferon (Roop et al., 1981; Schaffner et al., 1978; Nagata et al., 1980)], removal of intervening sequences by RNA splicing appears to be a general mechanism by which the majority of functional mRNAs are manufactured in eucaryotic cells.

Earlier studies have demonstrated that a large fraction of

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