aggregation of the proteins during the delipidation procedure. These results indicate that the antigenic structures of apoC-III between HDL and VLDL are similar. Since more than 85% of the immunoreactivity of apoC-III is located in the COOH-terminal half (residues 41-79), we suggest that the orientation of this part of the molecule in HDL and VLDL is probably identical.

References

Atassi, M. Z. (1975) *Immunochemistry 12*, 423.

Atassi, M. Z., & Lee, C. L. (1978) *Biochem. J. 171*, 429.

Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466.

Bethune, J. L., Ulmer, D. D., & Vallee, B. L. (1964) *Biochemistry 3*, 1964.

Bradley, W. A., & Gotto, A. M. (1978) in *Disturbances in Lipid* and *Lipoprotein Metabolism*. (Dietschy, J. M., Gotto, A. M., & Ontko, J., Eds.) pp 113-137, American Physiology Society, Bethesda, MD.

Brewer, H. B., Jr., Shulman, R. S., Herbert, P. N., Ronan, R., & Wehrly, K. (1974) *J. Biol. Chem. 249*, 4975.

Davies, B. J. (1964) *Ann. N.Y. Acad. Sci. 121*, 404.

Jackson, R. L., Morrisett, J. D., & Gotto, A. M. (1976) *Physiol. Rev. 56*, 259.

Jackson, R. L., Lin, H. Y., Mao, S. J. T., Chan, L., & Means, A. R. (1977) *Endocrinology 101*, 849.

Klee, W. A., & Richards, F. M. (1957) *J. Biol. Chem. 229*, 489.

MacFarlane, A. S. (1964) in *Mammalian Protein Metabolism* (Munro, M. W., & Allison, J. B., Eds.) p 331, Academic Press, New York.

Mao, S. J. T., Gotto, A. M., & Jackson, R. L. (1975) *Biochemistry 14*, 4127.

Mao, S. J. T., Sparrow, J. T., Gilliam, E. B., Gotto, A. M., & Jackson, R. L. (1977) *Biochemistry 16*, 4150.

Morrisett, J. D., David, J. S. K., Pownall, H. J., & Gotto, A. M. (1973) *Biochemistry 12*, 1290.

Morrisett, J. D., Jackson, R. L., & Gotto, A. M. (1977) *Biochim. Biophys. Acta 472*, 93.

Schonfeld, G., & Pfleger, B. (1974) *J. Clin. Invest. 54*, 236.

Schonfeld, G., Chen, J. S., McDonnell, W. F., & Jeng, I. (1977a) *J. Lipid Res. 18*, 645.

Schonfeld, G., Chen, J. S., & Roy, R. G. (1977b) *J. Biol. Chem. 252*, 6651.

Shulman, R. S., Herbert, P. N., Fredrickson, D. S., Wehrly, K., & Brewer, H. B., Jr. (1974) *J. Biol. Chem. 249*, 4969.

Smith, L. C., Pownall, H. J., & Gotto, A. M. (1978) *Annu. Rev. Biochem. 47*, 751.

Sparrow, J. T., Pownall, H. J., Hsu, F. J., Blumenthal, L. D., Culwell, A. R., & Gotto, A. M. (1977) *Biochemistry 16*, 5427.

Immunochemical Study of the Structure of Poly(adenylic acid)†

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ABSTRACT: Antibodies specific for poly(adenylic acid) (M_r ~700 000) [poly(A)] were generated by immunization of rabbits with poly(A) covalently linked to bovine serum albumin via the 3′-terminal residue of the polynucleotide. The antibodies precipitated with poly(A) as well as with nucleotide–protein conjugates, regardless of the purine or pyrimidine base, but not with conjugates of nucleosides. Precipitation was also seen with DNA and RNA and with polyribose phosphate. Agglutination of *Hemophilus influenzae* type b, which has a capsule of polyribose phosphate, could also be demonstrated. In a competitive binding assay, the antibodies bound poly(A) with an affinity at least 4 orders of magnitude greater than that of oligo(adenylic acids) as large as A_{10}. Cross-reaction was seen with poly(I), poly(C), and poly(G) and with polyribose phosphate. Unlike antibodies generated by poly(A)–MBSA, the specificity of these antibodies was directed at ribose phosphate residues, suggesting a conformation of poly(A) in solution in which the purine residues are stacked "inside" a single-stranded helix with the ribose phosphate residues extending outward. This conformation is apparently disrupted by reaction with MBSA. Earlier physicochemical studies are in agreement with the type of structure derived from our data. Poly(A) can, therefore, be envisaged as a dense cloud of negative charges arranged in a helical array. There is evidence that the conformation of poly(A) in RNA is the same; therefore, any theory for the role of the poly(A) "tail" in mRNA should take into account the contribution of a highly negatively charged segment at the 3′ end. These studies support the efficacy of using immunochemical methods to study nucleotide conformation if care is taken to ensure that the conformation of the nucleotide in the antigen is in the native state.

The ability of antibodies to recognize conformation in polypeptides and proteins is well established (Gerwing & Thompson, 1968; Young & Leung, 1970; Brown, 1962; Lehrer & Van Vunakis, 1965; Arnon, 1973). Similar recognition of polynucleotide conformation comes from immunological studies on ribosomal RNA (Souleil & Panijel, 1968) and on double- and triple-helical oligonucleotides (Stollar, 1975), as well as from experiments with antibodies to specific oligonucleotide sequences (D'Alisa & Erlanger, 1974, 1976). With respect to the latter, it was shown that antibodies specific for AAA cross-reacted only slightly with A and poly(A) and not at all with denatured DNA, despite the fact that AAA is the triplet coden for lysine. Thus, the conformation of AAA must be unlike that of the triplet in DNA or in poly(A) and it is

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not even recognized as a sequence containing A. Similar findings were made with antibodies to AUG (D'Alisa & Erlanger, 1974, 1976).

In an effort to understand more about the effect of nucleotide conformation on immunoreactivity, we prepared antibody to poly(A). Attempts by others to prepare anti-poly(A) by direct immunization with the homopolymer were unsuccessful [cf. Yachnin (1962)]. Immunization with a poly(A)–MBSA[1] complex (Plescia et al., 1964) yielded antibodies that could distinguish between single-stranded and multistranded polynucleotides and between deoxy- and ribonucleotides and DNA–RNA hybrids (Lacour et al., 1968; Seaman et al., 1965; Stollar & Raso, 1974). Other properties of these sera will be discussed later. We chose to follow a different methodology, namely, to immunize with a conjugate in which the 3′-terminal residue of poly(A) was covalently linked to a carrier protein. It was our working hypothesis that, under these circumstances, poly(A) would be more likely to assume its "native" conformation than when its negatively charged phosphates were neutralized by the polybasic protein MBSA. The findings described herein support these expectations and illustrate the potential of immunological techniques in the investigation of the conformation of polynucleotides in solution.

Experimental Procedures

Conjugation of Poly(A) to BSA. Poly(adenylic acid) (potassium salt; Miles) had an s_{20} value of 9.73 at pH 7, which corresponds to a molecular weight of 700 000 (Fresco & Doty, 1957). It was dialyzed against water overnight at 4 °C to remove low molecular weight contaminants and then lyophilized. Forty-one milligrams (0.059 μmol) was dissolved in 1 mL of water and to it was added 0.5 mL of 0.1 M $NaIO_4$. The solution was kept for 20 min in the dark at room temperature and then dialyzed against 700 mL of water for 45 min with vigorous stirring, to remove the excess periodate. The oxidized poly(A) was then transferred to a tube containing a solution of 5 mg of BSA (0.072 μmol) in 0.5 mL of water; the solution had been previously adjusted to pH 9.5 with 5% K_2CO_3. The reaction was allowed to proceed for 45 min with occasional mixing, the pH being maintained at 9.5 with 5% K_2CO_3. Then 2.4 mg of $NaBH_3CN$ (Aldrich) (0.038 mmol), freshly dissolved in 0.1 mL of water, was added. After 1 h at room temperature, the solution was dialyzed against several changes of water over a period of 48 h.

The extent of conjugation was determined by precipitation with anti-BSA prepared in sheep. Increasing volumes of antisera were added to solutions of either poly(A), BSA, or the presumptive conjugate and allowed to stand at 4 °C overnight. The solutions were then centrifuged in a Sorvall SS-1 rotor at 10000*g* for 10 min at 4 °C. The supernatants were decanted and diluted to 30 mL with PBS. The pellet was dissolved in 0.1 M glycine hydrochloride buffer, pH 2.8, to a final volume of 3 mL. The UV spectra of the supernatants and of the redissolved precipitates were examined, and the 260 nm/280 nm ratios were determined. When 400 μL of antiserum was added to 100 μL of conjugate [containing ca. 1.6 mg of poly(A)], almost no UV-absorbing material was observed in the supernatant. The poly(A) could be accounted for in the precipitate. In control experiments, no precipitation of poly(A) alone by anti-BSA occurred.

In the procedure described above for the synthesis of the conjugate, an 850-fold excess of periodate over poly(A) was used. This excess was necessary in order to allow the oxidation to take place within the 20-min period. Smaller quantities of periodate (for example, a 10-fold excess) resulted in the conjugation of less than 20% of poly(A), as determined by precipitation with anti-BSA.

Immunization Procedures. New Zealand white rabbits were immunized in each toepad with 0.1 mL of a 1:1 mixture of antigen (2 mg/mL) in 0.15 M NaCl and complete Freund's adjuvant (Difco) for 3 weeks at weekly intervals. Bleeding by cardiac puncture was begun on the fourth week and boosting was done at monthly intervals. Bleedings between boosts were pooled. The course of immunization was followed by observing whether precipitation occurred within 48 h after adding 20 μL of a poly(A) solution (1.9 mg/mL) to 200 μL of serum. Distinct precipitates were observed after 3 months of immunization.

Preparation of Globulin Fractions of Antisera. Sera were fractionated by using sodium sulfate precipitation (Strauss et al., 1960). The final precipitate was dissolved in water and dialyzed against repeated changes of saline until the addition of $BaCl_2$ to the dialysate did not produce a precipitate. The globulin fractions were sterile filtered and stored at 4 °C or frozen.

Immunoelectrophoresis and Gel Diffusion. Immunoelectrophoresis and gel diffusion were conducted in 1.5% agar gels prepared in 0.05 M sodium barbital buffer, pH 8.2. This buffer was also used as the electrolyte. Electrophoresis of anti-poly(A) was performed on a water-cooled plate at 5 mA per 7.5 × 5 cm slide and monitored by observing the migration of albumin stained with Evans blue. After the electrophoresis was completed, poly(A) and goat antirabbit serum were each placed in separate lateral troughs, and the plates were placed in a humid box at 4 °C. After 3 days, when precipitation was complete, the slides were washed and stained (Uriel, 1964). The poly(A)–anti-poly(A) precipitate appeared in the region of migration of IgG. It should be noted that the poly(A) preparation of M_r 700 000 did not seem to penetrate the gels sufficiently. Decreasing the agar concentration by half, substituting 0.5% agarose for agar, or using phosphate buffer (pH 7.6) did not help. Only shearing of the poly(A) by 20 passages through a 26-gauge needle allowed it to migrate sufficiently to produce a precipitate in the gel.

Quantitative Precipitin Analysis. Microquantitative precipitins were performed as previously described (Kabat, 1961). The ninhydrin procedure for protein determination could not be used because poly(A) reacted with ninhydrin, producing high background readings. Therefore, protein was determined by using a modification of the method of Bradford (1976). Two hundred microliters of distilled water was added to the washed precipitates and to various control tubes. Then, 3 mL of a 1:5 dilution of the Dye Reagent Concentrate (Bio-Rad) was added, vortexed, and incubated for 20 min at room temperature. The absorbance of the solution at 595 nm was then determined in a Gilford 2000 spectrophotometer. Known amounts of BSA were used as calibration standards. The preparation used in the radioimmunoassay studies was found to have 0.15 mg of Ab/mL.

Radioimmunoassays. [^{3}H]Poly(I) (19 mCi/mmol P; 50 000–100 000 M_r) was purchased from Schwarz/Mann;

[1] Abbreviations used: poly(A)–MBSA, a complex between poly(adenylic acid) and bovine serum albumin (BSA) in which the carboxyl groups of BSA have been esterified with methyl alcohol; TBS, 0.01 M Tris-HCl, pH 7, 0.15 M NaCl, and 0.1% gelatin; PBS, 0.01 M phosphate buffer–0.85% NaCl, pH 7.2; A–RSA, AMP–HSA, etc., conjugates in which 10–15 molecules of the cited ribonucleoside (-tide) are covalently attached to one molecule of rabbit serum albumin (RSA), human serum albumin (HSA), or ovalbumin (Ova); 5-MeCyt, 5-methylcytosine; SSC, 0.15 M NaCl–0.15 M trisodium citrate.

[^{3}H]poly(dA), [^{3}H]poly[d(A-T)] (21.5 Ci/mol P; about 700 000 M_r), A_2, and A_4 were obtained from Miles Laboratories; poly[[^{3}H](A-T)] (507 Ci/mol P) was from B. Lubit (Lubit & Erlanger, 1978); A_3 and A_5–A_{10} were purchased from Boehringer-Mannheim. Polyribose phosphate was a gift of Dr. J. Robbins of the National Institutes of Health. The oligoadenylates were kept in frozen stock solutions, the concentrations of which were determined from the absorbance at 257 nm. Extinction coefficients were taken from Table I and Figure 3 of Brahms et al. (1966).

Poly[^{3}H]adenylic acid sodium salt was purchased from Amersham, and the same lot was used throughout the experiments. A stock solution in 0.1% ethanol was stored frozen and diluted in water before use. Its specific activity was given as 580 mCi/mmol nucleotide residue, and it had a molecular weight range of 13 000–45 000 (38–137 nucleotide residues). Approximately 6000 cpm or (5–10) $\times 10^{-14}$ mol was used in the radioimmunoassay.

The binding of [^{3}H]poly(A) to a globulin fraction of anti-poly A (R 476 IV) was investigated by using a membrane binding procedure. To 100 μL of a solution of [^{3}H]poly(A) was added 25 μL of antiserum of various dilutions in TBS. Control tubes containing TBS instead of antiserum were set up in each experiment. The solutions were allowed to incubate at 37 °C for 30 min after which 4 mL of SSC was added to each tube. The contents of each tube was then filtered by suction through a HAWP (25 mm) Millipore filter, followed by two 4-mL washes with SSC and one with water. The filters were dried and placed in vials with 5 mL of a toluene–Omnifluor cocktail (New England Nuclear). Counting was usually for 10 min in a Searle Isocap 300 liquid scintillation counter. By use of the external standard ratio (ESR), the efficiency was calculated to be above 50%.

Maximal binding (20–24%) was found at 1:200 dilution. Unsuccessful attempts to increase binding included denaturation of the poly(A) by heating to 100 °C for 10 min in 1% formaldehyde, followed by rapid cooling, purification of the radioactive poly(A) on a P-2 column or by ethanol precipitation, or increasing the amount of poly(A) in the reaction tube by 50%. On the other hand, extending the incubation time from 30 min to 16 h at 37 °C resulted in binding of 45% of the poly(A); after 24 h, 54% was bound. No additional increase occurred after 48 h. These results can be explained by the kinetics and equilibrium of binding at low concentrations of both antigen and antibody. [Poly(A) was at 5×10^{-10} M and the antibody, at a dilution of 1:200, was at the same concentration.]

For the competitive binding experiments, a serum dilution of 1:400 was used, and the sequence of addition was as follows: hapten (50 μL) and then antiserum (25 μL, diluted in TBS); after a 5-min incubation at 37 °C, [^{3}H]poly(A) (50 μL) was added, followed by a 1-h incubation at 37 °C. The contents of the tubes were then transferred to filters, washed, and counted as described above. Any variation in the sequence of addition gave inconsistent results.

Results

Preparation and Characterization of the Poly(A)–BSA Conjugate. The poly(A)–BSA conjugate used for immunization was prepared by a modification of the periodate procedure of Erlanger & Beiser (1964) using BSA that was shown previously to have undetectable amounts of ribonuclease. By UV spectrophotometry, the conjugate was found to have one poly(A) molecule per molecule of BSA, assuming a molar absorbance of an A residue at 257 nm of 9000 (Brahms et al., 1966) and a molecular weight of 700 000. All of the poly(A) was precipitable by an anti-BSA serum.

Table I: Qualitative Tube Precipitation Tests[a]

positive	positive	negative
poly(A)	poly(I)	A-RSA
A_{10}	DNA[b] native	G-RSA
AMP-HSA	DNA[b] heat denatured	U-RSA
ATP-RSA	RNA[c] native	C-RSA
ApA-RSA	RNA[c] heat denatured	5-MeCyt-OVa
GMP-HSA	polyribose phosphate[d]	T-RSA
CpG-RSA		

[a] The precipitation tests were performed by addition of 20 μL of antigen (1–2 mg/mL) to 200 μL of globulin preparation of anti-poly(A). Tubes were examined after 24 and 48 h. Preimmune sera were negative. [b] Calf thymus, highly polymerized; denaturation was by heating to 100 °C for 10 min and then cooling in ice. [c] Yeast RNA; denaturation was as described in footnote *b*. [d] Capsular material from *H. influenzae*, type b.

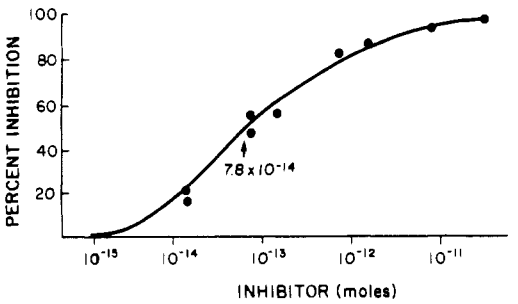

FIGURE 1: Inhibition of binding of [^{3}H]poly(A) by poly(A).

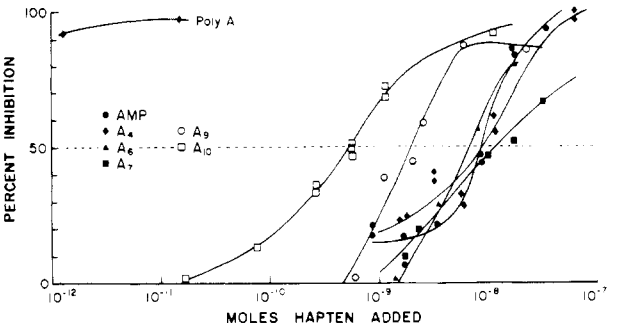

FIGURE 2: Inhibition of binding of [^{3}H]poly(A) by various oligonucleotides.

Shown in Table I are the results of precipitation tests between anti-poly(A) and a number of antigens. Precipitation was seen with poly(A) and A_{10}; with respect to protein conjugates, precipitation was seen with all nucleotide conjugates, regardless of the purine or pyrimidine base, but with none of the nucleoside conjugates including A–RSA. Precipitation was also seen with DNA and RNA and, qualitatively, more seemed to occur with the native forms. Finally, precipitation was also seen with polyribose phosphate. These results were confirmed by two-dimensional gel diffusion studies (not shown).

The antibody was mainly IgG (see Experimental Procedures).

Radioligand Binding Studies. The results of competitive binding studies between unlabeled poly(A) and [^{3}H]poly(A) are shown in Figure 1. From this curve, the 50% inhibition concentration is 7.8×10^{-14} mol of poly A (about 6×10^{-10} M). This value is in agreement with the amount of [^{3}H]-poly(A) bound in direct binding studies (see Experimental Procedures).

The results of competition studies with oligonucleotides are shown in Figure 2. A_{10} is the best inhibitor but it is about

Table II: Competitive Binding Studies with Oligonucleotides

inhibitor	M_r	amount for 50% inhibn (μg)
poly(A)	7×10^5	0.05 (0.6 nM)
poly(I)	5×10^5	0.06 (1.0 nM)
poly(G)	5×10^5	0.08 (ca. 1.3 nM)
polyribose phosphate	not known	0.3
poly(dA)	3.5×10^4	1.8 (0.4 μM)

4 orders of magnitude less potent than poly(A). A_9 is somewhat less active than A_{10}; the other oligoadenylates are clustered at a concentration ~1.5–2 orders of magnitude higher than that of A_{10}.

Competition could also be shown with other polynucleotides (Table II). Within experimental error, poly(I) and poly(G) bound to antibody as well as did poly(A). In agreement with the precipitation data in Table I, polyribose phosphate also interacted well with anti-poly(A). On the other hand, poly(dA) was a relatively poor inhibitor.

In direct binding studies, the antibody at 1:200 and 1:400 dilutions could be shown to bind poly(I) (in agreement with data in Table II) and poly[d(A-T)] but did not bind poly(A-T), poly(dA), adenosine, AMP, ADP, ATP, and deoxyadenosine (data not shown).

Reaction with Hemophilus influenzae Type b. Polyribose phosphate is a major component of the capsule of *H. influenzae* type b (Zamenhof et al., 1953). Since the polysaccharide precipitated with anti-poly(A), agglutination of the microorganism was tested for and found to be positive up to a dilution titer of 512.

Discussion

The immunological procedures used in these experiments were chosen with the aim of producing antibodies responsive to the conformation of poly(A) in solution. For this reason, poly(A) was covalently linked by its 3′-terminal nucleotide residue to a protein carrier rather than being complexed with MBSA, as was done by others. In polynucleotide–MBSA complexes, ion pairing occurs between the basic groups of MBSA and the phosphates of the polynucleotide, a reaction likely to affect the conformation of the polynucleotide. The experimental data show that the antibodies described in this paper differ markedly from those produced by immunization with MBSA complexes. For example, Lacour et al. (1968) reported that anti-poly(A)–MBSA reacted with poly(A) but not with poly(I) or poly(C). The same was reported for an anti-poly(A) serum isolated from rabbits immunized with anti-poly(A)–poly(U)–MBSA (Nahon et al., 1972). High specificity for the purine or pyrimidine base is the general rule for antibodies elicited by MBSA complexes of polynucleotides. An anti-poly(I)–MBSA did not react with poly(A), poly(U), or poly(C) (Lacour et al., 1968; Nahon et al., 1972; Guigues & Leng, 1976). Similarly, anti-poly(G)–MBSA was specific for poly(G) (Michelson et al., 1971).

The anti-poly(A) produced in these studies showed markedly different properties. (a) It was highly specific for a conformation of poly(A) that was not assumed by AMP, by short nucleotides, or by poly(dA). Even A_{10} was about 4 orders of magnitude less active an inhibitor than poly(A). The higher molecular weight of poly(A) (700 000 vs. 4100 for A_{10}) would give more immunodeterminants per molecule but at most this would account for a 150-fold difference, i.e., the ratio of the molecular weights. In reality, steric problems owing to the bulky antibody molecule would diminish this advantage markedly. (b) The adenine residues did not appear to be immunodeterminants since poly(I), poly(C), and poly(G) bound equally as well as poly(A). (c) The polyribose phosphate backbone was a major determinant as shown by the binding of polyribose phosphate and the agglutination of *H. influenzae* type b. The precipitation data in Table I also support the contribution of the phosphate groups; i.e., nucleotide–BSA conjugates are precipitated; nucleoside–BSA conjugates are not. Incidentally, the data in Table I showing precipitation with AMP–BSA do not contradict the data on AMP in the competitive binding experiments (Figure 1). It has been shown (Hornic & Karush, 1972; Golapakrishnan & Karush, 1974) that binding constants for a polyvalent antigen can be 3–4 orders of magnitude greater than for the monovalent hapten in solution. Precipitation with polyvalent antigens frequently occurs with sera that cannot be shown to bind the corresponding hapten by radioimmunoassay. Significant binding by radioimmunoassay can be demonstrated only with antibodies with relatively high binding constants.

The immunological data, therefore, point to a conformation of poly(A) near neutrality in which the adenine residues are not exposed, as, for example, if the bases are stacked on the "inside" of a structure from which the ribose phosphates extend outward. Physicochemical studies on solutions of poly(A) provide support for this conformation, as do X-ray crystallographic experiments. For example, Witz & Luzzati (1965) found that, at 25 °C and pH ≥ 6, poly(A) is a single-stranded helix with the bases stacked perpendicular to the axis. The same kind of structure is suggested by Leng & Felsenfeld (1966). Van Holde et al. (1965) came to a similar conclusion from CD measurements and proposed a model in which the ribose phosphates can be seen to extend outward along the helix. In this regard, it is interesting that A_9 and A_{10} are better inhibitors of anti-poly A than AMP, A_4, A_6, and A_7, all of which are clustered together in Figure 2. This enhanced activity coincides with the ability to assume one helical turn of the single-stranded coil. These results are reminiscent of earlier findings with oligopeptides in which reaction with an antibody to a polypeptide occurred only with oligopeptides of a size sufficient to allow assumption of a helical structure (Sela, 1969). Similarly, (dinitrophenyl)oligolysines were antigenic only if they were long enough to assume a helical structure (Schlossman et al., 1965).

The immunological cross-reactions observed with other homopolymers imply that their conformations are similar to that of poly(A). The physicochemical data are in reasonable agreement with our findings. Fasman et al. (1964) and Witz & Luzzati (1965) found that poly(C) is very much like poly(A), i.e., a single-stranded, base-stacked helix. With respect to poly(I), Thiele & Guschlbauer (1973), using CD measurements, found poly(I) to be a single-strand "poorly stacked" helix at 20 °C, in 0.1 M NaCl. Our data would indicate that the stacking interactions do not play a minor role in poly(I) structure. The cross-reaction between anti-poly(A) and poly(G) is not so easily understood. At room temperature, poly(G) has been found to exist as a four-stranded helix in which four guanine residues are mutually hydrogen bonded (Howard et al., 1977; Zimmerman et al., 1975; Arnott et al., 1974). A single-stranded form has been seen at 92 °C (Howard et al., 1977). However, it is more likely that anti-poly(A) is reacting with the ribose phosphate groups of the four-stranded helix.

Cross-reaction was also seen with poly[d(A-T)], which, by X-ray crystallography, is a double-strand polymer that forms a right-handed, eightfold antiparallel helix with an axial rise of 3.04 Å (Arnott et al., 1974). The bases are stacked so that

the purines and pyrimidines overlap and are hydrogen bonded. The helix is densely packed, more so than the B-DNA. If this structure also exists in solution, its phosphate residues will be available for reaction with antibody. It is interesting that antibodies to poly[d(A-T)] cross-reacted with poly(A) (Lubit & Erlanger, 1978).

Another finding in our studies is the lack of substantial cross-reaction with poly(dA). There is good evidence that its conformation differs from that of poly(A) (Ts'o et al., 1966; Adler et al., 1967). Our results indicate that the differences are marked and not just a matter of different stacking angles as suggested by others.

In summary, our immunochemical data, along with physicochemical evidence, indicate that the outer surface of poly(A) is made up of an array of closely packed ribose phosphate groups and, hence, can be seen as a dense cloud of negative charges. There is evidence that this also holds true for poly(A) sequences in RNA. In pioneering experiments (Panijel et al., 1966a,b; Souleil & Panijel, 1968), antibody specific for poly(A) was obtained from an antiribosome serum by precipitation with poly(A) and subsequent dissociation. This anti-poly(A) preparation, like ours, precipitated with poly(I), poly(C), and poly(G). In short, it showed specificity for "antigenic sites that are probably located along the polyribose phosphate backbone" (Panijel et al., 1966a). Thus, any explanation of the function of poly(A) in mRNA should take this conformation into account. It is interesting that most histone mRNAs do not have poly(A) at the 3′ end [cf. Borun et al. (1977)]. A dense cloud of negative charges at the 3′ end of histone mRNA could interfere with its 5′ to 3′ translation and release of the highly positively charged histone molecules. Preliminary experiments to test the ability of anti-poly(A) to bind various mRNAs were frustrated by the presence of ribonuclease in the antisera. The sera are being treated to remove all traces of ribonuclease in order to pursue this line of experimentation further.

In conclusion, immunization with a poly(A)–BSA conjugate, in which attachment is via the 3′ end of the nucleotide, results in antibody that recognizes a conformation of poly(A) in solution for which there is considerable physicochemical evidence. Our results, therefore, support the efficacy of using immunochemical procedures for the study of the structure of polynucleotides and nucleic acids but only if the antigen is prepared in a way that conserves the native conformation of the polynucleotide.

References

Adler, A., Grossman, L., & Fasman, G. D. (1967) *Proc. Natl. Acad. Sci. U.S.A. 57*, 423–430.

Arnon, R. (1973) in *The Antigens* (Sela, M., Ed.) pp 87–159, Academic Press, New York and London.

Arnott, S., Chandrasekaran, R., & Marttila, C. M. (1974) *Biochem. J. 141*, 537–543.

Borun, T. W., Ajiro, K., Zweidler, A., Dolby, J. W., & Stephens, R. E. (1977) *J. Biol. Chem. 252*, 173–180.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Brahms, J., Michelson, A. M., & Van Holde, K. E. (1966) *J. Mol. Biol. 15*, 467–488.

Brown, R. K. (1962) *J. Biol. Chem. 237*, 1162–1167.

D'Alisa, R., & Erlanger, B. F. (1974) *Biochemistry 13*, 3575–3579.

D'Alisa, R., & Erlanger, B. F. (1976) *J. Immunol. 116*, 1629–1634.

Erlanger, B. F., & Beiser, S. M. (1964) *Proc. Natl. Acad. Sci. U.S.A. 52*, 68–74.

Fasman, G., Lindblow, C., & Grossman, L. (1964) *Biochemistry 3*, 1015–1021.

Fresco, J. R., & Doty, P. (1957) *J. Am. Chem. Soc. 79*, 3928–3929.

Gerwing, J., & Thompson, K. (1968) *Biochemistry 7*, 3888–3892.

Gopalakrishnan, P. U., & Karush, F. (1974) *J. Immunol. 113*, 769–778.

Guigues, M., & Leng, M. (1976) *Eur. J. Biochem. 69*, 615–624.

Hornic, C., & Karush, F. (1972) *Immunochemistry 9*, 325–340.

Howard, F. B., Frazier, J., & Miles, H. T. (1977) *Biopolymers 16*, 791–809.

Kabat, E. A. (1961) *Experimental Immunochemistry*, 2nd ed., Charles C Thomas, Springfield, IL.

Lacour, F., Michelson, A. M., & Nahon, E. (1968) in *Nucleic Acids in Immunology* (Plescia, O. J., & Braun, W., Eds.) pp 32–46, Springer-Verlag, New York.

Lehrer, H. I., & Van Vunakis, H. (1965) *Immunochemistry 2*, 255–262.

Leng, M., & Felsenfeld, G. (1966) *J. Mol. Biol. 15*, 455–466.

Lubit, B. W., & Erlanger, B. F. (1978) *Immunochemistry 15*, 663–671.

Michelson, A. M., Lacour, F., & Nahon-Merlin, E. (1971) *C. R. Hebd. Seances Acad. Sci., Ser. D 272*, 669–672.

Nahon, E., Michelson, A. M., & Lacour, F. (1972) *Biochim. Biophys. Acta 149*, 127–139.

Panijel, J., Souleil, C., & Cayeux, P. (1966a) *Science 152*, 773–775.

Panijel, J., Souleil, C., & Cayeux, P. (1966b) *Biochim. Biophys. Acta 123*, 221–234.

Plescia, O. J., Braun, W., & Palczuk, N. C. (1964) *Proc. Natl. Acad. Sci. U.S.A. 52*, 279–285.

Schlossman, S. F., Yaron, A., Ben-Efraim, S., & Sober, H. A. (1965) *Biochemistry 4*, 1638–1645.

Seaman, E., Van Vunakis, H., & Levine, L. (1965) *Biochemistry 4*, 1312–1318.

Sela, M. (1969) *Science 166*, 1365–1374.

Souleil, C., & Panijel, J. (1968) *Biochemistry 7*, 7–13.

Stollar, B. D. (1975) *CRC Crit. Rev. Biochem. 3*, 45–69.

Stollar, B. D., & Raso, V. (1974) *Nature (London) 250*, 231–234.

Strauss, A. J. L., Seegal, B. C., Hsu, K. C., Burkholder, P. M., Nastuck, W. L., & Osserman, K. E. (1960) *Proc. Soc. Exp. Biol. Med. 105*, 184–191.

Thiele, D., & Guschlbauer, W. (1973) *Biophysik 9*, 261–271.

Ts'o, P. O. P., Rapoport, S. A., & Bollum, F. J. (1966) *Biochemistry 5*, 4153–4170.

Uriel, J. (1964) in *Immunoelectrophoretic Analysis* (Grabar, P., & Burtin, P., Eds.) pp 30–81, Elsevier, Amsterdam.

Van Holde, K. E., Brahms, J., & Michelson, A. M. (1965) *J. Mol. Biol. 12*, 726–739.

Witz, J., & Luzzati, V. (1965) *J. Mol. Biol. 11*, 620–630.

Yachnin, S. (1962) *Nature (London) 195*, 1319.

Young, J. D., & Leung, C. Y. (1970) *Biochemistry 9*, 2755–2762.

Zamenhof, S., Leidy, G., Fitzgerald, P. L., Alexander, H. E., & Chargaff, E. (1953) *J. Biol. Chem. 203*, 695–704.

Zimmerman, S. B., Cohen, G. H., & Davies, D. R. (1975) *J. Mol. Biol. 92*, 181–192.