Maelicke, A., Haar, F. v. d., Sprinzl, M., and Cramer, F. (1975), *Biopolymers 14,* 155.
Olson, W. (1975a), *Biopolymers 14,* 1775.
Olson, W. (1975b), *Biopolymers 14,* 1795.
Perahia, D., Pullman, B., and Saran, A. (1974), *Biochim. Biophys. Acta 340,* 299.
Quigley, G. J., and Rich, A. (1976), *Science 194,* 796.
Remin, M., and Shugar, D. (1972), *Biochem. Biophys. Res. Commun. 48,* 636.
Rosenberg, J. M., Seeman, N. C., Kim, J. J. P., Suddath, F. L., Nicholas, H. B., and Rich, A. (1973), *Nature (London) 243,* 150.
Rubin, J., Brennan, T., and Sundaralingam, M. (1972), *Biochemistry 11,* 3112.
Sarma, R. H., Lee, C. H., Evans, F. E., Yathindra, N., and Sundaralingam, M. (1974), *J. Am. Chem. Soc. 96,* 7337.
Sarma, R. H., Mynott, R. J., Wood, D. J., and Hruska, F. E. (1973), *J. Am. Chem. Soc. 95,* 6457.
Schleich, T., Cross, B. P., and Smith, I. C. P. (1976), *Nucleic Acids Res. 3,* 355.
Schweizer, M. P., Broom, A. D., Ts'o, P. O. P., and Hollis, D. P. (1968), *J. Am. Chem. Soc. 90,* 1042.
Schweizer, M. P., and Kreishman, G. P. (1973), *J. Magn. Resonance* 9, 334.
Schweizer, M. P., Witkowski, J. T., and Robins, R. K. (1971), *J. Am. Chem. Soc. 93,* 277.
Secrist, J. A., III, Barrio, J. R., Leonard, N. J., and Weber, G. (1972), *Biochemistry 11,* 3499.
Singh, H., Herbut, M. H., Lee, C. H., and Sarma, R. H. (1976), *Biopolymers 15,* 2167.
Smith, I. C. P., Mantsch, H. H., Lapper, R. D., Deslauriers, R., and Schleich, T. (1973), Proceedings of the International Symposium on Conformation of Biological and Molecular Polymers, Symposium on Quantum Chemistry and Biochemistry, Bergmann, E. D., and Pullman, B., Ed., Vol. 5, New York, N.Y., Academic Press, p 345.
Son, T. D., and Guschlbauer, W. (1975), *Nucleic Acids Res. 2,* 873.
Suck, D., Manor, P. C., Germain, G., Schwalbe, C. H., Weimann, G., and Saenger, W. (1973), *Nature (London), New Biol. 246,* 161.
Sundaralingam, M., Mizuno, H., Stout, C. D., Rao, S. T., Liebman, M., and Yathindra, N. (1976), *Nucleic Acids Res. 3,* 2471.
Sussman, J. L., Seeman, N. C., Kim, S. H., and Berman, H. M. (1972), *J. Mol. Biol. 66,* 403.
Tolman, G. L., Barrio, J. R., and Leonard, N. J. (1974), *Biochemistry 13,* 4869.
Ts'o, P. O. P., Kondo, N. S., Schweizer, M. P., and Hollis, D. P. (1969), *Biochemistry 8,* 997.
Warshaw, M. M., and Cantor, C. R. (1970), *Biopolymers 9,* 1079.
Warshaw, M. M., and Tinoco, I., Jr. (1965), *J. Mol. Biol. 13,* 54.
Warshaw, M. M., and Tinoco, I., Jr. (1966), *J. Mol. Biol. 20,* 29.
Watts, M. T. (1977), Ph.D. Thesis, University of California, Berkeley.
Wood, D. J., Hruska, F. E., Mynott, R. J., and Sarma, R. H. (1973), *Can. J. Chem. 51,* 2571.

Interaction of Oligoribocytidylates with T7 DNA in Neutral and Acid Media†

Marie-Thérèse Sarocchi-Landousy,* Barbara L. Haas,‡ and Wilhelm Guschlbauer*

ABSTRACT: Oligoribocytidylates of chain length 4 to 12 were found to interact with native T7 DNA at neutral and slightly acid pH. The results suggest that binding occurred at deoxycytosine clusters which may be displaced by the oligomers at neutral pH, while a local triple-stranded structure would be formed at acid pH. Transcription of DNA-$(Cp)_n$ complexes by *Escherichia coli* RNA polymerase showed a decrease in level without affecting the specificity of the transcription, suggesting that oligocytidylate binding did not occur on the promoters.

Many reports concern the binding of short oligonucleotides to single-stranded nucleic acids: for example, to 5S RNA to determine the length and location of loops in the secondary structure (Lewis & Doty, 1970); to charged or uncharged tRNA to reproduce interactions similar to the codon-anticodon associations (Uhlenbeck et al., 1970; Danchin & Grunberg-Manago, 1970; Schimmel et al., 1972); or to denatured DNA to study interactions between the complementary strands (McConaughy & McCarthy, 1967). Niyogi & Thomas (1967) and Rüger & Bautz (1968) have also used RNA fragments varying in length to study the recurrence of specific sequences in natural DNA.

It would be interesting to deal with a double-stranded polymer or DNA—instead of a single-stranded one as previously shown—to mimic replicative or transcriptional processes. Only a few reports have appeared on that topic. Holloman et al. (1975) have observed the binding of deoxyribooligonucleotides to ϕX 174 supercoiled DNA. They suggest that such complexes may simulate early steps in genetic recombination. More recently, it has been shown that RNA can hybridize to double-stranded DNA in the presence of formamide (Thomas et al., 1976). One of the DNA strands is displaced and a stable RNA-DNA structure, called R-loop, is formed.

† From the Service de Biochimie, Centre d'Etudes Nucléaires de Saclay, F-91190, Gif sur Yvette, France. *Received January 10, 1977; revised manuscript received July 21, 1977.* This paper is part 21 in the series Protonated Polynucleotide Structures. Part 20 is by Haas et al. (1976).

‡ Present address: Department of Pathology, Washington University School of Medicine, St. Louis, Mo. 63110.

It remains after removal of formamide or treatment of the DNA by restriction endonucleases.

We have observed that oligoribocytidylates of short length can bind to native DNA under neutral conditions. Many indications led us to suppose that the interaction takes place mainly at dG and dC clusters and that, according to the pH and ionic conditions, there may be a displacement of the dC sequences by the oligonucleotides. With synthetic polymers of the G-C series, we have observed a similar displacement reaction (Haas et al., 1976). In fact, mixtures of poly(dG)·poly(dC) and poly(rC) lead, at neutral pH and high ionic strength, to the formation of a poly(dG)·poly(rC) hybrid. The typical triple-stranded structure observed for poly(purine)·poly(pyrimidine) polymers, i.e. one purine strand for two pyrimidine strands, was also found at lower pH. Moreover, these triple-stranded complexes remain stable, even if the purine strand is formed of monomeric elements (Sarocchi et al., 1970). For the A-T or A-U series, however, Morgan and Wells (1968) observed only hybrid triplexes in which poly(rU) interacted specifically with poly(dA)·poly(dT) and poly(rUC) with poly(dT-dC)·poly(dG-dA).

As a working hypothesis, we supposed that dG and dC clusters of natural DNAs would behave like synthetic dG·dC oligomers and could thus react with short oligocytidylates. For this reason, we chose T7 DNA which is known to be rich in repetitive dG and dC sequences (Summers & Szybalski, 1968; Mushynski & Spencer, 1970). The biological significance of such clusters is not yet demonstrated. They are thought to be involved in enzymatic reactions, possibly in transcription, since the transcribed strand is enriched in dC sequences (Summers & Szybalski, 1968). With synthetic polymers, a decrease in transcription by *Escherichia coli* RNA polymerase was observed when the third strand was added (Morgan & Wells, 1968; Murray & Morgan, 1973). We therefore also studied RNA synthesis with the T7 DNA-$(Cp)_n$ complexes as matrix.

Materials and Methods

Polymers. T7 DNA was prepared by phenol treatment of T7 phages according to Richardson (1966). It contained neither nicks nor gaps as shown by alkaline sucrose density gradient analysis, S1 nuclease assays, and tests with different RNA polymerases (*E. coli* holoenzyme and core enzyme, yeast enzyme B), the matrix specificities of which are well known.

Labeled and unlabeled oligoribocytidylates were obtained by alkaline hydrolysis of [^{3}H]poly(rC) (Miles, Elkhart) and poly(rC) (Choay, Paris), respectively, and subsequent column chromatography on DEAE[1]-cellulose (Haas, 1975). Oligouridylates came from Miles.

Enzymes. E. coli RNA polymerases were gifts from A. Ruet (Saclay). Both the holoenzyme (E^{σ}) and the core enzyme (E) were pure proteins as seen by gel electrophoresis. Yeast enzyme B was from S. Dezélée (Saclay) who performed the tests. RNase III was prepared by J. L. Darlix (Genève) (Darlix, 1975).

Methods. Mixtures of T7 DNA, at concentrations of about 10^{-3} M, and oligomers were made in Tris-HCl buffer (20 mM, pH 8.0) and NaCl of different molarities. For melting experiments a sodium acetate buffer was used as indicated in the figure legends.

[1] Abbreviations for synthetic polynucleotides conform to the IUPAC-IUB Rules ((1971) *J. Mol. Biol. 55,* 299). Other abbreviations are: CD, circular dichroism; Cl_3AcOH, trichloroacetic acid; DEAE, diethylaminoethyl; EDTA, ethylenediaminetetraacetic acid.

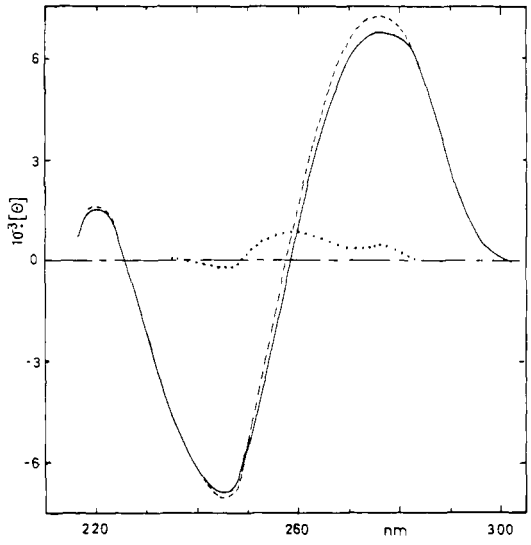

FIGURE 1: CD spectra at pH 7.5 and 0.05 M NaCl for the interaction between T7 DNA and $(Cp)_5$. $(Cp)_5$/DNA = 2%: (- - -) sum of components; (—) experimental spectrum of the complex; (• • •) difference spectrum.

Sucrose density gradient analysis was performed on T7 DNA samples (50 μg) plus [^{3}H]oligoribocytidylates ($(Cp)_n$/DNA = 11.5%; 27.9 μCi/μmol of P) in linear concentration gradients, 5–15% (w/v) sucrose and 0.05 M NaCl. Centrifugations were run in an SW-65 Ti rotor, for 3.5 h at 35 000 rpm. Fractions of 320 μL were collected and counted in toluene-based scintillation fluid containing 30% Triton X-100.

Enzymatic assays were carried out in standard buffer (40 mM Tris-HCl (pH 7.9)–10 mM $MgCl_2$–0.1 mM EDTA–6 mM 2-mercaptoethanol–50 mM NaCl) and with the four nucleoside triphosphates (0.1 mM) with [^{3}H]UTP or [^{32}P]-GTP (35 000 counts min^{-1} $nmol^{-1}$). RNA synthesis was measured after 15 min of incubation at 37 °C by acid precipitation with 5% Cl_3AcOH and filtration through nitrocellulose filters (HAWP 025 Millipore).

Circular dichroism was measured on a Jobin-Yvon dichrograph III. Absorbance spectra were recorded on a Zeiss DMR 10 spectrophotometer.

Results

(I) *Physico-chemical Study of the Interaction between T7 DNA and Oligoribocytidylates.* At neutral pH, interaction between T7 DNA and oligoribocytidylates was evidenced by circular dichroism measurements and sucrose density gradient analysis. Dichroic spectra of mixtures between T7 DNA and oligocytidylates differed from those of the sum of the reference components (Figure 1). If oligocytidylates were replaced by oligouridylates, both spectra superposed exactly. Neutral sucrose density gradient analyses, performed under the same ionic and pH conditions, clearly demonstrated that binding of oligocytidylates to DNA took place (Figure 2).

This interaction persisted at lower pH. It was, however, less pronounced if the mixture was directly made at a pH between 3.0 and 6.0. Small variations were observed as a function of ionic strength (in the range of 0.01 to 0.5 M NaCl) and oligocytidylate chain length. We therefore chose oligocytidylates, between 4 and 12 residues long, in order to reduce self-association (Haas, 1975) and to get fragments of length about equal to T7 dG and dC clusters (Mushynski & Spencer, 1970). From the amplitude of CD difference spectra it was concluded

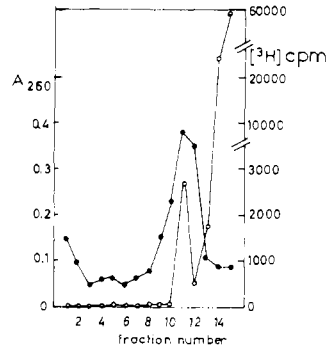

FIGURE 2: Sucrose gradient analysis of T7 DNA-$[^3H](Cp)_6$ complexes. DNA = 50 μg; $[^3H](Cp)_6$/DNA = 11.5%. Fractions were collected from the bottom of the gradient: (●) A_{260}; (○) ^3H cpm.

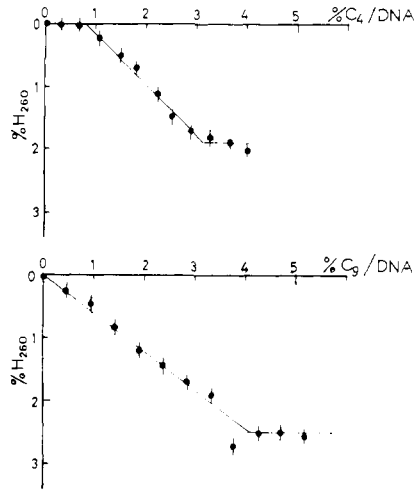

FIGURE 3: Percentage hypochromicity of T7 DNA as a function of $(Cp)_n$ at pH 7.5 and 0.05 M NaCl.

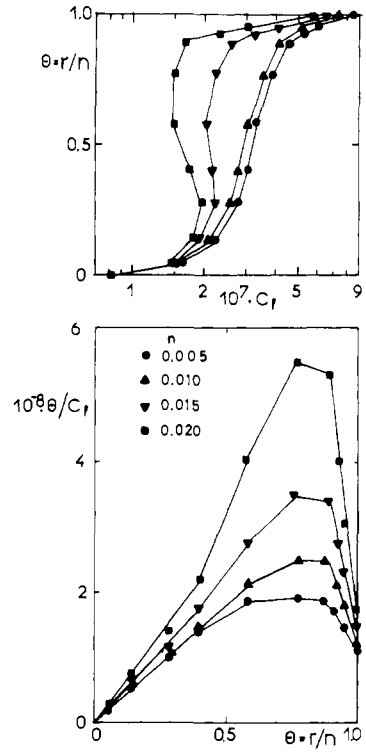

FIGURE 4: Hill (a, top) and Scatchard plot (b, bottom) of interaction of $(Cp)_4$ with T7 DNA using the data in Figure 3 at different values of saturation binding n: (●) $n = 0.005$; (▲) $n = 0.01$; (▼) $n = 0.015$; (■) $n = 0.02$. For the convenience of plotting all the data on the same scale, θ/C_f was plotted against θ, instead of r/C_f vs. r in the Scatchard plot, and θ vs. C_f in the Hill plot.

that oligomers with 4 or 5 residues interacted better with DNA than longer ones at high ionic strength and neutral pH.

The variations of the DNA spectrum with varying amounts of oligomers were followed by ultraviolet differential absorbance. Binding of oligomers to DNA provoked a hypochromic change. The percentage of hypochromicity at 260 nm vs. relative concentrations of oligocytidylates to DNA (Figure 3) reached a saturation plateau at about 3–4% both for $(Cp)_4$ and $(Cp)_9$. This is in the range of the known percentage and length of repetitive dG and dC sequences in T7 DNA (Mushynski & Spencer, 1970). In addition, it should be noted that the nonamers interacted with DNA even at very low $(Cp)_9$/DNA ratios while $(Cp)_4$–DNA complexes could only form at $(Cp)_4$/DNA ratios beyond 1%. This was also corroborated by enzymatic assays (see below).

If the hypochromicity data were presented on a semilogarithmic plot, sigmoid curves were obtained, characteristic of cooperative interactions, i.e. that oligomers in excess were necessary for the binding to take place. For this reason, no quantitative binding data could be obtained from the sucrose density gradient data (Figure 2).

In order to obtain the binding constant from Hill or Scatchard plots, the amount of bound and free oligocytidylates at each point has to be known, i.e. the saturation binding value (n) must be determined. This value is, however, inaccessible by experimentation. An upper estimate can be obtained from the break point in Figure 3, i.e. it must be less than 3% for $(Cp)_4$, or $n < 0.03$. Hill plots with $n = 0.005$, 0.01, 0.015, and 0.02 are shown in Figure 4a. Clearly, the two higher values of n yield physically meaningless results. Similarly, the Scatchard plots (Figure 4b) show a break in the linear part of the plot. On the other hand, $n = 0.005$ or 0.01 give very similar Hill and Scatchard plots, indicating that $n \simeq 0.01$ is probably correct. Both values give a $K \simeq 3 \times 10^8$ M^{-1} at the midpoint of the transition (Schwarz, 1976).

The nonamer, which showed binding already at very low concentrations, yielded Hill and Scatchard plots which indicated two steps: a noncooperative followed by a cooperative one (the cooperativity of which was much smaller than for $(Cp)_4$, and $n \simeq 0.02$). As pointed out by Schwarz (1976), such data can be analyzed "under favorable circumstances". The precision of the data certainly does not warrant such analysis.

What is the nature of the interaction at neutral pH? Two hypotheses may be proposed, keeping in mind our suggestion that the sequences concerned are the dG and dC clusters.

(1) A hybrid deoxyribo-ribopolymer is formed. This implies that the system $(Cp)_n$–DNA behaves like the synthetic polymer poly(dG)·poly(dC) plus poly(rC) under high ionic conditions (Haas et al., 1976), i.e. that repetitive dG and dC sequences behave like oligomers. While oligocytidylates did not displace the poly(dC) strand of the poly(dG)·poly(dC) (Haas et al., 1976), we should consider that the ratios of length between the displaced sequence and the oligomer which displaces may intervene. Holloman et al. (1975) have shown that single-stranded fragments of DNA could associate to superhelical φX 174 DNA by a displacement reaction. And it is known that, in the G-C series, RNA–DNA hybrids are stronger than DNA–DNA duplexes (Chamberlin, 1965).

(2) A local three-stranded $(Cp)_n$–DNA complex is formed. Under our experimental conditions this triplex does not exist for synthetic polymers (Haas et al., 1976). But one cannot

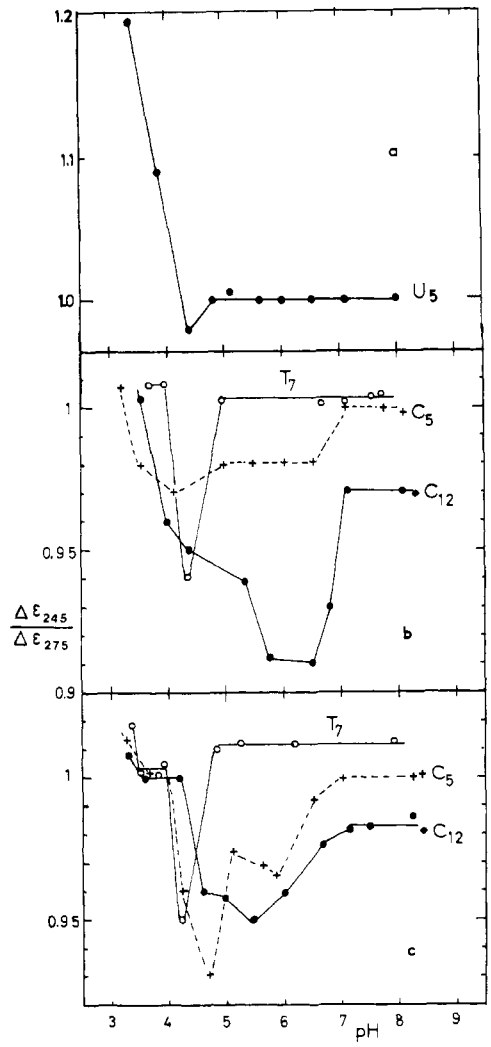

FIGURE 5: Variations of CD spectra at 245 and 275 nm as a function of pH, at 0.05 M (a,b) or 0.25 M NaCl (c): (a) DNA-$(Up)_5$; (b and c) (○) T7 DNA; (+) DNA-$(Cp)_5$; (●) DNA-$(Cp)_{12}$. $(Up)_5$/DNA or $(Cp)_n$/DNA = 2%.

assume that pairing in natural DNAs is quite the same as in polynucleotides and therefore that such an association does not occur. Small conformational changes related to the fine structure of the nucleic acid do exist (Arnott et al., 1975) and probably intervene in the binding reactions.

The shape of the CD difference spectra was a good tool to deduce the nature of the interaction between poly(dG)·poly(dC) and poly(rC) (Haas et al., 1976). The amplitudes here were, however, too small to allow comparison of spectral shape. Nevertheless, variations between the DNA reference and the complex DNA-$(Cp)_n$ were observed by plotting the ratios of the dichroic signals at 245 and 275 nm as a function of pH (Figure 5). (In this range of pH, this ratio is almost zero for oligocytidylates alone.) For example, at 0.05 or 0.25 M NaCl and between pH 7.2 and 6.0, in the case of the DNA-$(Cp)_n$ solution, a transition was obtained which might correspond to the protonation of free dC sequences. The same change in titration behavior was observed with poly(dG)·poly(dC) and poly(rC) for solutions in which hybrid formation had occurred (Haas et al., 1976). This suggested that, at neutral pH, some dC sequences might be displaced by oligocytidylates with a local opening of the DNA helix, while a three-stranded complex could form at acid pH.

Figure 5a shows that, under the same conditions, mixtures of oligouridylates and T7 DNA did not present any variation

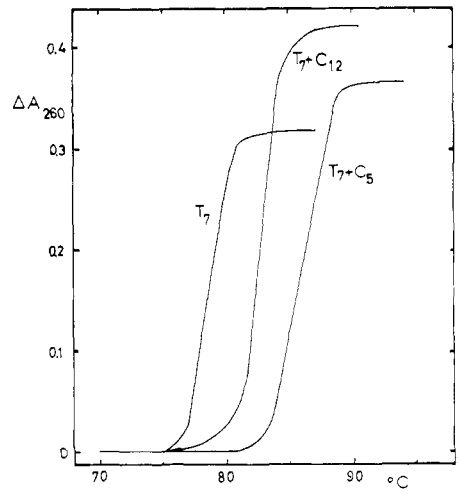

FIGURE 6: Melting curves of T7 DNA and DNA-$(Cp)_n$ complexes at pH 5.8 and 0.05 M NaCl. $(Cp)_n$/DNA = 2%.

beyond pH 4.5 when DNA itself varied. This confirms the binding of oligocytidylates to DNA and argues in favor of an interaction at dG and dC sequences.

The melting curves of the mixtures of DNA plus oligocytidylates agree with the two forms of complexes, according to pH values, as suggested before. At neutral pH (pH 7.0) there was no difference in melting points between DNA and DNA-$(Cp)_n$ solutions, which is not surprising if we assume that short ribo sequences displace short deoxyribo sequences. On the contrary, an increase was observed for DNA plus oligoribonucleotide samples at acid pH (Figure 6). At pH 5.8 and 0.05 M NaCl, the T_m was 79 °C for T7 DNA, 83 °C for DNA-$(Cp)_{12}$, and 86 °C for DNA-$(Cp)_5$. This result suggests that a triplex did exist locally when pH was below 6.0. The fact that DNA-$(Cp)_5$ "melts" at higher temperature than DNA-$(Cp)_{12}$ probably reflects the more perfect fit of the smaller oligoribocytidylates to the dG and dC clusters: the mean length of the repetitive sequences is closer to 5 than to 12 (Mushynski & Spencer, 1970). Nevertheless, the nature of the interaction at neutral pH remained uncertain. Enzymatic assays on T7 DNA-$(Cp)_n$ complexes could possibly distinguish between the two hypotheses suggested. We tried to determine it by measuring their transcription.

(II) *Enzymatic Tests on T7 DNA-$(Cp)_n$ Complexes.* Enzymatic assays on T7 DNA-$(Cp)_n$ complexes evidenced a decrease in level of the transcription without affecting the specificity. For convenient enzymatic conditions we studied only the effect of oligocytidylate binding upon transcription above pH 7.0.

Transcription of DNA-$(Cp)_n$ complexes varied according to the length of the oligomers. The longer ones, $(Cp)_7$, $(Cp)_9$, and $(Cp)_{12}$, provoked an inhibition of RNA synthesis (Figure 7)—the longer the oligomer, the stronger the decrease of transcription. With short oligonucleotides, $(Cp)_4$ and $(Cp)_5$, a slight increase of RNA synthesis was observed at low concentrations while higher concentrations also showed inhibition of transcription. Taking into account the data in Figure 3, this increase in RNA synthesis corresponds to the "nonbinding" of small oligomers at low concentrations while higher amounts induced binding of oligocytidylates to DNA, which provoked a decrease in the level of RNA synthesized.

Control experiments were performed in order to verify that enzymes were not trapped on DNA-$(Cp)_n$ complexes or bound to the free oligonucleotides, which would explain the decrease in transcription. RNA polymerase was first preincubated with

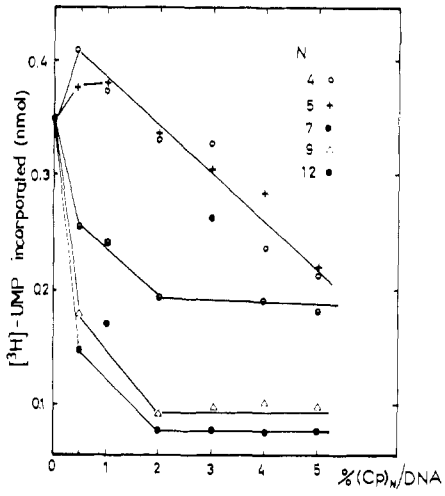

FIGURE 7: Transcription of DNA-$(Cp)_n$ complexes by *E. coli* RNA polymerase (DNA = 3.5 μg; E^{σ} = 0.75 μg).

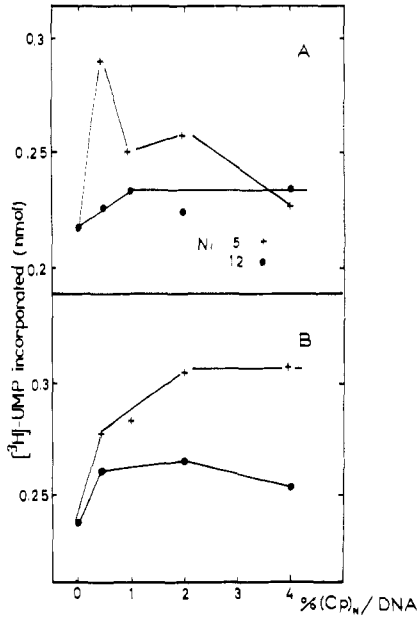

FIGURE 8: Transcription of T7 DNA (2.5 μg) after preincubation of the enzyme (E^{σ}) (0.75 μg) with $(Cp)_n$ (A) and with T7 DNA (B).

the oligomers for 5 min; then DNA and the four nucleoside triphosphates were added (Figure 8A). In another assay, RNA polymerase was preincubated with DNA alone; then the oligocytidylates and the four nucleoside triphosphates were added (Figure 8b). A stimulation of RNA synthesis was observed in both cases; this increase was larger with $(Cp)_4$ than with $(Cp)_9$ or $(Cp)_{12}$. It demonstrated that the enzyme was not trapped locally on the DNA molecule. Furthermore, oligonucleotides which were not bound to the DNA were shown to increase the transcription level.

Our results resemble those of Morgan & Wells (1968) and Murray & Morgan (1973) on triplexes formed with synthetic polymers. This is rather in favor of a local triple-stranded complex. Similar results would be expected, however, if the DNA was opened at precise sequences. Experiments with *E. coli* RNA polymerase without σ factor (core enzyme) or with yeast enzyme B clearly demonstrated that DNA strand separation took place and that hybrid duplexes were formed. In fact, neither enzyme can transcribe an intact double-stranded DNA matrix (Burgess et al., 1969; Vogt, 1969; Dezélée et al.,

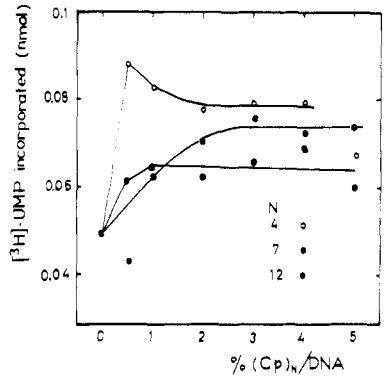

FIGURE 9: Transcription of T7 DNA-$(Cp)_n$ complexes by *E. coli* core enzyme (DNA = 3.5 μg; E = 0.75 μg).

1974). When oligomers were bound to DNA (Figure 9) we observed an increase in the level of transcription by *E. coli* core enzyme. Similarly, RNA synthesis by yeast enzyme B was raised by a factor of 3 (not shown). This suggested that binding of oligoribocytidylates at neutral pH induced an opening of the DNA helix where oligomers might replace some dC sequences to form RNA-DNA duplexes. (Control experiments with oligomers alone ($n \leq 12$) showed that they were not transcribed.)

E. coli RNA polymerase with σ factor binds to T7 DNA at specific sites (Dausse et al., 1972; Minkley & Pribnow, 1973; Bordier & Dubochet, 1974). Polyacrylamide gel electrophoresis in the presence of RNase III was performed on RNA synthesized by *E. coli* holoenzyme to determine if the observed inhibition of RNA synthesis was related to one specific site of T7 DNA (Studier, 1973; Darlix, 1975; Dausse et al., 1975). The three small initiator RNAs proceeding from the three strong promoters of T7 DNA early region and the RNA fragments resulting from specific transcription were observed both for the DNA reference and for the complexes in similar proportions (not shown). We may thus conclude that oligoribocytidylates did not interact specifically at T7 promoters.

Dinucleoside phosphates are known to allow RNA initiation when the concentration of the four nucleoside triphosphates is quite reduced in the reaction mixture (Minkley & Pribnow, 1973). It was also shown that, sometimes, short oligomers may serve as primers for *E. coli* RNA polymerase and thus increase the transcription level (Niyogi, 1969; Yefimova et al., 1975). Under the same conditions, oligoribocytidylates bound to DNA were almost not used as primers. There was only a slight increase in RNA synthesis, about 30% for $(Cp)_4$ and 15% for $(Cp)_7$ when the transcription of DNA-$(Cp)_n$ complexes was compared with that of the DNA reference. This finding is understandable if one supposes that oligocytidylates were complexed to the dG sequences, while the transcribed strand is richer in dC sequences (Summers and Szybalski, 1968).

Discussion

Small oligoribocytidylates, 4 to 12 residues long, were shown to interact with duplex T7 DNA. Under the same ionic and pH conditions, complexes were not formed with oligouridylates. In addition, binding curves of the DNA-oligocytidylates interaction showed that binding took place at about 1% of the DNA. This suggests that interaction might essentially take place at dG and dC clusters (Mushynski & Spencer, 1970).

The nature of the association varied according to the pH conditions. The similar behavior of the titration curves both for T7 DNA-$(Cp)_n$ complexes (Figure 5) and for the system poly(dG)·poly(dC) plus poly(rC) (Haas et al., 1976) suggests

that, at neutral pH, oligoribocytidylates might displace repetitive deoxyribocytosines with local strand separation. This hypothesis was supported by assays with RNA polymerases initiating RNA synthesis preferentially at nonperfect duplex sequences (Figure 9). At lower pH, a triple-stranded complex could form according to the classical scheme: one purine strand/two pyrimidine strands.

Ionic strength influenced binding at neutral pH. At low NaCl concentrations, oligomers were easily complexed since the DNA structure is more flexible, but a minimal concentration was necessary for short oligonucleotides ($n = 4$ or 5) to bind. At high ionic strength, the DNA molecule is more compact, which would favor the binding of short oligomers because of a decrease in charge barrier, but at the same time disadvantaged DNA-$(Cp)_{n>7}$ complexes since the strands open less easily. In all cases an excess of oligomers was necessary to obtain maximal interaction.

The dichroic difference signals obtained were not very intense, but they exceeded largely what was expected for a structural modification affecting about 1 or 2% of the DNA molecule. This suggests that the interaction not only affected the bound sequences themselves, but also perturbed the structure on both sides of these sequences. This agrees with the work of Burd et al. (1975) on synthetic polymers $(dC_{20}dA_{10})\cdot(dT_{10}dG_{20})$ and $(dC_{20}dA_{15})\cdot(dT_{15}dG_{20})$ and Early et al. (1977) on $(dC_{15}dA_{15})\cdot(dT_{15}dG_{15})$. They demonstrated that (dA)·(dT) sequences are influenced by the conformational stability of close-by (dG)·(dC) sequences. In the same line, locally formed hybrids or triplexes may act on both sides of the interaction as structural inducers. While natural DNAs are in the B form, all polynucleotide triplexes (Arnott and Selsing, 1974) and hybrid duplexes (Milman et al., 1967) have been found to be in the A form.

RNA synthesized by *E. coli* RNA polymerase on T7 DNA-$(Cp)_n$ matrices showed a decreased level, but the specificity of the transcription process was not affected. The same results were obtained with [^{32}P] GTP or [^{3}H]UTP. This is strong evidence that the three promoter sites were not concerned with the binding of oligoribocytidylates. Pribnow (1975) has recently determined the sequence and the localization of two of the three T7 early promoters. They do not show a particular enrichment in dG or dC sequences, which would explain why no change in specific initiation was observed.

On the contrary, one cannot exclude that dG and dC clusters may intervene in processes concerned with late RNA synthesis. Most of the chains initiated by T7 RNA polymerase begin precisely with the triplet pppGpGpA (McAllister et al., 1973; Bishayee et al., 1976). In addition, it was shown by Chamberlin & Ring (1973) that this enzyme possesses particular affinity for poly(dG)·poly(dC). It therefore should interact with repetitive dG and dC sequences of T7 DNA and it would be interesting to test T7 DNA-$(Cp)_n$ complexes with T7 RNA polymerase.

Acknowledgments

We are indebted to A. Ruet and S. Dezélée for their gift of *E. coli* RNA polymerases and yeast enzyme B, respectively, and to J. L. Darlix for RNase III. A. Sentenac is gratefully acknowledged for reading this manuscript.

References

Arnott, S., Chandrasekharan, R., & Selsing, E. (1975) *Struct. Conform. Nucleic Acids Protein-Nucleic Acid Interact., Proc. Annu. Harry Steenbock Symp., 4th, 1974,* 577-596.

Arnott, S., & Selsing, E. (1974) *J. Mol. Biol. 88,* 509-521.

Bishayee, S., Yudelevich, A., & Maitra, U. (1976) *Biochem. Biophys. Res. Commun. 68,* 626-633.

Bordier, C., & Dubochet, J. (1974) *Eur. J. Biochem. 44,* 617-624.

Burd, J. F., Larson, J. L., & Wells, R. D. (1975) *J. Biol. Chem. 250,* 6002-6007.

Burgess, R. R., Travers, A. A., Dunn, J. J., and Bautz, E. K. F. (1969) *Nature (London) 221,* 43.

Chamberlin, M. (1965) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 24,* 1446-1457.

Chamberlin, M., & Ring, J. (1973) *J. Biol. Chem. 248,* 2235-2244.

Danchin, A., & Grunberg-Manago, M. (1970) *FEBS Lett. 9,* 327-330.

Darlix, J. L. (1975) *Eur. J. Biochem. 51,* 369-376.

Dausse, J. P., Sentenac, A., & Fromageot, P. (1972) *Eur. J. Biochem. 26,* 43-49.

Dausse, J. P., Sentenac, A., & Fromageot, P. (1975) *Eur. J. Biochem. 57,* 569-578.

Dezélée, S., Sentenac, A., & Fromageot, P. (1974) *J. Biol. Chem. 249,* 5978-5983.

Early, T. A., Kearns, D. R., Burd, J. F., Larson, J. E., & Wells, R. D. (1977) *Biochemistry 16,* 541-551.

Haas, B. L. (1975) Thèse de Doctorat 3rd cycle, Paris sud-Orsay, France.

Haas, B. L., & Guschlbauer, W. (1975) *Nucleic Acids Res. 2* (Suppl 1), s89-s92.

Haas, B. L., Sarocchi, M. T., & Guschlbauer, W. (1976) *Nucleic Acids Res. 3,* 1549-1559.

Holloman, W. K., Wiegand, R., Hoessli, C., & Radding, C. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72,* 2394-2398.

Lewis, J. B., & Doty, P. (1970) *Nature (London) 225,* 510-512.

McAllister, W. J., Kupper, H. A., & Bautz, E. K. F. (1973) *Eur. J. Biochem. 34,* 481-501.

McConaughy, B. L., & McCarthy, B. J. (1967) *Biochim. Biophys. Acta 149,* 180-189.

Milman, G., Langridge, R., & Chamberlin, M. J. (1967) *Proc. Natl. Acad. Sci. U.S.A. 57,* 1804-1810.

Minkley, E., & Pribnow, D. (1973) *J. Mol. Biol. 77,* 255-277.

Morgan, A. R., & Wells, R. D. (1968) *J. Mol. Biol. 37,* 63-80.

Murray, N. L., & Morgan, A. R. (1973) *Can. J. Biochem. 51,* 436-449.

Mushynski, W. E., & Spencer, J. H. (1970) *J. Mol. Biol. 52,* 91-106

Niyogi, S. K. (1969) *J. Biol. Chem. 244,* 1576.

Niyogi, S. K., & Thomas, C. A. (1967) *Biochem. Biophys. Res. Commun. 26,* 51-57.

Pribnow, D. (1975) *J. Mol. Biol. 99,* 419-443.

Richardson, C. C. (1966) *J. Mol. Biol. 15,* 49-61.

Rüger, W., & Bautz, E. K. F. (1968) *J. Mol. Biol. 31,* 83-90.

Sarocchi, M. T., Courtois, Y., & Guschlbauer, W. (1970) *Eur. J. Biochem. 14,* 411-421.

Schimmel, P. R., Uhlenbeck, O. C., Lewis, J. B., Dickson, C. K., Eldred, E. W., & Schreier, A. A. (1972) *Biochemistry 11,* 642-646.

Schwarz, G. (1976) *Biophys. Struct. Mech. 2,* 1-12.

Studier, F. W. (1973) *J. Mol. Biol. 79,* 237-248.

Summers, W. C., & Szybalski, W. (1968) *Biochim. Biophys. Acta 166,* 371-380.

Szybalski, W., Kubinski, H., & Sheldrick, P. (1966) *Cold*

Spring Harbor Symp. Quant. Biol. 31, 123-128.
Thomas, M., White, R. L., & Davis, R. W. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73,* 2294-2298.
Uhlenbeck, O. C., Baller, J., & Doty, P. (1970) *Nature (London) 225,* 508-510.
Vogt, V. (1969) *Nature (London) 223,* 854.
Yefimova, L. Y., Knorre, V. L., Savinkova, L. K., & Salganik, R. I. (1975) *FEBS Lett. 58,* 359-362.

Phase Separation of Acidic and Neutral Phospholipids Induced by Human Myelin Basic Protein†

J. M. Boggs,* M. A. Moscarello, and D. Papahadjopoulos

ABSTRACT: Differential scanning calorimetry was used to detect lipid phase separation induced in mixtures of acidic and neutral phospholipids by myelin basic protein from human central nervous system myelin. Phosphatidic acid, phosphatidylglycerol, and phosphatidylserine mixtures with phosphatidylcholine (PC) were used and were shown to be nearly randomly mixed in the absence of the protein. Incorporation of basic protein into these mixtures caused a shift in the phase transition temperature toward the temperature of the PC component, indicating that it binds and separates out the acidic lipid leaving a PC-enriched phase. In some cases, a transition due to the acidic lipid-basic protein complex was also observed. The shift toward the transition temperature of the PC component occurred regardless of whether the PC was the lower melting or the higher melting component of the mixture. Thus, the protein does not just bind to the lipid which melts first, but binds to the acidic lipid even if it melts at a much higher temperature than the neutral lipid. If enough acidic lipid is available, the protein can bind to 27-34 molecules of acidic lipid per molecule of protein. At pH 7.4 basic protein has 38 basic residues; thus, nearly all of these can be involved in electrostatic binding to acidic lipid polar head groups resulting in lipid phase separation.

There is abundant evidence which indicates that both lipids and proteins are asymmetrically distributed across certain cell membranes, but it is not clear how this asymmetry is induced and maintained (Rothman and Lenard, 1977). Early x-ray diffraction studies showed that the repeat distance of myelin was equivalent to the thickness of two membranes, indicating an asymmetric arrangement within each of the membranes (Schmitt et al., 1941; Blaurock and Worthington, 1969). Determination of the electron density profiles of myelin suggested that this asymmetry occurs both at the polar head group-aqueous interface and in the hydrocarbon region of the membrane (Caspar and Kirshner, 1971). It was suggested that the asymmetry in the hydrocarbon region was due to an asymmetric distribution of cholesterol, although it is equally possible that it is caused by an asymmetric distribution of other lipids and/or intrinsic proteins. Specific binding of proteins to certain lipid classes resulting in phase separation could be one way in which lipid asymmetry is induced and maintained. In order to investigate this possibility, we are studying the ability of myelin proteins to induce lipid phase separation when incorporated individually into lipid vesicles containing both acidic and neutral phospholipids randomly mixed. We reported earlier that a hydrophobic intrinsic protein, lipophilin, isolated from the proteolipid of human CNS myelin, is embedded in the hydrocarbon region and immobilizes some lipid surrounding it in the form of "boundary lipid" (Boggs et al., 1976). This protein causes phase separation by binding the acidic lipid preferentially to its boundary layer (Boggs et al., 1977).

In this study, we present evidence using differential scanning calorimetry (DSC) which indicates that the basic protein or encephalitogenic component from myelin, which interacts with lipid primarily by electrostatic interactions, can also cause lipid phase separation in a mixture of acidic and neutral lipids by binding to the polar head groups of acidic lipids.

Materials and Methods

Preparation of Basic Protein. Myelin was isolated from normal human white matter and basic protein extracted from it by the method of Lowden et al. (1966). The protein was stored in the lyophilized form.

Lipids. Phosphatidylserine (PS)[1] was isolated from bovine brain, phosphatidic acid (PA) was obtained from egg yolk phosphatidylcholine, and dimyristoylphosphatidic acid (DMPA) from dimyristoylphosphatidylcholine (DMPC) as described earlier (Papahadjopoulos and Miller, 1967; Papahadjopoulos et al., 1976). Phosphatidylglycerol (PG) was prepared enzymatically from egg phosphatidylcholine by a modification of the method of Dawson (1967). Dipalmitoylphosphatidylglycerol (DPPG) and dimyristoylphosphatidylcholine (DMPC) were synthesized as described earlier (Papahadjopoulos et al., 1973).

These lipids were stored in chloroform under nitrogen in sealed ampules at −50 °C. Dipalmitoylphosphatidylcholine (DPPC) was purchased from Serdary Research Laboratory

† From the Department of Biochemistry, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada (J.M.B. and M.A.M.), and the Department of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, New York (D.P.). *Received July 5, 1977.* This work was supported by grants from the Medical Research Council of Canada and the National Institutes of Health (U.S.A.) (No. 6M-18921) and by a postdoctoral fellowship from the Multiple Sclerosis Society of Canada for J.M.B.

[1] Abbreviations used: PS, phosphatidylserine; PA, phosphatidic acid; DMPA, dimyristoylphosphatidic acid; DMPC, dimyristoylphosphatidylcholine; PG, phosphatidylglycerol; DPPG, dipalmitoylphosphatidylglycerol; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; DSC, differential scanning calorimetry.