Biochemistry

Volume 15, Number 4 February 24, 1976

Polynucleotide Displacement Reactions: Detection by Interferon Induction†

Erik De Clercq, Paul F. Torrence, and Bernhard Witkop*

ABSTRACT: A large variety of displacement reactions between homopolynucleotides and complexes thereof has been demonstrated by interferon induction data obtained in primary rabbit kidney cell cultures superinduced with metabolic inhibitors. The polymers involved in these helix–coil displacement studies were: poly(adenylic acid), poly(inosinic acid), poly(cytidylic acid), poly(uridylic acid), poly(ribothymidylic acid), polylaurusin, poly(7-deazaadenylic acid), poly(7-deazainosinic acid), poly(5-bromocytidylic acid), and poly(5-bromouridylic acid). As monitored by ultraviolet absorbance–temperature profiles, all displacement reactions were directed toward the formation of the helix with the higher thermal stability. Concomitantly, the resulting helix was invariably more active as interferon inducer than the reactant helix, except for some reactions in which poly(7-deazaadenylic acid) was involved. For the latter reactions both the reactant and resultant helices were inactive as interferon inducer. The interferon induction data revealed that all displacement reactions proceeded to completion within 1 h even at temperatures well below the T_m of the reactant helix. The helix–coil displacement reaction could also be monitored by sucrose velocity gradient analysis, and, as evidenced for poly(A)·2poly(I) + 2poly(C) → 2 poly(I)·poly(C) + poly(A), readily occurred at the cellular level, presumably at the cell surface.

Interactions among polynucleotides[1] have been established by a wide variety of physicochemical techniques (e.g., Michelson et al., 1967). In contrast, no description exists of any biochemical or biological system which is generally applicable to the study of such nucleic acid interactions. In a preliminary account (De Clercq et al., 1974a), we presented evidence that interferon induction in primary rabbit kidney cells "superinduced" with inhibitors (actinomycin D and cycloheximide) could be effectively employed to monitor polynucleotide triplex formation and polynucleotide displacement reactions. These latter findings are further documented and extended in the present report. The following conclusions were reached. (a) Interferon induction in primary rabbit kidney cells "superinduced" with actinomycin D and cycloheximide provides a sensitive assay to follow the course of polynucleotide displacement reactions, and it may indicate the nature of the product as well. In certain instances, the assay discriminates between possibilities which are difficult to resolve by temperature–absorbance profiles alone. (b) The polynucleotide displacement reaction is uniformly directed toward the formation of the helix with the higher T_m as demonstrated with a large number of systems that differ substantially in chemical structure as well as physicochemical and biological properties. (c) Since all displacement reactions were accompanied by a rise in interferon production, our studies emphasize the importance of T_m in the interferon inducing capacity of polyribonucleotide duplexes. (d) All displacement reactions were demonstrated at temperatures far beneath the T_m of the least stable helix. They were also found to occur at the cellular level, presumably at the cell surface.

The displacement reactions to be described are of interest not only in that they provide a method for directly comparing the stabilities of different helices (Felsenfeld and Miles, 1967), but more specifically because such displacements may occur in living systems: for instance, during the transcription of mRNA from DNA by RNA polymerase (Chamberlin, 1965; Kornberg, 1974).

† From the Rega Institute for Medical Research, University of Leuven, B-3000 Leuven, Belgium (E.D.C.), and the Laboratory of Chemistry, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014 (P.F.T. and B.W.). *Received Auust 25, 1975.* The work was supported in part by grants from the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek and the Katholieke Universiteit te Leuven (Fonds Derde Cyclus).

[1] Abbreviations for synthetic polynucleotides conform to the recommendations of the IUPAC-IUB Commission (*J. Mol. Biol. 55*, 299, 1971). Less commonly used abbreviations are: poly(c^7A), poly(7-deazaadenylic acid); poly(c^7I), poly(7-deazainosinic acid); poly(rT), poly(ribothymidylic acid); poly(br^5U), poly(5-bromouridylic acid); poly(br^5C), poly(5-bromocytidylic acid); poly(io^5C), poly(5-iodocytidylic acid); poly(L), polylaurusin or poly(formycin B), the polynucleotide derived from the nucleoside, 1,6-dihydro-3-β-D-ribofuranosyl-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one. Other abbreviations are: MEM, Eagle's minimal essential medium; PBS, Dulbecco's phosphate-buffered saline.

Table I: Characteristics of Synthetic Polynucleotides.

Polymer	Source or Preparation Method	s_{20}[a]	ϵ_{max}	Buffer[c]
Poly(A)	*d*	9.5(*f*)	10 000(*p*)	0.1 M NaCl–0.01 M cac[b] (pH 6.85)
Poly(U)	*d*	9.4(*l*)	9 430(*q*)	0.195 M NaCl–0.01 M cac (pH 7.5)
Poly(I)	*d*	9.4(*l*)	10 400(*p*)	0.1 M NaCl–0.01 M cac (pH 6.85)
Poly(C)	*d*	8.8(*l*)	6 300(*p*)	0.1 M NaCl–0.1 M cac (pH 6.85)
Poly(c^{7}A)	*e,f*	*m*	8 900(*r*)	0.1 M NaCl–0.01 M cac (pH 7)
Poly(br^{5}U)	*f,g*	*m*	8 250(*p*)	0.1 M NaCl–0.01 M cac (pH 7)
Poly(rT)	*h*	*m*	9 170(*h*)	0.02 M cac (pH 7)
Poly(br^{5}C)	*i*	11.5(*n*)	5 510(*i*)	0.2 M cac (pH 7)
Poly(c^{7}I)	*j*	4.3(*n*)	9 430(*j*)	0.2 M NaCl–0.01 M cac (pH 7)
Poly(L)	*k*	9.5(*o*)	8 000(*r*)	0.2 M NaCl–0.01 M cac (pH 7)

[a] Expressed as Svedbergs. [b] cac, sodium cacodylate. [c] t = 25 °C. [d] P-L Biochemicals (Milwaukee, Wis.). [e] Ikehara and Fukui, 1968. [f] Torrence and Witkop, 1975. [g] Riley and Paul, 1970. [h] Howard et al., 1971. [i] Howard et al., 1969. [j] Torrence et al., 1974. [k] Torrence et al., 1975. [l] $s_{20,w}$ values supplied by manufacturer. [m] $s_{20,w}$ values not determined. Polymers were judged to be of high molecular weight by the fact that they were voided from a Sephadex G-200 column and had melting profiles identical with samples of known high molecular weight ($s_{20,w} > 6$ S). [n] Determined in 0.15 M NaCl–0.02 M Tris–0.001 M EDTA (pH 7.5). [o] Determined in 0.05 M NaCl–0.01 M sodium cacodylate (pH 7). [p] Sigler et al., 1962. [q] Blake et al., 1967. [r] P. F. Torrence and B. Witkop, unpublished observations.

Materials and Methods

Polynucleotides. The sources or methods of preparation, sedimentation values, and spectral data employed to determine the concentration of the polynucleotides are presented in Table I. The radiolabeled polynucleotides [5-^{3}H]poly(C) ($s_{20,w}$ 6.5 S; specific activity 65.5 Ci/mol of P) and [8-^{3}H]poly(A) ($s_{20,w}$ 12.4 S; specific activity 85.6 Ci/mol of P) were obtained from Miles Laboratories. They were used in the sucrose gradient sedimentation experiments after they had been mixed 1:9 with the corresponding unlabeled polymers. These preparations were referred to as poly(C*) and poly(A*), respectively.

Methods employed for the determination of *ultraviolet absorbance-temperature profiles* have been previously described (Torrence et al., 1973). When the displacement reactions were monitored by thermal profile, the general procedure was to mix the homopolymer constituents at concentrations (expressed as P) of about 5×10^{-4} M in the selected buffer. After adequate time to ensure duplex formation (2–7 days at 4 °C), the third homopolymer was added in the appropriate stoichiometric quantity. The polynucleotides were allowed to react at a concentration of about 5×10^{-4} M for 2–10 days at 4 °C and then diluted (~10×) with appropriate buffer. After an additional 1–7 days at 4 °C, the melting profile was determined.

Methods employed for measuring *interferon production in primary rabbit kidney (PRK) cell cultures* "superinduced" with cycloheximide and actinomycin D have also been described (De Clercq et al., 1975). For this purpose stock solutions of the homopolymers and their complexes were prepared at 1 mg/ml in 0.1 M Tris-HCl–0.2 M NaCl (pH 7.0) and stored at 4 °C. Prior to use, the polymers were diluted in Eagle's minimal essential medium (MEM) and mixed at the appropriate stoichiometric ratios to give final concentrations of 5 μg/ml (homopolymers), 10 μg/ml (homopolymer duplexes), or 15 μg/ml (homopolymer triplexes), respectively. The mixtures were incubated for 1 h at 37 °C, and, unless stated otherwise, immediately thereafter applied onto the cells.

The reactions poly(A)·2poly(I) + 2poly(C) and poly(A) + 2-poly(I)·poly(C) were monitored by sucrose velocity gradient analysis using either poly(A*) or poly(C*) as the radioactive marker. Homopolymers and homopolymer complexes were mixed in Dulbecco's phosphate-buffered saline (PBS, 0.01 M sodium phosphate–0.15 M NaCl–0.001 M Ca^{2+}–0.001 M Mg^{2+} (pH 7.0)) at the appropriate stoichiometric ratios to give final concentrations of 5 μg/ml (homopolymers), 10 μg/ml (homopolymer duplexes), or 15 μg/ml (homopolymer triplexes), respectively. The mixtures were incubated for 1 h at 37 °C and then analyzed by sucrose velocity gradient as described before (De Clercq et al., 1975).

Results

Ultraviolet Absorbance-Temperature Profiles. The helix-coil displacement reactions demonstrated herein will be presented by the reaction itself, the t_m's of the reactant and product helices (determined separately), and finally by the salt concentration at which the thermal profile of the resultant products (as well as the individual helices) was determined. All of the duplexes (or triplexes) involved in this study have been previously reported (see Table I) and the t_m's obtained in this study correspond well to earlier reported values. For the thermal profile measurements, all samples contained, in addition to the indicated salt concentration, 0.01 M sodium cacodylate and all were buffered to pH 7.

The displacement reaction

$$\text{poly(A)} \cdot 2\text{poly(I)} + 2\text{poly(C)} \xrightarrow[t_m = 44\ ^\circ\text{C}]{} 2\text{poly(I)} \cdot \text{poly(C)} + \text{poly(A)} \quad t_m = 63\ ^\circ\text{C} \quad (0.15\ \text{M NaCl}) \qquad \text{(a)}$$

was first observed by Sigler et al. (1962) employing ultraviolet and infrared spectroscopy and sucrose gradient centrifugation, and was subsequently confirmed by Singer and Tolbert (1965) using ribonuclease III from *E. coli*. The melting profile (Figure 1a) obtained when 1 mol (3 μmol of P) of poly(A)·2poly(I) was mixed with 2 mol (2 μmol of P) of poly(C) confirmed the occurrence of the above displacement since the only transition witnessed was that corresponding to poly(I)·poly(C) (t_m = 63 °C in 0.15 M NaCl–0.01 M sodium cacodylate (pH 7); data not illustrated; see also Michelson and Pochon, 1966). There was no evidence of any transition due to poly(A)·2poly(I) (t_m = 44 °C under the same conditions; data not illustrated; see also De Clercq et al., 1975). The small (~5%) hyperchromic drift in

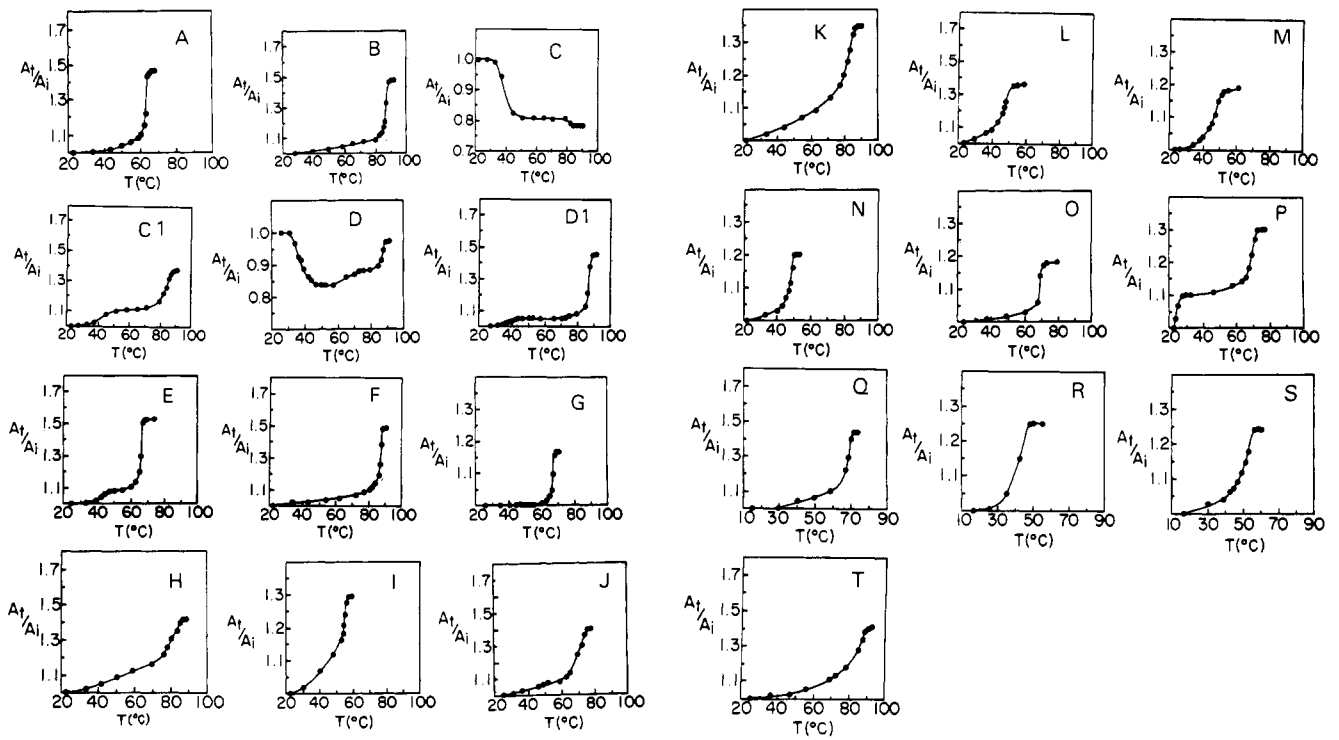

FIGURE 1: Polynucleotide displacement reactions as monitored by temperature–absorbance profile. For each profile, the reaction under scrutiny, the buffer employed, and the wavelength (λ) monitored are indicated. A_t/A_i is the ratio of absorbance at temperature t to that at the initial temperature. Sodium cacodylate is abbreviated cac. (A) Poly(A)·2poly(I) + 2poly(C), 0.15 M NaCl–0.01 M cac (pH 7), λ 250 nm; (B) poly(A)·2poly(I) + 2poly(br^{5}C), 0.15 M NaCl–0.01 M cac (pH 7), λ 250 nm; (C) poly(L)·poly(br^{5}C) + poly(c^{7}I), 0.15 M NaCl–0.01 M cac (pH 7), λ 300 nm; (C1) poly(L)·poly(br^{5}C) + poly(c^{7}I), 0.15 M NaCl–0.01 M cac (pH 7), λ 250 nm; (D) poly(L)·poly(br^{5}C) + poly(I), 0.15 M NaCl–0.01 M cac (pH 7), λ 300 nm. (D1) poly(L)·poly(br^{5}C) + poly(I), 0.15 M NaCl–0.01 M cac (pH 7), λ 250 nm; (E) poly(L)·poly(C) + poly(I), 0.20 M NaCl–0.01 M cac (pH 7), λ 250 nm; (F) poly(I)·poly(C) + poly(br^{5}C), 0.15 M NaCl–0.01 M cac (pH 7), λ 250 nm; (G) poly(c^{7}I)·poly(C) + poly(I), 0.20 M NaCl–0.01 M cac (pH 7), λ 250 nm; (H) poly(c^{7}I)·poly(C) + poly(br^{5}C), 0.15 M NaCl–0.01 M cac (pH 7), λ 250 nm; (I) poly(c^{7}A)·poly(U) + poly(A), 0.10 M NaCl–0.01 M cac (pH 7), λ 260 nm; (J) poly(c^{7}A)·poly(rT) + poly(A), 0.10 M NaCl–0.01 M cac (pH 7), λ 260 nm; (K) poly(A)·2poly(c^{7}I) + 2poly(br^{5}C), 0.15 M NaCl–0.01 M cac (pH 7), λ 260 nm; (L) poly(A)·2poly(c^{7}I) + 2poly(C), 0.15 M NaCl–0.01 M cac (pH 7), λ 250 nm; (M) poly(L)·poly(C) + poly(c^{7}I), 0.20 M NaCl–0.01 M cac (pH 7), λ 250 nm; (N) poly(c^{7}A)·poly(U) + poly(rT), 0.10 M NaCl–0.01 M cac (pH 7), λ 260 nm; (O) poly(c^{7}A)·poly(U) + poly(br^{5}U), 0.10 M NaCl–0.01 M cac (pH 7), λ 260 nm; (P) poly(c^{7}A)·poly(rT) + poly(br^{5}U), 0.10 M NaCl–0.01 M cac (pH 7), λ 260 nm; (Q) poly(c^{7}A)·poly(I) + poly(C), 0.45 M NaCl–0.01 M cac (pH 7), λ 250 nm; (R) poly(c^{7}A)·poly(I) + poly(U), 0.45 M NaCl–0.01 M cac (pH 7), λ 260 nm. (S) 2poly(c^{7}A)·poly(I) + poly(A), 0.45 M NaCl–0.01 M cac (pH 7), λ 250 nm. (T) poly(c^{7}A)·poly(br^{5}U) + poly(A), 0.10 M NaCl–0.01 M cac (pH 7), λ 260 nm.

the 30–55 °C range probably corresponds to the melting of neutral poly(A) (e.g., see Bobst et al., 1969).

$$\text{poly(A)} \cdot 2\text{poly(I)} + 2\text{poly(br}^5\text{C)} \rightarrow \quad t_m = 44\ ^\circ\text{C}$$
$$2\text{poly(I)} \cdot \text{poly(br}^5\text{C)} + \text{poly(A)} \quad t_m = 87\ ^\circ\text{C} \quad (0.15\ \text{M NaCl}) \qquad \text{(b)}$$

This reaction is analogous to the previous displacement. The thermal profile analysis (Figure 1B) demonstrates the absence of poly(A)·2poly(I) and clearly shows the presence of poly(I)·poly(br^{5}C) in addition to a small temperature dependent hyperchromicity due to neutral poly(A).

$$\text{poly(L)} \cdot \text{poly(br}^5\text{C)} + \text{poly(c}^7\text{I)} \rightarrow \quad t_m = 72\ ^\circ\text{C}$$
$$\text{poly(c}^7\text{I)} \cdot \text{poly(br}^5\text{C)} + \text{poly(L)} \quad t_m = 83\ ^\circ\text{C} \quad (0.15\ \text{M NaCl}) \qquad \text{(c)}$$

This displacement can be advantageously monitored at two different wavelengths: at 300 nm where the major transition is due to the melting of free poly(L) (~40 °C (Torrence, Waters and Witkop, unpublished observations) and at 250 nm where the dissociation of the poly(c^{7}I)·poly(br^{5}C) duplex is witnessed (Figure 1c).

$$\text{poly(L)} \cdot \text{poly(br}^5\text{C)} + \text{poly(I)} \rightarrow \quad t_m = 72\ ^\circ\text{C}$$
$$\text{poly(I)} \cdot \text{poly(br}^5\text{C)} + \text{poly(L)} \quad t_m = 87\ ^\circ\text{C} \quad (0.15\ \text{M NaCl}) \qquad \text{(d)}$$

As in the previous displacement reaction, both free poly(L) and poly(I)·poly(br^{5}C) are clearly observable in the melting profiles determined at 300 and 250 nm, respectively (Figure 1D). There is no evidence for the presence of poly(L)·poly(br^{5}C).

$$\text{poly(L)} \cdot \text{poly(C)} + \text{poly(I)} \rightarrow \quad t_m = 47\ ^\circ\text{C}$$
$$\text{poly(I)} \cdot \text{poly(C)} + \text{poly(L)} \quad t_m = 67\ ^\circ\text{C} \quad (0.20\ \text{M NaCl}) \qquad \text{(e)}$$

Although this reaction was not monitored by its thermal profile at 300 nm, the profile at 250 nm (Figure 1E) reveals a transition with $t_m \sim 43$ °C which most likely corresponds to free poly(L) (also witnessed at 250 nm in Figure 1C) as well as the melting of poly(I)·poly(C) itself.

$$\text{poly(I)} \cdot \text{poly(C)} + \text{poly(br}^5\text{C)} \rightarrow \quad t_m = 63\ ^\circ\text{C}$$
$$\text{poly(I)} \cdot \text{poly(br}^5\text{C)} + \text{poly(C)} \quad t_m = 87\ ^\circ\text{C} \quad (0.15\ \text{M NaCl}) \qquad \text{(f)}$$

Massoulié and Michelson (1967) used absorbance-temperature profiles when they demonstrated reaction f and their result is confirmed here since only the dissociation of poly(I)·poly(br^{5}C) can be seen (Figure 1F).

$$\text{poly(c}^7\text{I)} \cdot \text{poly(C)} + \text{poly(I)} \xrightarrow[t_m = 49\ ^\circ\text{C}]{} \text{poly(I)} \cdot \text{poly(C)} + \text{poly(c}^7\text{I)} \quad t_m = 67\ ^\circ\text{C} \quad (0.20\ \text{M NaCl}) \tag{g}$$

Determination of the melting profile of the products of this reaction (Figure 1G) reveals the presence of poly(I)·poly(C) only.

$$\text{poly(c}^7\text{I)} \cdot \text{poly(C)} + \text{poly(br}^5\text{C)} \xrightarrow[t_m = 47\ ^\circ\text{C}]{} \text{poly(c}^7\text{I)} \cdot \text{poly(br}^5\text{C)} + \text{poly(C)} \quad t_m = 83\ ^\circ\text{C} \quad (0.15\ \text{M NaCl}) \tag{h}$$

This reaction may be considered analogous to example f above. The presence of poly(c^{7}I)·poly(br^{5}C) is clear from the thermal profile analysis of the resultant products (Figure 1H). The noncooperative temperature-dependent hyperchromicity seen between 20 and 70 °C may be due, at least in part, to the "melting" of neutral poly(C) (Fasman et al., 1964).

$$\text{poly(c}^7\text{A)} \cdot \text{poly(U)} + \text{poly(A)} \xrightarrow[t_m = 32.5\ ^\circ\text{C}]{} \text{poly(A)} \cdot \text{poly(U)} + \text{poly(c}^7\text{A)} \quad t_m = 56\ ^\circ\text{C} \quad (0.10\ \text{M NaCl}) \tag{i}$$

This displacement was carried out in 0.10 M NaCl to avoid the complicating duplex to triplex rearrangement which can occur at ionic strengths >0.15 M NaCl. The presence of poly(A)·poly(U) is clear from the temperature-absorbance profile (Figure 1I). The significant noncooperative hyperchromic change seen from 20 to 50 °C is largely due to the melting of poly(c^{7}A) (Ikehara and Fukui, 1968; Bobst, Torrence and Witkop, unpublished observations).

$$\text{poly(c}^7\text{A)} \cdot \text{poly(rT)} + \text{poly(A)} \xrightarrow[t_m = 47\ ^\circ\text{C}]{} \text{poly(A)} \cdot \text{poly(rT)} + \text{poly(c}^7\text{A)} \quad t_m = 72\ ^\circ\text{C} \quad (0.10\ \text{M NaCl}) \tag{j}$$

In this instance, the transition witnessed (Figure 1J) at t_m ~71 °C is due to the melting of poly(A)·2poly(rT) which is formed from the strandwise rearrangement of poly(A)·poly(rT) (Howard et al., 1971). Again, the noncooperative melting of neutral poly(c^{7}A) is apparent.

$$\text{poly(A)} \cdot 2\text{poly(c}^7\text{I)} + 2\text{poly(br}^5\text{C)} \xrightarrow[t_m = 47\ ^\circ\text{C}]{} 2\text{poly(c}^7\text{I)} \cdot \text{poly(br}^5\text{C)} + \text{poly(A)} \quad t_m = 83\ ^\circ\text{C} \quad (0.15\ \text{M NaCl}) \tag{k}$$

Ikehara et al. (1974) obtained evidence that poly(c^{7}I) forms a triplex with poly(A). Our data (not shown) confirm that a complex is indeed formed and this complex has a t_m which is similar to that reported by Ikehara et al. (1974). When 1 mol of this triplex is mixed with 2 mol of poly(br^{5}C), there occurs a displacement reaction corresponding to eq k above since only poly(c^{7}I)·poly(br^{5}C) and neutral poly(A) can be detected in the thermal profile (Figure 1K).

$$\text{poly(A)} \cdot 2\text{poly(c}^7\text{I)} + 2\text{poly(C)} \xrightarrow[t_m = 47\ ^\circ\text{C}]{?} 2\text{poly(c}^7\text{I)} \cdot \text{poly(C)} + \text{poly(A)} \quad t_m = 47\ ^\circ\text{C} \quad (0.15\ \text{M NaCl}) \tag{l}$$

In this instance, since the t_m's of the reactant helix and the potential product helix are the same (within experimental error), the thermal profile of this reaction mixture does not provide any information as to whether or not reaction has occurred (Figure 1L).

$$\text{poly(L)} \cdot \text{poly(C)} + \text{poly(c}^7\text{I)} \xrightarrow[t_m = 47\ ^\circ\text{C}]{?} \text{poly(c}^7\text{I)} \cdot \text{poly(C)} + \text{poly(L)} \quad t_m = 49\ ^\circ\text{C} \quad (0.20\ \text{M NaCl}) \tag{m}$$

The outcome of this reaction is also impossible to judge conclusively on the basis of thermal profile (at 250 nm) alone (Figure 1M). The thermal profile at 300 nm (not illustrated) showed evidence for the melting of free poly(L), but was not unambiguous.

$$\text{poly(c}^7\text{A)} \cdot \text{poly(U)} + \text{poly(rT)} \xrightarrow[t_m = 32.5\ ^\circ\text{C}]{} \text{poly(c}^7\text{A)} \cdot \text{poly(rT)} + \text{poly(U)} \quad t_m = 47\ ^\circ\text{C} \quad (0.10\ \text{M NaCl}) \tag{n}$$

$$\text{poly(c}^7\text{A)} \cdot \text{poly(U)} + \text{poly(br}^5\text{U)} \xrightarrow[t_m = 32.5\ ^\circ\text{C}]{} \text{poly(c}^7\text{A)} \cdot \text{poly(br}^5\text{U)} + \text{poly(U)} \quad t_m = 69\ ^\circ\text{C} \quad (0.10\ \text{M NaCl}) \tag{o}$$

$$\text{poly(c}^7\text{A)} \cdot \text{poly(rT)} + \text{poly(br}^5\text{U)} \xrightarrow[t_m = 47\ ^\circ\text{C}]{} \text{poly(c}^7\text{A)} \cdot \text{poly(br}^5\text{U)} + \text{poly(rT)} \quad t_m = 69\ ^\circ\text{C} \quad (0.10\ \text{M NaCl}) \tag{p}$$

The existence of the three displacement reactions n, o, and p, is demonstrated by thermal profile measurements (Figures 1N, O, and P; note melting of free poly(rT) in Figure 1P). Analogous displacements cannot be demonstrated with the poly(A)·poly(U) and poly(A)·poly(rT) systems. When a displacement reaction (analogous to n or o above) does occur, the displaced strand adds to the double helix to form a triplex (Torrence, De Clercq and Witkop, in preparation).

$$\text{poly(c}^7\text{A)} \cdot \text{poly(I)} + \text{poly(C)} \xrightarrow[t_m = 23\ ^\circ\text{C}]{} \text{poly(I)} \cdot \text{poly(C)} + \text{poly(c}^7\text{A)} \quad t_m = 69\ ^\circ\text{C} \quad (0.45\ \text{M NaCl}) \tag{q}$$

$$\text{poly(c}^7\text{A)} \cdot \text{poly(I)} + \text{poly(U)} \xrightarrow[t_m = 23\ ^\circ\text{C}]{} \text{poly(c}^7\text{A)} \cdot \text{poly(U)} + \text{poly(I)} \quad t_m = 43\ ^\circ\text{C} \quad (0.45\ \text{M NaCl}) \tag{r}$$

$$2\text{poly(c}^7\text{A)} \cdot \text{poly(I)} + \text{poly(A)} \xrightarrow[t_m = 23\ ^\circ\text{C}]{} \text{poly(A)} \cdot 2\text{poly(I)} + 2\text{poly(c}^7\text{A)} \quad t_m = 51\ ^\circ\text{C} \quad (0.45\ \text{M NaCl}) \tag{s}$$

For the latter displacement reaction reactants were mixed at 0 °C and the resulting solutions were maintained at 0 °C until the melting profiles were determined. If poly(c^{7}A)·poly(I) merely melted to constituent homopolymers and then one homopolymer reannealed with the other compo-

Table II: Polynucleotide Displacement Reactions as Monitored by Interferon Production in Primary Rabbit Kidney Cell Cultures Superinduced with Cycloheximide and Actinomycin D.[a]

Reaction	Interferon Titer (U/ml) Helix as such	Helix + Coil
a. Poly(A)·2-poly(I) + 2-poly(C)	<10	20 000
2-Poly(I)·poly(C) + poly(A)	20 000	20 000
2-Poly(C) → poly(A)·2-poly(I)	<10	3 000
Poly(A) → 2-poly(I)·poly(C)	6 000	6 000
Poly(A)·2-poly(I) → 2-poly(C)	<10	10 000
2-Poly(I)·poly(C) → poly(A)	3 000	3 000
b. Poly(A)·2-poly(I) + 2-poly(br^{5}C)	<10	6 000
2-Poly(I)·poly(br^{5}C) + poly(A)	6 000	6 000
c. Poly(L)·poly(br^{5}C) + poly(c^{7}I)	<10	3 000
d. Poly(L)·poly(br^{5}C) + poly(I)	<10	6 000
e. Poly(L)·poly(C) + poly(I)	<10	6 000
f. Poly(I)·poly(C) + poly(br^{5}C)		
1:1	6 000	6 000
1:10	1 000	2 000
1:100	100	600
g. Poly(c^{7}I)·poly(C) + poly(I)		
1:1	800	6 000
1:10	150	3 000
1:100	10	600
h. Poly(c^{7}I)·poly(C) + poly(br^{5}C)		
1:1	800	3 000
1:10	150	2 000
1:100	10	1 000
i. Poly(c^{7}A)·poly(U) + poly(A)	<10	30
Poly(A)·poly(U) + poly(c^{7}A)	1 000	60
j. Poly(c^{7}A)·poly(rT) + poly(A)	10	60
Poly(A)·poly(rT) + poly(c^{7}A)	2 000	200
k. Poly(A)·2-poly(c^{7}I) + 2-poly(br^{5}C)	<10	3 000
2-poly(c^{7}I)·poly(br^{5}C) + poly(A)	4 500	4 500
l. Poly(A)·2-poly(c^{7}I) + 2-poly(C)	<10	<10
2-Poly(c^{7}I)·poly(C) + poly(A)	1 000	1 000
m. Poly(L)·poly(C) + poly(c^{7}I)	<10	20
Poly(c^{7}I)·poly(C) + poly(L)	600	20
n. Poly(c^{7}A)·poly(U) + poly(rT)	<10	<10
o. Poly(c^{7}A)·poly(U) + poly(br^{5}U)	<10	<10
p. Poly(c^{7}A)·poly(rT) + poly(br^{5}U)	<10	<10
q. Poly(c^{7}A)·poly(I) + poly(C)	<10	250
poly(I)·poly(C) + poly(c^{7}A)	2 500	60
r. poly(c^{7}A)·poly(I) + poly(U)	<10	<10
s. 2-poly(c^{7}A)·poly(I) + poly(A)	<10	<10
t. poly(c^{7}A)·poly(br^{5}U) + poly(A)	<10	<10

[a] Interferon titers are defined as the reciprocal of the highest dilution of sample that reduced virus-induced cytopathogenicity by 50% (De Clercq et al., 1974, 1975). Final concentrations of the helices in the assay mixtures: 10 μg/ml (duplexes) or 15 μg/ml (triplexes). Final concentrations of the coils in the assay mixtures: 5 μg/ml. For reactions f, g, and h the assay mixtures were tested as such (1:1) and after being diluted 1:10 and 1:100 in MEM. All assay mixtures were first incubated for 1 h at 37 °C and then exposed to the cells for 1 h at 37 °C, except mixtures n, o, p, q, r and s, which were incubated for 1 h at 4 °C and then exposed to the cells for 1 h at 4 °C. For reaction a the homopolymers and homopolymer complexes were either mixed (and then applied onto the cells) or administered sequentially with 1-h interval to the cells. For reactions l and m the assay mixtures were incubated 1 h at 37 °C and tested (1) immediately thereafter and (2) after an additional incubation period of 1 week at 4 °C; identical results were obtained whether the mixtures were assayed immediately or after 1 week at 4 °C. All data represent average values for two or more experiments with different rabbit kidney cell preparations.

nent, some transition ought to be evident in the thermal profiles around 23 °C. No such transitons were witnessed; instead, the profiles of all three displacements showed only one transition corresponding to the melting of the postulated reaction products (Figures 1Q, R, and S).

$$\text{poly(c}^7\text{A)} \cdot \text{poly(br}^5\text{U)} + \text{poly(A)} \xrightarrow[t_m = 69\ ^\circ\text{C}]{} \text{poly(A)} \cdot \text{poly(br}^5\text{U)} + \text{poly(c}^7\text{A)} \quad (t_m = 87\ ^\circ\text{C},\ 0.10\ \text{M NaCl}) \qquad \text{(t)}$$

The thermal profile obtained for reaction t indicates the presence of poly(A)·poly(br^{5}U) [melting as poly(A)·2poly(br^{5}U) due to strandwise rearrangement (Riley and Paul, 1970)]. In addition, the presence of free poly(c^{7}A) is indicated by the noncooperative hyperchromicity displayed before the melting of poly(A)·poly(br^{5}U) helix (Figure 1T).

Interferon Production in Primary Rabbit Kidney Cells Superinduced with Cycloheximide and Actinomycin D. In accord with previous findings (De Clercq et al., 1974; Torrence et al., 1975), poly(A)·2 poly(I), poly(L)·poly(C), and poly(L)·poly(br^{5}C) failed to induce interferon in rabbit kidney cell cultures superinduced with cycloheximide and actinomycin D (Table IIa–e). However, when poly(A)·2poly(I) was mixed with poly(C) or poly(br^{5}C), interferon production was raised to the levels generally observed for poly(I)·poly(C) and poly(I)·poly(br^{5}C) (Table IIa and b) (Torrence et al., 1974), confirming the existence of the displacement reactions a and b. Addition of poly(A) to poly(I)·poly(C) or poly(I)·poly(br^{5}C) did not affect the interferon inducing capacity of the latter (Table IIa and b). Poly(C) was equally effective in elevating the interferon inducing activity of poly(A)·2poly(I) whether it was added to the cells 1 h before, together with, or 1 h after the triplex (Table IIa), suggesting that at the cellular level an identical displacement occurred as in the test tube. In perfect agreement with the displacement reactions c, d, and e is the rise in interferon titer noted upon addition of poly(c^{7}I) to poly(L)·poly(br^{5}C) (Table IIc) and of poly(I) to either poly(L)·poly(br^{5}C) or poly(L)·poly(C) (Table IId and e). The interferon titers obtained for the helix–coil mixtures c, d, and e coincide quite well with the titers normally observed for poly(c^{7}I)·poly(br^{5}C), poly(I)·poly(br^{5}C), and poly(I)·poly(C) (Torrence et al., 1974).

The displacement reactions f, g, and h were advantageously monitored at 1:10 and 1:100 dilutions of the initial mixtures since the interferon inducing activity of the reactant helices and product helices diverged quantitatively more at lower (0.1 μg/ml) than at higher (10 μg/ml) concentrations (Table IIf–h). Dismutation reaction f would not be noticed if the reaction was carried out at 10 μg/ml only. Poly(I)·poly(C) and poly(c^{7}I)·poly(C) became markedly more active in inducing interferon after they had been mixed with either poly(br^{5}C) or poly(I) (Table II–h), pointing to the formation of poly(I)·poly(br^{5}C), poly(I)·poly(C), and poly(c^{7}I)·poly(br^{5}C) according to reaction schemes f, g, and h.

Although poly(c^{7}A)·poly(U) and poly(c^{7}A)·poly(rT) appear to dismutate to poly(A)·poly(U) and poly(A)·poly(rT) upon addition of poly(A) (reactions i and j), there was only a small increase in interferon production (Table IIi and j). A much higher increase should have been expected if poly(c^{7}A)·poly(U) and poly(c^{7}A)·poly(rT) were integrally converted to poly(A)·poly(U) and poly(A)·poly(rT) (De Clercq et al., 1974). Yet, the relatively low rise in interferon titer would not seem inconsistent with a complete displacement, if one takes into account that both poly(A)·poly(U) and poly(A)·poly(rT) lost a significant part of their interferon inducing activities when mixed with poly(c^{7}A) (Table IIi and j). The detrimental effect of poly(c^{7}A) is most probably due to an inhibition of cellular RNA synthesis (De Clercq et al., 1976).

Since the t_m values of poly(A)·2poly(c^7I) and poly(c^7I)·poly(br^5C) are ca. 36 °C apart (reaction k), it is not surprising that in the mixture poly(A)·2poly(c^7I) + 2poly(br^5C), poly(A) is exchanged for 2poly(br^5C) to form the more stable complex 2poly(c^7I)·poly(br^5C). As expected (Torrence et al., 1974), formation of poly(c^7I)·poly(br^5C) is also evidenced by a marked increased in interferon production (Table IIk). However, the situation is quite different for poly(A)·2poly(c^7I), poly(c^7I)·poly(C), and poly(L)·poly(C). The t_m values of these complexes are almost identical, so that thermal profiles alone do not allow a decision whether any of the following reactions occurs:

$$\text{poly(A)} \cdot 2\text{poly}(c^7\text{I}) + 2\text{poly(C)} \rightarrow 2\text{poly}(c^7\text{I}) \cdot \text{poly(C)} + \text{poly(A)} \quad \text{(l)}$$

$$\text{poly(L)} \cdot \text{poly(C)} + \text{poly}(c^7\text{I}) \rightarrow \text{poly}(c^7\text{I}) \cdot \text{poly(C)} + \text{poly(L)} \quad \text{(m)}$$

The interferon induction data obtained for these systems demonstrate (1) the occurrence of the displacement reaction m, the equilibrium of which appears to be shifted toward the formation of poly(L)·poly(C) (Table IIm), and (2) the absence of any interaction between poly(A)·2poly(c^7I) and poly(C), and between poly(c^7I)·poly(C) and poly(A) (Table III). Identical results were obtained whether the mixtures poly(A)·2poly(c^7I) + 2poly(C), 2poly(c^7I)·poly(C) + poly(A), poly(L)·poly(C) + poly(c^7I), and poly(c^7I)·poly(C) + poly(L) were investigated immediately after 1-h incubation at 37 °C or after an additional incubation at 4 °C for 1 week.

Displacement reactions n, o, and p as well as r, s, and t could not be adequately monitored by interferon induction (Table IIn-t), since both the reactant helices and the postulated product helices have been shown inactive as interferon inducers [poly(c^7A)·poly(U), poly(c^7A)·poly(rT), poly(c^7A)·poly(br^5U), poly(A)·poly(br^5U), De Clercq et al., 1974; poly(A)·2poly(I), see Table IIa)]. Poly(c^7A)·poly(I) was also found ineffective as interferon inducer (Table IIq-s). Since poly(c^7A)·poly(I) would dissociate under physiological conditions (37 °C, 0.15 M NaCl) because of its low t_m (23 °C, 0.45 M NaCl), interferon induction experiments involving poly(c^7A)·poly(I) were run at 4 °C instead of 37 °C (Table IIq-s). Reactions n, o, and p were also monitored for interferon induction at 4 °C (Table IIn, o, and p). Displacement reaction q was confirmed in the interferon induction assay (Table IIq). That the amount of interferon produced by the mixture poly(c^7A)·poly(I) + poly(C) remained below the interferon level observed with poly(I)·poly(C) itself is not surprising, since poly(I)·poly(C) proved markedly less effective as interferon inducer in the presence of free poly(c^7A) (Table IIq).

Sucrose Velocity Gradient Sedimentation. As reaction a was considered representative for all other displacement reactions, it was further analyzed by sucrose gradient sedimentation using radiolabeled poly(A*) or poly(C*) to follow the reaction. In the first experiment (data not shown), the sedimentation profile of poly(A*)·2poly(I) was shifted upward to the region of free poly(A*) when poly(A*)·2poly(I) was mixed with 2poly(C). In the second experiment, the sedimentation behavior of 2-poly(I)·poly(C*) was not substantially altered upon addition of poly(A). Yet, 2poly(C*) sedimented in the region occupied by poly(I)·poly(C*) after it had been reacted with poly(A)·2poly(I). These sucrose sedimentation data confirm the occurrence of displacement reaction a. They further establish that, also in accord with Sigler et al. (1962), Singer and Tolbert (1965) and the interferon induction data presented in Table IIa, the reaction proceeds to completion within 1 h at 37 °C.

Discussion

As originally established by Sigler et al. (1962) for reaction a, poly(A)·2poly(I) + 2poly(C) → 2poly(I)·poly(C) + poly(A), a stable helical structure can be completely dissociated at a temperature well below its T_m, when mixed with an equivalent of the proper random-coil homopolymer, to form a more stable helix (with a T_m higher than that of the reactant helix). The present report extends these findings to a large variety of polynucleotide helix-coil displacements (cf. reactions a-t). The occurrence of these displacement reactions was evidenced by physicochemical (ultraviolet absorbance-temperature profiles) as well as biological (interferon induction) assay systems. The displacement reaction was invariably directed toward the formation of the helix with greater thermal stability. A similar conclusion was reached by Inman (1964) (shown in eq viii), Chamberlin and Patterson (1965) (shown in eq iv-vii), and Massoulié and Michelson (1967) (shown in eq i-iii) for the following helix-coil reactions.

$$\text{poly(I)} \cdot \text{poly(C)} + \text{poly}(br^5\text{C}) \rightarrow \text{poly(I)} \cdot \text{poly}(br^5\text{C}) + \text{poly(C)} \quad \text{(i)}$$

$$\text{poly(I)} \cdot \text{poly(C)} + \text{poly}(io^5\text{C}) \rightarrow \text{poly(I)} \cdot \text{poly}(io^5\text{C}) + \text{poly(C)} \quad \text{(ii)}$$

$$\text{poly(I)} \cdot \text{poly}(br^5\text{C}) + \text{poly}(io^5\text{C}) \rightarrow \text{poly(I)} \cdot \text{poly}(io^5\text{C}) + \text{poly}(br^5\text{C}) \quad \text{(iii)}$$

$$\text{poly(dI)} \cdot \text{poly(C)} + \text{poly(dC)} \rightarrow \text{poly(dI)} \cdot \text{poly(dC)} + \text{poly(C)} \quad \text{(iv)}$$

$$\text{poly(I)} \cdot \text{poly(dC)} + \text{poly(C)} \rightarrow \text{poly(I)} \cdot \text{poly(C)} + \text{poly(dC)} \quad \text{(v)}$$

$$\text{poly(dI)} \cdot \text{poly(C)} + \text{poly(I)} \rightarrow \text{poly(I)} \cdot \text{poly(C)} + \text{poly(dI)} \quad \text{(vi)}$$

$$\text{poly(dI)} \cdot \text{poly(dC)} + \text{poly(I)} \rightarrow \text{poly(I)} \cdot \text{poly(dC)} + \text{poly(dI)} \quad \text{(vii)}$$

$$\text{poly(dI)} \cdot \text{poly(dC)} + \text{poly}(dbr^5\text{C}) \rightarrow \text{poly(dI)} \cdot \text{poly}(dbr^5\text{C}) + \text{poly(dC)} \quad \text{(viii)}$$

When the melting (or formation) of a polynucleotide helix is defined as an equilibrium situation (helix ⇌ coils), then $\Delta G = 0$ and $T_m = \Delta H/\Delta S$. Assuming that ΔH and ΔS are independent of temperature, then

$$\Delta G_{helix} = \Delta S_{helix}(T_m - T)$$

To compare the stabilities of two helices (y and z) at some temperature T, one may write

$$\frac{\Delta G_y}{\Delta G_z} = \frac{\ln K_y}{\ln K_z} = \frac{\Delta S_y(T_{m_y} - T)}{\Delta S_z(T_{m_z} - T)} \quad (1)$$

Thus, in order to compare duplex stabilities on the basis of T_m, not only must ΔH_y, ΔH_z, ΔS_y, and ΔS_z be independent of temperature, but ΔS_y and ΔS_z must be equal. Then

$$\frac{\Delta G_y}{\Delta G_z} = \frac{(T_{m_y} - T)}{(T_{m_z} - T)} \quad (2)$$

All displacement reactions described herein (a-t) as well as those described before (a, i-viii) follow the generalization that the helix with the higher T_m is the product, thus

confirming the applicability of eq 2 and the assumptions involved. That eq 2 may be generally applicable is somewhat surprising in view of the fact that ΔH may, in certain instances, vary with temperature (Bloomfield et al., 1974). Furthermore, the assumption that $\Delta S_1 = \Delta S_2$ (eq 1) must also be valid, indicating that the entropic changes between two different helices (duplex vs. duplex) are minimal and that enthalpic changes are the principal driving force. This latter consideration may not apply to reactions like a, b, k, and l where considerable differences in ΔS are expected when comparing two-stranded and three-stranded helices. Yet, enthalpic changes may still predominate in these cases as well as in case s where the triplex is the reaction product. Finally, the concept of predicting the course of a helix–coil displacement on the basis of helix T_m assumes that the homopolymer reactant and homopolymer product are in the same state. While this is obviously not always the situation (e.g., m and n), the assumption seems to hold.

Not only are all displacement reactions directed toward the formation of the helix with the higher T_m, but the resultant helices are also superior in interferon inducing capacity as compared to the reactant helices [at least if their T_m values are sufficiently spaced: reactions a–k and q—this rule does not apply for reactions n–p and r–t, since both the resultant and reactant helices of these reactions are ineffective as interferon inducers]. Hence, our data reemphasize the importance of T_m in the interferon inducing activity of helical RNA complexes (De Clercq and Merigan, 1969).

As demonstrated with reaction a (Table IIa), helix–coil displacement also occurs at the cellular level: poly(C) added to the cells 1 h before or after poly(A)·2poly(I) brought about a similar increase in interferon titer as did poly(C) added to the cells simultaneously with poly(A)·2poly(I). Thus, poly(C) and poly(A)·2poly(I) added to the cells with 1-h interval are still available for interaction, as might be anticipated if both poly(C) and poly(A)·2poly(I) were integrally retained at the cell surface. That displacement reactions may occur at the cell surface is not surprising. It has been previouly established that poly(I) and poly(C), when administered sequentially, reunite at the cellular level, most probably at the outer cell membrane (De Clercq and De Somer, 1972).

While polynucleotide interactions can be studied by a wide variety of physicochemical techniques such as sucrose gradient ultracentrifugation, infrared and ultraviolet spectroscopy, etc., the number of biological and biochemical systems available for such studies are to date few in number. Often such methods which have been employed previously are either lacking in specificity or generality. For instance, polynucleotide phosphorylase has been used to ascertain complex formation between poly(A) and poly(U) (Grunberg-Manago, 1958). Ribonuclease A can be used to differentiate duplexes or triplexes from single strands (Barnard, 1969). Yet, both methods suffer from a lack of total specificity and could not be used to differentiate one duplex from another or a triplex from a duplex as is required in the study of displacement reactions. Singer and Tolbert (1965) employed a potassium ion activated phosphodiesterase (RNase II) from *E. coli* to demonstrate the displacement a above. This enzyme represented a considerable improvement over pancreatic ribonuclease A, since the former does not hydrolyze helical polyribonucleotide at all. Although untried as yet, there is every possibility that antibodies directed against specific forms of nucleic acids (Stollar, 1973; Stollar and Raso, 1974) may be of considerable use in the investigation of polynucleotide interactions. The data presented in this paper illustrate the utility of the interferon system in analyzing polynucleotide displacement reactions. Not only can this assay be used to determine whether or not reaction occurs, but it also provides useful information regarding the nature of the product. Its specificity is derived from the highly specific interferon induction system which requires an intact double-helical structure with unaltered 2′-OH groups. In this assay system, duplexes may be readily differentiated from single strands and/or triplexes. The assay requires that reactants and products maintain their strandedness under physiological conditions and that either reactant or product triggers a significant interferon response. This interferon system can be employed as a screening procedure, often providing information in days that may require weeks to obtain by physicochemical methods.

Acknowledgments

The authors are indebted to Dr. James A. Waters (National Institutes of Health) for the generous gift of the 5′-diphosphates employed in the preparation of poly(c^7I) and poly(L) and to Mrs. Anita Van Lierde for excellent technical assistance.

References

Barnard, E. A. (1969), *Annu. Rev. Biochem. 38,* 677–732.
Blake, R. D., Massoulié, J., and Fresco, J. R. (1967), *J. Mol. Biol. 30,* 291–308.
Bloomfield, V. A., Crothers, D. M., and Tinoco, Jr., I. (1974), Physical Chemistry of Nucleic Acids, New York, N.Y., Harper and Row, pp 313–314.
Bobst, A. M., Rottman, F., and Cerutti, P. A. (1969), *J. Mol. Biol. 46,* 221–239.
Chamberlin, M. J. (1965), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 24,* 1446–1457.
Chamberlin, M. J., and Patterson, P. L. (1965), *J. Mol. Biol. 12,* 410–428.
De Clercq, E., Billiau, A., Torrence, P. F., Waters, J. A., and Witkop, B. (1976), *Biochem. Pharmacol.,* in press.
De Clercq, E., and Merigan, T. C. (1969), *Nature (London) 222,* 1148–1152.
De Clercq, E., and De Somer, P. (1972), *J. Virol. 9,* 721–731.
De Clercq, E., Torrence, P. F., De Somer, P., and Witkop, B. (1975), *J. Biol. Chem. 250,* 2521–2531.
De Clercq, E., Torrence, P. F., and Witkop, B. (1974a), *Proc. Natl. Acad. Sci. U.S.A. 71,* 182–186.
De Clercq, E., Torrence, P. F., Witkop, B., Stewart II, W. E., and De Somer, P. (1974b), *Science 186,* 835–837.
Fasman, G. D., Lindblow, C., and Grossman, L. (1964), *Biochemistry 3,* 1015–1021.
Felsenfeld, G., and Miles, H. T. (1967), *Annu. Rev. Biochem. 36,* 407–448.
Grunberg-Manago, M. (1958), *Biokhimiia 23,* 307–317.
Howard, F. B., Frazier, J., and Miles, H. T. (1969), *J. Biol. Chem. 244,* 1291–1302.
Howard, F. B., Frazier, J., and Miles, H. T. (1971), *J. Biol. Chem. 246,* 7073–7086.
Ikehara, M., and Fukui, T. (1968), *J. Mol. Biol. 38,* 437–441.
Ikehara, M., Fukui, T., Koide, T., and Inaba, J. (1974), *Nucleic Acids Res. 1,* 53–62.
Inman, R. B. (1964), *J. Mol. Biol. 9,* 624–637.
Kornberg, A. (1974), DNA Synthesis, San Francisco,

Calif., W. H. Freeman, p 340.
Massoulié, J., and Michelson, A. M. (1967), *Biochim. Biophys. Acta 134,* 22–26.
Michelson, A. M., Massoulié, J., and Guschlbauer, W. (1967), *Prog. Nucleic Acid Res. Mol. Biol. 6,* 83–141.
Michelson, A. M., and Pochon, F. (1966), *Biochim. Biophys. Acta 114,* 469–480.
Riley, M., and Paul, A. V. (1970), *J. Mol. Biol. 50,* 439–455.
Sigler, R. B., Davies, D. R., and Miles, H. T. (1962), *J. Mol. Biol. 5,* 509–517.
Singer, M. F., and Tolbert, G. (1965), *Biochemistry 4,* 1319–1330.
Stollar, B. D. (1973), in The Antigens, Sela, M., Ed., New York, N.Y., Academic Press, pp 1–85.
Stollar, B. D., and Raso, V. (1974), *Nature (London) 250,* 231–234.
Torrence, P. F., Bobst, A. M., Waters, J. A., and Witkop, B. (1973), *Biochemistry 12,* 3962–3972.
Torrence, P. F., De Clercq, E., Waters, J. A., and Witkop, B. (1974), *Biochemistry 13,* 4400–4408.
Torrence, P. F., De Clercq, E., Waters, J. A., and Witkop, B. (1975), *Biochem. Biophys. Res. Commun. 62,* 658–664.
Torrence, P. F., and Witkop, B. (1975), *Biochim. Biophys. Acta 395,* 56–66.

Triple-Helical Polynucleotides. Mixed Triplexes of the Poly(uridylic acid)·Poly(adenylic acid)·Poly(uridylic acid) Class†

Paul F. Torrence, Erik De Clercq, and Bernhard Witkop*

ABSTRACT: By the techniques of interferon induction in primary rabbit kidney cells "superinduced" with metabolic inhibitors, ultraviolet absorbance–temperature profiles, sensitivity to pancreatic ribonuclease A, and sucrose velocity gradient ultracentrifugation, a number of reactions between double-helical RNA and single-stranded RNA or DNA homopolymers were investigated. The polymers involved in these studies were poly(adenylic acid), poly(uridylic acid), poly(ribothymidylic acid), poly(5-bromouridylic acid), poly(deoxythymidylic acid), poly(deoxyuridylic acid), poly(3-methyluridylic acid), poly(2′-*O*-methyluridylic acid), and poly(2′-azido-2′-deoxyuridylic acid). Two different reaction courses, both leading to the formation of triple helices, were noted: (1) poly(Ux)·poly(A) + poly(Uy) → poly(Ux)·poly(A)·poly(Uy) if the T_m of poly(Ux)·poly(A) was higher than the T_m of poly(Uy)·poly(A); (2) poly(Ux)·poly(A) + poly(Uy) → poly(Uy)·poly(A)·poly(Ux) if the T_m of poly(Ux)·poly(A) was lower than the T_m of poly(Uy)·poly(A). In these equations, the homopolymer written to the left of poly(A) implies Watson–Crick hydrogen bonding whereas the polymer to the right of poly(A) is involved in Hoogsteen hydrogen bonding.

The term "recognition" has been used largely to denote the process in which a protein combines with a specific section of a nucleic acid (Yarus, 1969). Nonetheless, it is clear that the interaction of single-stranded nucleic acid with double-stranded nucleic acid can also give rise to a recognition system based on specific affinity. For instance, hybridization studies have established that the eukaryotic genome contains significant amounts of dA-rich and poly(dA)[1] sequences and dG-rich and poly(dG) sequences (Shenkin and Burdon, 1974, and references cited therein). Just as lysine-rich histones possess a greater affinity for the (dA + dT)-rich regions in DNA (Ohba, 1966; Mazen and Champagne, 1968) and arginine-rich histones have a greater affinity for (dG + dC)-rich regions in DNA (Clark and Felsenfeld, 1972), it may be expected that a polynucleotide comprised of the base uracil (or its derivatives) would possess a specific affinity for a poly(dT)·poly(dA) sequence in the DNA duplex by virtue of the formation of a triplex helix analogous to poly(U)·poly(A)·poly(U). In this connection, it is of interest that dA-dT clusters have been found near the origin of DNA replication in *Escherichia coli* 15T⁻ cells (Baril and Kubinski, 1975).

Although our knowledge of the factors that govern formation and stability of polynucleotide triplexes is quite limited (Bloomfield et al., 1974), and in spite of the fact that no nucleic acid triple helix[2] has yet been observed to occur naturally in any biological system, a variety of hypotheses

† From the Laboratory of Chemistry, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014 (P.F.T. and B.W.), and the Rega Institute, University of Leuven, B-3000 Leuven, Belgium (E.D.C.). *Received August 25, 1975.*

[1] Abbreviations for synthetic polynucleotides conform to the recommendations of the IUPAC–IUB Commission [*J. Mol. Biol. 55,* 299 (1971)]. Less commonly used abbreviations are: poly(br⁵U), poly(5-bromouridylic acid); poly(rT), poly(ribothymidylic acid) or poly(5-methyluridylic acid); poly(Um), poly(2′-*O*-methyluridylic acid); poly(m³U), poly(3-methyluridylic acid); poly(dUf), poly(2′-fluoro-2′-deoxyuridylic acid). Other abbreviations are: MEM, Eagle's minimal essential medium; PBS, Dulbecco's phosphate-buffered saline; poly(U*), [5-³H]poly(uridylic acid).

[2] It is important to differentiate here between a nucleic acid triple helix and a nucleic acid base triple. The former may be defined (for purposes of this paper) as the formation of a three-stranded polynucleotide for a least one helical turn. In contrast, the latter, arising from tertiary hydrogen bonding interactions, may be defined as formed from three hydrogen-bonding bases and does not result in a triple helix. Triples of this latter type have been demonstrated in yeast phenylalanine tRNA (Kim et al., 1974).