Kinetic Investigation of the Random-Coil to Double-Helix Conformation Change of Poly[d(A-T)]†

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ABSTRACT: Rate studies were undertaken to investigate the mechanism of double-helix formation of the synthetic DNA analog, poly[d(A-T)]. The results indicate that the formation of the double-helical poly[d(A-T)] involves the twisting of a single polymer chain into a double-stranded helical structure. This conformation change consists of three distinct first-order processes with typical half-life values of 1 msec, 35 msec, and 3 sec, respectively. The effects on the rate of double-helix formation of such factors as: initial and final pH values of the solutions, molecular weight of poly[d(A-T)], limited hydroxymethylation of the adenine bases on the polymer and the presence of acridine orange or glycerin in the solution, were studied. According to the mechanism proposed for the first phase of the reaction the double-helix formation starts with the development of nuclei (consisting of a chain loop and a few paired bases) on the poly[d(A-T)] molecule; the subsequent propagation of helical regions occurs by the rapid twisting of the nuclei, and local helix growth is terminated by the convergence of two helical branches.

In order to carry out its biological function, double-stranded DNA must be able to unwind and rewind in a very efficient manner in living cells. Although various schemes have been proposed (Cairns and Davern, 1967; Becker and Hurwitz, 1970), the details of this remarkable process remain essentially unknown. In recent years, several synthetic DNA analogs have been prepared which can undergo a reversible conformation change from a random-coil state to double-helical form (Schachman *et al.*, 1960; Wells *et al.*, 1965). Naturally, in the presence of a variety of enzymes, the *in vivo* unwinding–rewinding mechanism of native DNA could be very different from the conformation change of these simple analogs. It appears, however, that certain important features of the mechanism (*e.g.*, the rapid rotation of the macromolecule around its long axis) would be common for both systems. As a consequence of such similarities, studies of the conformation change of DNA analogs could be of great help in understanding the *in vivo* process.

We have undertaken the kinetic investigation of the mechanism of the double-helix formation of poly[d(A-T)]. This synthetic nucleic acid is an alternating copolymer of deoxyadenosine 5′-phosphate and thymidine 5′-phosphate which will undergo a reversible conformation change in aqueous solution upon a suitable temperature or pH change (Schachman *et al.*, 1960). In poly[d(A-T)], the strictly alternating primary structure assures that when one base pairing occurs anywhere along the polymer chain, all the neighboring bases will be automatically in register. This special characteristic of the system suggested to us that the mechanism of double-helix formation of this synthetic nucleic acid might be a relatively simple and kinetically tractable process. The rate of *unwinding* of double-helical poly[d(A-T)] has been studied (Spatz and Baldwin, 1965) but, prior to our experiments, the rapid rate of the *formation* of double-helical structure has been investigated neither for this polymer nor for the other nucleic acids.

The purpose of our studies has been twofold. In the first place, we wanted to measure the rates involved in the double-helix formation of poly[d(A-T)] and propose a mechanism for this conformation change. Secondly, we planned to establish by our investigation whether a kinetic technique of this kind could be useful for detecting very minor changes in nucleic acids brought about by addition of reagents, solvents, etc.

Materials and Methods

Materials. Poly[d(A-T)] was purchased from Miles Laboratories, Inc., Kanakakee, Ill. According to the manufacturer, it was prepared following the procedures of Schachman *et al.* (1960). All chemicals used were reagent grade except where noted otherwise. Solutions were made using double-distilled water which was filtered through a fine porosity sintered glass filter and was degassed under vacuum.

pH Determinations. The pH of the polymer solutions was measured using a Beckman Expandomatic pH meter (Model 76A) which was standardized before each series of measurements at pH 7.00 and 10.00 with standard buffers (Sargent-Welch Scientific Co.) and at pH 12.45 with a saturated $Ca(OH)_2$ solution according to procedures previously described (Senior *et al.*, 1971). The pH adjustment of the solutions was carried out in a specially constructed glove box thermostated at 10.0 ± 0.1° (Hickey, 1972). To exclude CO_2, the box was continuously flushed with nitrogen. When adjusting the pH of the solutions for absorbance measurements, microliter quantities of 1 N NaOH were added from a microliter syringe (Hamilton Co., Inc., 0.05-ml capacity). The pH values were determined before and after each absorbance measurement (the differences were always less than 0.03 pH unit).

Absorbance Measurements. For these determinations, a Cary 14 recording spectrophotometer equipped with thermostated cell blocks was used. The temperature of the solutions was maintained at 10.0 ± 0.1°. In order to eliminate possible CO_2 absorption and to prevent fogging of the glass surfaces, dry nitrogen was blown into the cell compartments. All ab-

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sorbances were determined at 260 nm using stoppered silica cells (Pyrocell No. 6001J) of 1-cm light path. The solutions containing poly[d(A-T)] were placed in the sample cell, and the blank cells in the reference compartment contained the same solutions but no polymer. The cells were filled and stoppered in the glove box and were allowed to stand for about 15 min in the cell block of the spectrophotometer before measurements. The final absorbance value was recorded after two successive constant values were obtained at 10-min intervals.

Hydroxymethylation of Poly[d(A-T)]. A stock solution of 1% (w/w) formaldehyde was prepared using an aqueous solution of approximately 37% formaldehyde (Matheson, Coleman & Bell; Reagent, A. C. S.). The desired concentration of formaldehyde in the polymer solutions was attained by adding aliquots of the 1% formaldehyde stock. The concentration of poly[d(A-T)], PO_4^{3-}, and Na^+ in the solutions was the same as to be described below. The pH was 11.7. The formaldehyde-containing polymer solutions were allowed to stand overnight at room temperature, and their pH was adjusted to 12.1 before they were used in the kinetic experiments.

Viscosity Measurements. The intrinsic viscosities of poly[d(A-T)] solutions were determined under the same conditions (*i.e.*, 37°, 0.2 M NaCl–0.1 M sodium citrate, pH 7.0) as those reported by Schachman *et al.* (1960) using a Cannon-Ubbelohde dilution viscometer (No. 100, K 532) and a Neslab constant-temperature circulating bath (Model T9). The absolute viscosities of the solutions containing 0, 20, or 40% glycerin were determined at 10° in the same viscometer by measuring the efflux times of the solutions and that of water.

Kinetic Measurements. The rate of double-helix formation of poly[d(A-T)] was measured by monitoring the hypochromicity change at 260 nm in a Durrum-Gibson stopped-flow apparatus (Durrum Instruments, Inc.). In this instrument the chemical reaction to be studied is initiated by a rapid mixing of the components, and the resulting concentration change is recorded spectrophotometrically. For all experiments the temperature of the solutions in the apparatus was maintained at 10.00 ± 0.05° by a refrigerated circulator bath (Forma, Model 2096). The light source for the monochromator of the stopped-flow instrument was a Beckman deuterium lamp (no. 96280) powered by a Beckman hydrogen lamp power supply (Model B). The observed transmission changes were displayed and recorded on one or both of the following two storage oscilloscopes: Tektronix 549 with a high-gain differential amplifier (type D) and a Tektronix 5103N/D13 dual-beam model equipped with a high-gain differential amplifier (5A20N) and two independent time base generators (5B10N). Using both oscilloscopes, it was possible to display and record the transmission change of the reaction with three different time sweeps simultaneously in a single experiment. For the evaluation of the data the oscilloscope traces were photographed (Hewlett Packard oscilloscope camera no. 197A or Tektronix Camera C-5), corrected for parallax error and replotted on logarithmic graphs.

The reaction leading to the formation of double-helical poly[d(A-T)] was initiated by a pH-jump technique. One of the components to be mixed in the stopped-flow instrument was a poly[d(A-T)] solution at an alkaline pH favoring the random-coil conformation of the polymer, and the other a dilute HCl solution containing NaCl but no poly[d(A-T)]. The concentration of the HCl in the latter solution was adjusted in a manner such that, after mixing, the pH of the combined solutions was at a value favoring the double-helical conformation of poly[d(A-T)]. The conformation of the polymer at the final pH could be inferred from the pH *vs.* absorbance curves determined separately. In all kinetic experiments the sodium ion concentration of the mixed solutions was kept at 25 mM. The polymer solutions used in the stopped-flow instrument contained 13.0 mM NaCl, 4.0 mM Na_3PO_4, 25 μg/ml of polymer and, depending on the desired pH, 2.8–8.0 mM NaOH. For the polymer solutions which contained 20 and 40% (v/v) glycerin (Fisher Spectranalyzed grade) the NaOH concentration range was 14.0–28.0 mM. The acid solutions with which the polymer solutions were mixed contained 17.0–22.2 mM NaCl and 0.0–10.0 mM HCl for the water solutions, and 2.0–20.0 mM NaCl and 2.0–20.0 mM HCl for the water–glycerin solutions. The concentration of the HCl in the acid solutions depended on the desired final pH of the mixed solutions. This HCl concentration was determined experimentally by separate mixing experiments carried out in the nitrogen atmosphere of the glove box. (In these experiments equal volumes of alkaline polymer solutions and NaCl solutions were mixed and the pH was lowered to the desired value by the addition of microliter quantities of 1 N HCl.) During the stopped-flow work, after each mixing, the solution was emptied into a flask stoppered with a Mallcosorb carbon dioxide absorbing tube (Mallinckrodt Chemical Works). After a given series of mixing experiments, the flask was disconnected, sealed, and placed in the glove box. After temperature equilibration, the final pH of the solution was measured. These pH values agreed within 0.03 unit with those determined by the separate mixing experiments described above.

In the stopped-flow method the efficiency of the mixing process is measured by the "dead time" of the instrument, which is the time required for the mixed solution to flow from the mixing jet into the observation chamber. In our apparatus, the dead time was determined to be between 1.5 and 2.0 msec by a calibration method using 0.01 M $Fe(NO_3)_3$ (in 0.1 N H_2SO_4) and 0.01 M KCNS solutions. The details of this procedure together with the thorough description of the stopped-flow instrument is being published elsewhere (Hickey, 1972).

Results

Molecular Weights. According to Scheraga and Mandelkern (1953) there is a linear relationship between the logarithms of molecular weight and the quantity $s[\eta]^{1/3}$, where s and $[\eta]$ are the sedimentation coefficients and intrinsic viscosities of macromolecular fractions. This relationship was utilized in estimating the average molecular weights of our poly[d(A-T)] samples from intrinsic viscosity and sedimentation coefficient data in the following manner. On a logarithmic $s[\eta]^{1/3}$ *vs.* M_w plot (Figure 1) a straight line was drawn based on the results of Schachman *et al.* (1960), and the molecular weights corresponding to the $s[\eta]^{1/3}$ values of our samples were read off on the abscissa of the plot. Table I shows the results and the data utilized. In principle, this procedure should yield accurate molecular weight data for homogeneous polymer fractions. In our case, however, due to the known molecular weight heterogeneity of Miles poly[d(A-T)] samples (Jang and Bartl, 1971) an uncertain molecular weight average is obtained. For this reason we consider our molecular weight results only as estimates.

Hypochromicity Curves. The random-coil to double-helix conformation change of poly[d(A-T)] can be detected by the observation of the absorbance change of the system at 260 nm. The hypochromicity of nucleotides changes very sharply at this wavelength during the transition, and either the thermal or the pH-induced melting (or formation) of the double-helical structure can be conveniently followed in this manner

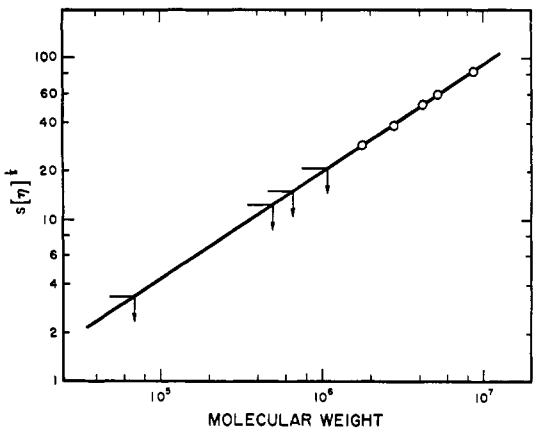

FIGURE 1: Estimation of the molecular weights of poly[d(A-T)] samples from hydrodynamic data using the Scheraga–Mandelkern relationship. The line drawn is defined by the data (○) of Schachman *et al.* (1960) and the arrows indicate molecular weights corresponding to the $s[\eta]^{1/3}$ values of the poly[dA-T)] samples studied in the present work.

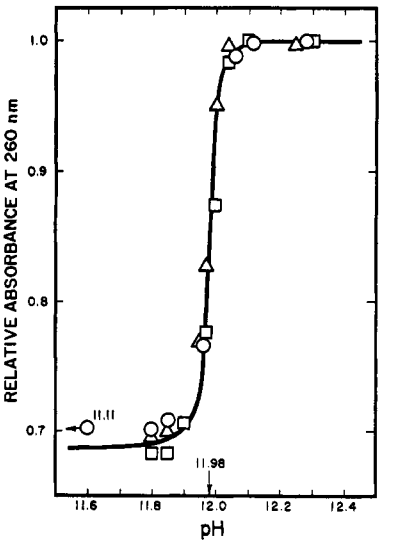

FIGURE 2: Absorbance *vs.* pH curve of poly[d(A-T)] samples of different molecular weight. (○) 69 × 10³; (△) 490 × 10³; (□) 660 × 10³. The transition of the polymer from a random-coil conformation (at high pH) to double-helical structure is indicated by the large change in hypochromicity at pH 11.98. See text for details of experimental conditions.

(Inman and Baldwin, 1962a). Since for our kinetic studies we needed to know the conformation of poly[d(A-T)] at various pH values, we had to establish absorbance *vs.* pH curves for the system under our experimental conditions. The results obtained at 10° on three poly[d(A-T)] samples of different molecular weight, using 25 mM Na^+ and 12.5-μg/ml polymer concentrations are shown in Figure 2. The high values of relative absorbance (right side) correspond to the random-coil form of the polymer and the low values to the double-helical conformation. It can be seen on this graph that the transition point for all three samples is close to pH 11.98. Figure 3 shows the hypochromicity curves for sample 3 in 20 and 40% aqueous glycerin solutions (the other concentrations used were the same as those of Figure 2). The observed total absorbance change for the 0 and 20% glycerin solutions was 31 ± 2% in good agreement with the reported value of 32% for the hypochromicity of aqueous poly[d(A-T)] solutions (Scheffler *et al.*, 1970). The polymer solution, however, which contained 40% glycerin indicated only a 29 ± 2% total absorbance change.

Rate Studies. Oscilloscope records of a typical absorbance change which follows the sudden lowering of the pH of poly[d(A-T)] solutions are shown in Figure 4. From such data, it was established that under our experimental conditions double-helical poly[d(A-T)] is formed by a three-phase kinetic process. It was found, furthermore, that each of the three phases could be described by the first-order rate law. This is demonstrated in Figure 5 which is the customary logarithmic plot of the first phase of the conformation change (fast reaction). The logarithmic plots of the other two phases (to be referred to as intermediate and slow reactions in further discussions) also indicated good linear behavior (Hickey, 1972). Although the rates change with experimental conditions (see details below), half-life values of 1 msec, 35 msec, and 3 sec can be considered as typical for the fast, intermediate, and slow reactions, respectively. It must be noted that due to limitations of the instrument the first (0–2 msec) portion of the fast reaction could not be recorded (Figure 5). It is sig-

TABLE I: Physicochemical Constants of Poly[d(A-T)] Samples.

Sample No.	Intrinsic Viscosity, [η] (dl/g)	Sedimentation Constant ($s^0_{20,w}$),[a] S	Mol Wt (10³)	Deg of Polymerization (Nucleotides/Molecule)
1	0.35	4.79	69	220
2	3.91	7.85	490	1600
3	4.22	9.23	660	2100
4	4.10	13.07	1100	3500

[a] Supplied by Miles Laboratories.

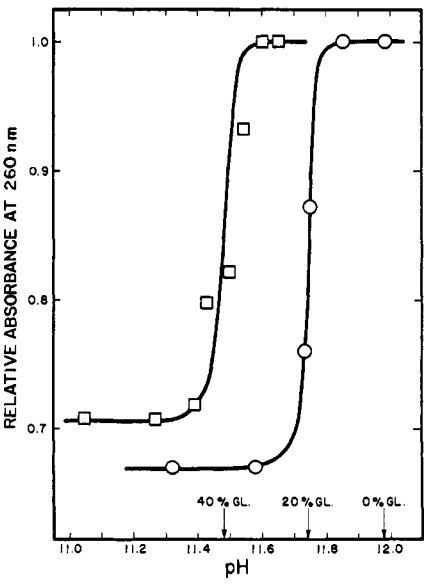

FIGURE 3: Hypochromicity curves of a poly[d(A-T)] sample (3) in aqueous solutions containing 20% (○) and 40% (□) glycerin. See text for further details. The arrows indicate the transition pH values including that of the 0% glycerin system (Figure 2).

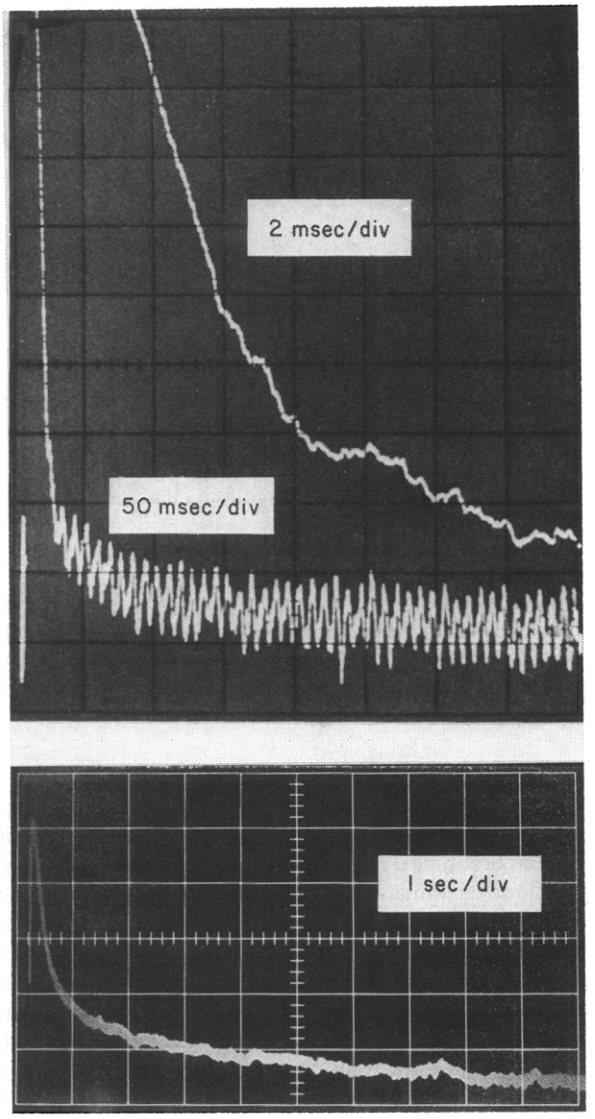

FIGURE 4: Oscillograms indicating the fast, intermediate, and slow phases in the double-helix formation of poly[d(A-T)]. The vertical axis represents the transmission of the solution at 260 nm (increasing linearly from top to bottom). The horizontal axis is time according to the scale shown on the inserts. These traces were recorded during a single stopped-flow experiment using three different oscilloscope sweep rates simultaneously. The experimental conditions were: 10°, 25 μg/ml. of polymer (sample 4), 25 mM Na^+, and 4 mM $(PO_4)^{3-}$. The pH values before and after mixing were 12.10 and 11.86, respectively.

nificant, however, that the linear extrapolation of this reaction back to zero time yielded the same absorbance for the system (within 3% experimental error) as the total absorbance of the two solution components used in the mixing. This must be interpreted to mean that the fast reaction observed is *not* preceded by a sizable reaction of even faster rate (which, of course, would be undetectable due to the dead time of the instrument). Typical values for the ratios of the absorbance changes due to the fast, intermediate and slow reactions are 93:5:2, respectively.

Among the three reactions observed, the fastest one was studied in the greatest detail. As was reported previously[1]

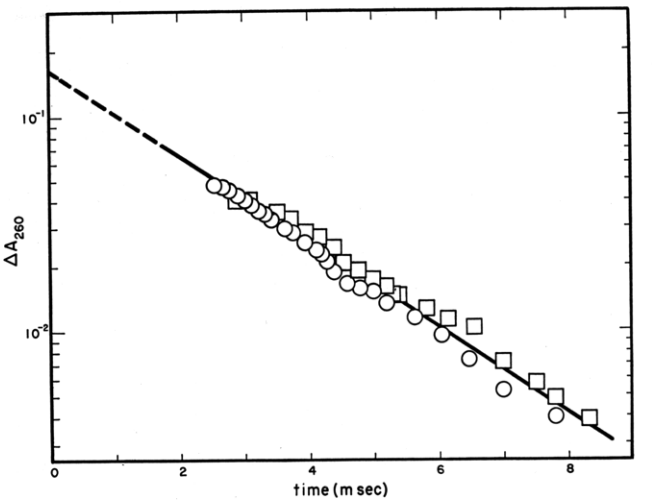

FIGURE 5: Logarithmic plot of the first phase (fast reaction) in the double-helix formation of poly[d(A-T)]. The circles and squares represent the results of two separate experiments. The pH of the solutions after mixing was 11.86. See text for further experimental details.

(Hickey and Hamori, 1971) and confirmed by additional results (Hickey, 1972), the rate of this reaction is strictly independent of the polymer concentrations used. In order to establish the effects of the initial and final pH of the solutions on the rate constant of the fast reaction (k_f) a series of experiments was carried out. The results obtained on sample 3 are summarized in Figure 6a. It can be seen on this graph that the initial pH of the solutions cannot be correlated with changes in the rate constant but, on the other hand, a definite relationship exists between the final pH values of the solutions and the rate constant of the fast reaction. The same conclusion could be drawn from the similar results obtained on poly[d(A-T)] samples 1 and 4 (Hickey, 1972). It must be noted on Figure 6a that as the final pH of the solution is increased k_f decreases almost linearly and when the final pH reaches the transition pH (11.98) the rate constant apparently becomes zero. Due to experimental difficulties, however, no data could be collected yet at final pH values very close to or far below the transition point.

The effect of solvent composition on the rate of the fast reaction was investigated in a series of experiments using glycerin. Since the presence of glycerin changed the transition pH of the polymer (see Figures 2 and 3), the initial and final pH values of the solutions were chosen such that they were the same distance from the transition pH of the system (*i.e.*, they were "symmetrical" pH jumps of equal size). This provision assured that the effect of solvent composition and not that of the pH would be measured. The results summarized in Table II show that k_f decreases with increasing glycerin addition.

Table II also shows the results of experiments carried out to test the effect of the molecular weight of the polymer on the fast rate. From the limited results obtained, it appears that molecular weight changes at high molecular weights have no effect, but the rate decreases significantly if the molecular weight of the polymer becomes very low.

The reaction of formaldehyde with DNA has been investigated in detail by other workers (Stollar and Grossman,

[1] When comparing data reported in our preliminary communication to those presented in this paper, it must be remembered that the pH values previously given were determined at 25.0° (and not at 10.0° as in this paper) and the slopes of first-order plots were reported as rate constants without being multiplied by the factor −2.303.

TABLE II: The Effect of Glycerin Addition and Polymer Molecular Weight on the Rate Constant of the Fast Reaction of the Double-Helix Formation of Poly d[(A-T)].[a]

% Glycerin (v/v)	Viscosity of Solvent (cP)	k_f (sec^{-1})	Mol Wt (10^3)	k_f (sec^{-1})
0	1.34	755	69	410
20	2.76	470	660	755
40	6.33	440	1,100	735

[a] In each kinetic experiment, the final pH of the solutions was 0.22 pH unit below the transition pH of the system. In the glycerin series sample 3 was used; in the molecular weight series the solvent contained no glycerin.

1961; Utiyama and Doty, 1971). It was shown that this reagent hydroxymethylates the amino groups of the polymer bases, which in this blocked state cannot participate in complementary base pairing and double-helix formation (Grossman *et al.*, 1961; Lewin, 1966). In order to study the effect of base substitution on the rate of double-helix formation, we hydroxymethylated a poly[d(A-T)] sample (3) using a mild treatment with formaldehyde (see Materials and Methods section). A very low degree of base substitution was achieved as indicated by the fact that the polymer solution treated with formaldehyde showed the same hypochromicity change at the transition pH as the untreated solution. Apparently, the presence of the few blocked bases would not observably decrease the capacity of the polymer to assume a completely double-helical conformation. The results of kinetic experiments using hydroxymethylated poly[d(A-T)] are shown in Table III. It can be seen that a progressive lowering of k_f occurred when the formaldehyde concentration was increased in the solutions. Interestingly, the proportion of the fast reaction in the overall process also decreased slightly with increasing hydroxymethylation (Table III, last column). In these experiments, in order to allow sufficient time for the hydroxymethylation reaction, the formaldehyde was mixed into the polymer solutions the day before the kinetic measurements. Another series of experiments was performed by adding the formaldehyde not into the polymer solution, but into the acid solution used for lowering the pH of the polymer system in the stopped-flow apparatus. In this arrangement, the polymer solutions were brought into contact with formaldehyde only a few milliseconds before double-helix formation and, presumably, not enough time was allowed for the hydroxymethylation reaction to take place. Since these experiments resulted in exactly the same k_f as those without any formaldehyde addition, it must be concluded that it is the (slow) reaction of the polymer with formaldehyde (*i.e.*, hydroxymethylation) which is responsible for the observed lowering of rates and not some other (fast) interaction of this reagent with poly[d(A-T)].

Aminoacridines (proflavine, acridine orange, etc.) are known to interact with nucleic acids by intercalating between the bases inside the double helix and by associating with the charged phosphate groups of the nucleotides outside the helix (Blake and Peacocke, 1968; Armstrong *et al.*, 1970; Sakoda *et al.*, 1971; Roth and Kochen, 1971). We investigated the effect of acridine orange on the fast rate of double-helix formation using conditions under which only a very limited

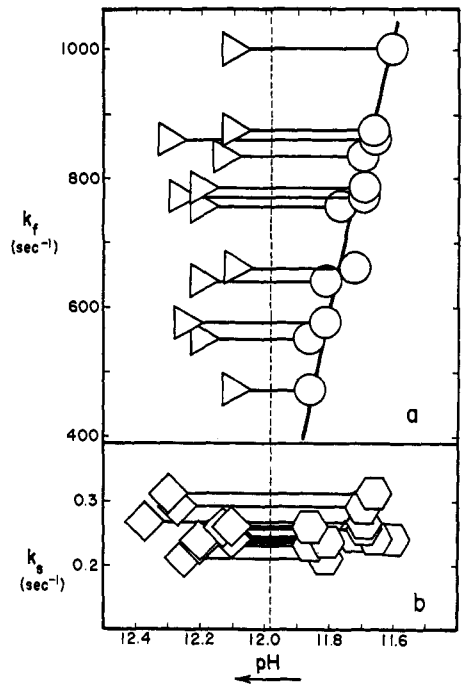

FIGURE 6: The effect of pH on the fast (a) and slow (b) phases of the double-helix formation process. The reaction, initiated by a sudden pH change in the stopped-flow apparatus, is represented by horizontal lines in this graph. The left ends (▷ or ◇) show the initial pH of the polymer solutions before mixing, and the right ends (○ or ○) the final pH after mixing. The ordinate values of the lines represent the rate constants observed. The vertical dotted line indicates the transition pH of the system from the random-coil form (left) to the double-helical conformation under the experimental conditions used (10°, 25 μg/ml of poly[d(A-T)], sample 3, 25 mM Na^+ and 4 mM $(PO_4)^{3-}$).

binding occurred (*e.g.*, 7 dye molecules/100 nucleotide units, at 0.010 mM acridine orange concentration; Hickey, 1972). The results of these studies are summarized in Table III. It can be seen that binding of acridine orange by poly[d(A-T)] decreases significantly both the rate and the extent of the first phase of double-helix formation.

TABLE III: Effect of Hydroxymethylation with Formaldehyde or Acridine Orange Addition on the Initial Phase of the Double-Helix Formation of Poly[d(A-T)].[a]

Formaldehyde (%)	Acridine Orange (mM)	k_f (sec^{-1})	Proportion of the Fast Reaction in the Total Change (%)[b]
0	0	480	93
0.004	0	300	91
0.008	0	240	90
0	0.010	425	88
0	0.025	330	83

[a] The pH of the solutions before and after mixing was 12.10 and 11.86, respectively. See the legend of Figure 6 and the Materials and Methods section for details on experimental conditions. [b] The estimated error of these values is ±2%.

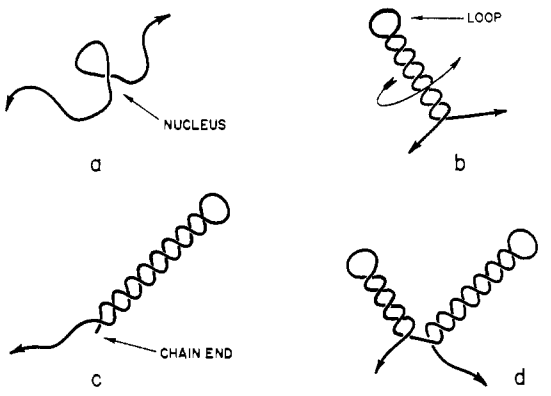

FIGURE 7: Double-helix formation of poly[d(A-T)]. Schematic representation of the postulated mechanism of the initial phase of the reaction. (a) Formation of a nucleus containing two or three base pairs; (b) propagation of the double-helical structure by the rapid twisting of a nucleus; (c) termination of growth by an encounter with a chain end; (d) termination by a convergence of two growing helical segments.

Discussion

Our observation (Hickey and Hamori, 1971; Hickey, 1972), that in dilute solutions and at low salt concentrations, the rate of double-helix formation of poly[d(A-T)] is independent of polymer concentration, confirmed the suggestion made by other workers (Inman and Baldwin, 1962b, 1964; Scheffler *et al.*, 1968, 1970) that under these conditions, double-helical poly[d(A-T)] is formed not by the entwining of two separate polymer molecules but by an intramolecular process involving the folding and twisting of the polymer chain within a single macromolecule. Our present results show that the actual mechanism of this conformation change is a complicated process, composed of three distinct phases.

This discussion will be limited mainly to the major phase, the fast reaction. It appears that the detailed interpretation of the other two phases of the double-helix formation will have to wait until more experimental details become available in our laboratory on the intermediate and slow reactions.

During our kinetic experiments, the double-helix formation of poly[d(A-T)] is initiated by the sudden lowering of the pH of the system, causing a protonation of the charged pyrimidine rings of the thymidine residues. This process, being an ionization reaction, must be very fast (Eigen and de Maeyer, 1963), and it should run practically concurrently with the mixing process in the stopped-flow instrument. Our findings, that the fast reaction is not preceeded by an even faster absorbance change, indicate that the ionization reaction is not accompanied by a significant absorbance change at 260 nm. (If it were, the fast reaction seen would not extrapolate to the initial absorbance of the system but to a lower value.) This result provides a direct evidence for the conclusion drawn from the spectroscopic studies of free nucleotides and DNA (Ageno *et al.*, 1970) that at 260 nm the observed hypochromicity of the bases is due to their base pairing and stacking and not to their ionization.

We believe that the fast reaction observed in the stopped-flow experiments is due to a conformation change proceeding in the following manner. When the number of charged thymine groups is reduced by the applied pH change the conditions will become suddenly favorable for helix formation. The folding of a single poly[d(A-T)] chain into double-helical structure must start with the formation of helix nuclei containing a few (perhaps 2–3) nucleotide units, in which the bases are paired and oriented according to the geometry of the double helix.[2] Figure 7a is a schematic drawing of a nucleus so defined. It appears that these nuclei will develop most readily from chain segments located relatively close to one another on the polymer backbone (Hickey, 1972). The next step, the propagation of the double-helical structure must involve the rapid twisting of these helix nuclei, which in this manner will grow into long double-helical segments (Figure 7b). Due to the unfavorable steric orientation of the polymer backbone, the loops at the ends of the nuclei will contain several nucleotides not in double-helical form. Four appears to be a good estimate for the number of such unpaired nucleotides in these loops (Scheffler *et al.*, 1970). A termination in the rapid growth of a given helical branch must occur when the propagating structure encounters a chain end or converges to another growing helical branch (Figure 7c,d). Due to the large local entropy decrease involved, the rate of nucleation appears to be much slower than the propagation of helical structure by the twisting mechanism. For this reason, the rate-determining step in the entire conformation change is probably the slow formation of helix nuclei.

The effects of various parameters tested on the fast rate give support to the mechanism proposed in the following manner. If the end point of the pH jump is lowered more and more below the transition pH of the polymer, the fraction of thymine bases which will be charged at the onset of double-helix formation is less and less.[3] Since these charged bases cannot participate in base pairing and double-helix formation their presence will hinder the nucleation of helical regions. Thus, the observed increase in the fast rate with decreasing final pH of the solutions (Figure 6a) can be explained by the diminishing of charges with decreasing pH. Notice, however (Figure 2), that the total absorbance change of the system remains practically constant when the final pH is varied from 11.6 to 11.9, and it is only k_f which changes drastically in this pH region (Figure 6). This, of course, indicates that the effect of charged thymine bases is kinetic and not thermodynamic. These observations suggest that the presence of charges on the polymer backbone at the onset of the conformation change is a rate-determining factor of the fast phase of the double-helix formation of poly[d(A-T)]. (As can be seen on Figure 6b, this is probably not true for the slow phase.)

It can be seen on Figure 6a, that the fast rate is independent of the initial pH of the solution, meaning, that the structure of the polymer at high pH is relatively insensitive to the hydrogen ion concentration of the solvent. This result would appear to confirm the random-coil conformation of poly[d(A-T)] under these conditions.

Since glycerin lowers the transition pH of the polymer (see Figure 3) it is apparently interacting with the nucleic acid in such a way that the tendency of the polymer toward helix formation is diminished. The kinetic effect observed (Table II) can be due to the retardation of the nucleation and/or propagation process in terms of the mechanism proposed. Since k_f is not decreasing in proportion to the viscosity increase of the solvent, glycerin is apparently interfering with the forma-

[2] The exact structure of the poly[d(A-T)] helix in solution is not known (Bram, 1971) but it is believed to be similar to the B form of the Watson–Crick double helix (Davies and Baldwin, 1963).

[3] It must be borne in mind that since the pK value of these bases is significantly less in the random-coil form than in the double-helical conformation the degree of ionization of the thymine groups in the former state will change significantly with pH even in the range 0.5 pH unit below the transition pH (Hickey, 1972).

tion of the base pairs during nucleation rather than retarding the rapid spinning of the nuclei by its effect on solvent viscosity.

Table II shows that a decrease in the molecular weight of the polymer decreases the rate significantly at low molecular weights. This can be explained in terms of the mechanism proposed by considering that at constant polymer concentration an increased concentration of chain ends will introduce increasing concentration of termination points for the propagation phase of the reaction (Figure 7c). This, of course, would have the consequence of decreasing the rate of double-helix formation. At high molecular weights, this effect is unimportant (Table II), because apparently under these conditions, the great majority of growing helical segments terminate by convergence (Figure 7d).

The effect of limited hydroxymethylation of poly[d(A-T)] (Table III) can also be explained in a similar manner. Since the bases blocked by this substitution cannot be incorporated into the double-helical structure, the growth of a propagating nucleus will always stop if it encounters a hydroxymethylated adenine base.

The fact that very low levels of base substitutions show a significant effect on the rate suggests that rate measurements such as these could be used in general for the detection of minor irregularities on the polymer chains. The potential of kinetic studies of this kind is also indicated by the results of our experiments with acridine orange (Table III).

In conjunction with studies on the other two phases of poly[d(A-T)] double-helix formation, work is under way to refine the mechanism presented here by the inclusion of events such as, helix growth at the convergence point of two helical branches ("Y" formation), the slow absorption of short helical regions by larger ones, etc. Also, work was started on a digital computer simulation of this double-helix formation process. The encouraging preliminary results indicate that the main feature of the fast phase of the reaction (*i.e.*, first-order behavior) can be readily reproduced by the simple mechanism put forth above (Hamori *et al.*, in preparation).

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