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Preparation and Characterization of Monodisperse, Cross-Linked Low Molecular Weight Deoxyribonucleic Acid*

Robert J. Cohen† and Donald M. Crothers

ABSTRACT: Fractional precipitation in an aqueous buffer–isopropyl alcohol mixture was used to fractionate calf thymus DNA according to molecular weight below about 2×10^6 daltons and a modification of this technique led to a convenient molecular weight determination. Interstrand cross-linking *via* mitomycin C, inducing reversible melting, can be accomplished down to 1.0×10^5 daltons. The T_m's and melting breadths agree qualitatively with the theoretical description of the helix–coil transition of low molecular weight DNA. Hg(II)–DNA Cs_2SO_4 density gradient ultracentrifugation led to fractionation of small molecules by base composition but did not appreciably sharpen the melting transition of the calf thymus DNA. We conclude from this latter observation that there is considerable deviation from random base sequence in sections of calf thymus DNA of 10^5 molecular weight.

The study of the kinetics and mechanism of the helix–coil transition of low molecular weight DNA (R. J. Cohen and D. M. Crothers, paper in preparation) necessitated the development of some new techniques felt to be of interest to the nucleic acid biochemist. The proposed fractional precipitation of DNA in an isopropyl alcohol–BPES[1] buffer is believed to be the first adequate molecular weight fractionation for short DNA molecules. One might mention that solubility differences in mixed solvents have long been used to fractionate synthetic polymers according to molecular weight.

Interstrand cross-linking of DNA *via* mitomycin C has heretofore been only reported for large DNA (>10^7 daltons) (Iyer and Szybalski, 1963, 1964; Szybalski and Iyer, 1964a,b; Summers and Szybalski, 1967). Mitomycin C was chosen for preparative cross-linking for two reasons: (1) among the various reagents, mitomycin C seems to produce the most thermostable links (Szybalski and Iyer, 1964a,b; Iyer and Szybalski, 1964), and (2) compared with other alkylating substances, a greater proportion of bound mitomycin is in the form of interstrand cross-link, about one-fifth to one-tenth (Szybalski and Iyer, 1964a,b). The helix–coil transition of mammalian DNA is considerably broader than that expected of an individual molecule. Marmur and Doty (1962) have found that the T_m's of various DNAs increases linearly with G + C content. So this broadening was thought to arise from the sum of the transitions of fairly large regions of differing base composition, each exhibiting a different temperature profile. It follows that for sheared DNA, there could appear a series of small molecules of varying G + C content each with a rather sharp melting transition.

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[1] Abbreviations used are: BPE, 6 mM Na_2HPO_4–2 mM NaH_2PO_4–1 mM Na_2EDTA; BPES, also has 0.179 M NaCl; HMP, 5 mM Na_2HPO_4 + 5 mM NaH_2PO_4 adjusted to an appropriate pH by 1 M NaOH. T_m is the midpoint of the helix–coil transition using temperature as the disrupting parameter.

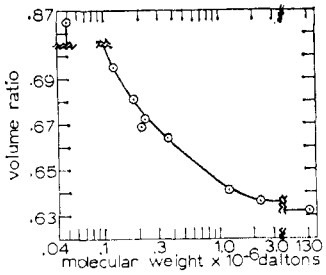

FIGURE 1: The isopropyl alcohol titration of calf thymus DNA in BPES. The ordinate is the volume of alcohol added to 1.00 ml of 0.2 mg/ml of DNA in BPES to precipitate material of various molecular weights.

The binding of Hg^{2+} to DNA increases with increased thymidine content; this binding is somewhat cooperative. Nandi *et al.* (1965) reported that Cs_2SO_4 density gradient equilibrium centrifugation can use this selectivity to fractionate DNA of varying base composition because the buoyant density of DNA in Cs_2SO_4 increases greatly with bound Hg^{2+}. This separation is four to six times that encountered in G + C fractionation of uncomplexed DNA in CsCl. We applied this method in an attempt to fractionate according to base composition our preparation of short molecular weight fractionated calf thymus DNA.

Experimental Section

The sodium salt of calf thymus DNA purchased from Sigma Chemical Company was dissolved in either BPES or BPE and subjected to degradation, usually with sonication by a Branson S125. The smallest DNA was sonicated at highest power for a total of 1 hr, alternating 5-min sonication with 5-min bubbling with nitrogen. This invariably resulted in DNA of about 1.2×10^5 daltons. Intermediate sizes were obtained by using less power and a shorter duration. Calf thymus DNA was sheared in a Virtus "45" to obtain samples of a few million daltons. Every degradation took place at 0°. The resultant DNA was purified by extraction with freshly distilled phenol, neutralized with 0.2 M sodium phosphate buffer (pH 6.8). The phenol was removed by extensive dialysis into BPES.

Concentrations were read spectrophotometrically taking the optical density of 10 μg/ml of DNA to be 0.213 (Reichmann *et al.*, 1954).

Molecular Weight Determination. A Spinco Model E analytical ultracentrifuge equipped with ultraviolet optics was used for all sedimentation velocity runs. Photographs were examined with a Joyce-Loebl microdensitometer. All runs were made in 12-mm cells with a concentration of 50 μg/ml of DNA in BPES.

The Doty, McGill, and Rice equation (1958), $s_{20,w} = 0.063\ M_w^{0.37}$, was used to determine M_w, the weight-average molecular weight. The relationship is based on light scattering and becomes increasingly uncertain at both high and very low molecular weights. Nevertheless, in the absence of data utilizing the more recent advances in light scattering, we have used it to calculate molecular weight.

Melting Curves. Calf thymus DNA was melted in water-jacketed quartz spectrophotometer cells (Hellma QS 160) after having been dialyzed at least twice into an alkaline buffer[2] consisting of 100 ml of 0.1 M NaH_2PO_4–0.1 M Na_2HPO_4–1 mM Na_2EDTA plus 19 ml of 1 M NaOH ($[Na^+]$ = 0.41 M, pH 11.6). The T_m occurs at about 28 to 30°. Hyperchromicity at 270 nm was observed in a Beckman DU-2 spectrophotometer equipped with a thermostated cell compartment. For both denaturation and renaturation, at least 10 min was allowed for temperature equilibration. The temperatures indicated are uncorrected water bath temperatures. The T_m of a particular DNA was repeatable to 0.5–1.0° from run to run.

Fractional Precipitation Procedure. (1) To a known volume of sonicated or sheared DNA (2–10 mg/ml) in BPES was added just enough isopropyl alcohol to completely precipitate it. The volume of alcohol was noted. (A previous titration of a small amount will make this easier; see the next paragraph.) (2) The mixture was equilibrated at least 4 hr at 25.0° with constant stirring. (3) A BPES–isopropyl alcohol stock solution at least ten to twenty times the volume of the equilibrating solution was prepared. The alcohol concentration was slightly less than that of the initial solution. The exact amount depended on how many fractions were desired. We usually chose to decrease the (volume) percentage of alcohol by 0.7% at each step, using, for example, successive extraction concentrations of 68, 67.3, 66.6, etc. The procedure yielded six to eight fractions. (4) After equilibration the precipitate was spun down and the supernatant was decanted and saved. (5) A known volume of stock solution comparable with the initial volume in 1 was used to resuspend the precipitate. (6) The mixture was equilibrated as in 2. (7) The apparent volume of the remaining stock solution was calculated and enough BPES added to lower the alcohol concentration so as to collect the number of fractions desired. (8) Steps 4–7 were repeated dissolving more and more DNA until no DNA precipitate remained. Succeeding fractions contain larger DNA. (9) The isopropyl alcohol was evaporated out of each fraction and the remaining solution was dialyzed into BPES. The first and last fractions were generally discarded. Ethanol may also be used (unpublished data).

A Titrometric Method for Molecular Weight Determination. The above suggests a simple and accurate analytic determination of molecular weight. Aliquots (10.00 ml) of about 0.2 mg/ml of calf thymus DNA in BPES were titrated with isopropanol until a precipitate started to form (the "cloud point"). Figure 1 shows the cloud point volume plotted against the molecular weight from sedimentation runs. Each titration was repeated at least three times and averaged. Titration reproducibility is about 0.5% of titrant volume.

Cross-Linking via Mitomycin C. The published method (Szybalski and Iyer, 1964a) for *in vitro* cross-linking had to be considerably modified for small DNA. Appropriate concentrations of mitomycin C (purchased from the Sigma Chemical Co.) were mixed with from 0.5 to 1.0 mg per ml of calf thymus DNA in BPES. A semiempirical equation will be discussed in the Results indicating the exact proportions. Nitrogen was bubbled through the solution for at least 10 min. Then a volume of $Na_2S_2O_4$ also in BPES con-

[2] This buffer has been referred to as "medium salt buffer" by Spatz and Crothers (1969) and by R. J. Cohen and D. M. Crothers (paper in preparation). We shall use this designation here.

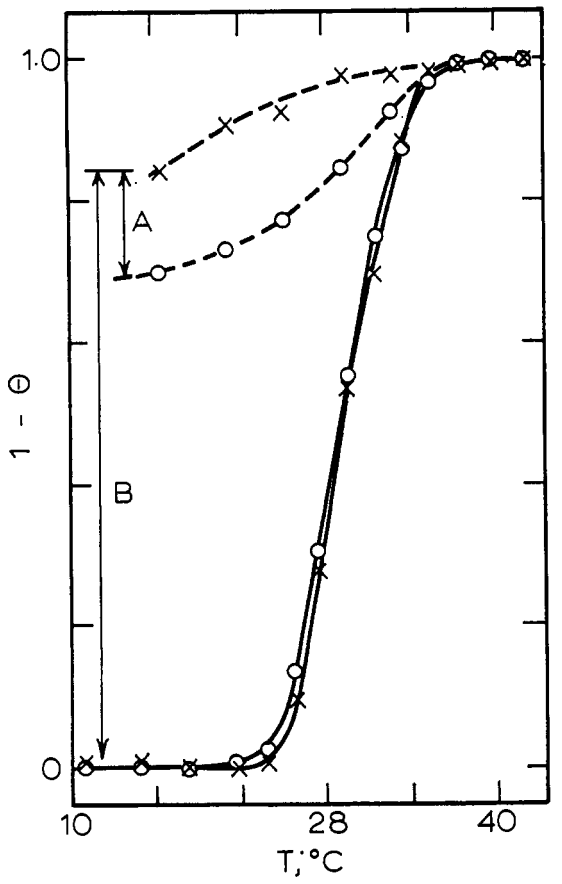

FIGURE 2: The melting and renaturation of a sample of partially cross-linked calf thymus DNA (2.3×10^6 daltons) in medium salt buffer. (O) Cross-linked sample; (×) control sample, not cross-linked; (——) increasing temperature; (---) decreasing temperature. The ratio A : B is the fraction cross-linked.

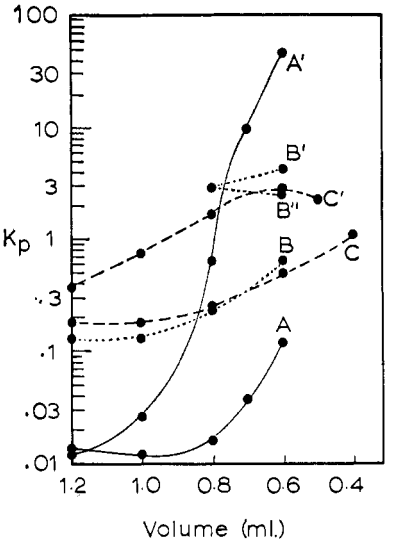

FIGURE 3: The partition coefficient, K_p, of native and denatured DNA of different molecular weight under various conditions. The abscissa represents the volume of polymer stock added to 1.0 ml of DNA solution in HMP.

Curve	DNA State	DNA Mol Wt × 10^{-5}	pH	Concn (μg/ml)
A	Denatured	(1/2) 23.0	7.0	210
A'	Native	23.0	7.0	100
B	Denatured	(1/2) 2.2	7.0	460
B'	Native	2.2	7.0	510
B''	Native	2.2	7.0	120
C	Denatured	(1/2) 2.2	7.2	1500
C'	Native	2.2	7.2	1700

taining three to ten times the previously added moles of mitomycin was mixed with the anaerobic solution to initiate cross-linking. N_2 bubbling was continued for 5 min more. The DNA was then dialyzed into at least seven changes of BPES to remove the antibiotic. (Mitomycin cross-links only in the reduced form.)

We also wanted only one cross-link per molecule. Assuming random reaction, the number of links in a molecule should follow a Poisson distribution. One can then calculate that if 10–15% of the molecules are renaturable, about 90% of these have no more than one cross-link. Also, the monofunctionally linked and intrastrand mitomycin are distributed into noncross-linked DNA.

Parameters for One Cross-Link per Molecule. Each molecular weight was tested as above on a small scale to determine the relative concentrations of mitomycin and DNA to obtain 10–15% cross-linked molecules. The resulting DNA was melted and renatured. The control was DNA treated with 0 mg/ml of mitomycin C. The per cent not cross-linked was taken as the ratio of the hyperchromicity in the test DNA to that of the control well below the temperature the DNA initially started to melt. Figure 2 shows one such comparison.

Polyethylene Glycol–Dextran Partitioning. The renaturability of cross-linked DNA was used to separate it from uncross-linked DNA by simply melting and cooling the whole sample and removing the denatured material. A two-phase polyethylene glycol–dextran partition method has proven successful in separating native from denatured DNA of molecular weights 10^7–10^8 daltons (Summers and Szybalski, 1967; Albertsson, 1965; Alberts, 1967). The technique was extended to DNA as small as 1.0×10^5 daltons and to high concentrations, up to 1.0 mg/ml. (a) To find the optimal conditions, tests similar to those reported by Summers and Szybalski (1967) were conducted. The polymer stock contained 16.8% (w/w) Dextran T500 (Pharmacia, Inc., Piscataway, N. J.) and 9.2% (w/w) polyethylene glycol (Carbowax 6000, Union Carbide Co.). Six 1.00-ml solutions of native DNA in HMP adjusted to pH 6.8–7.2 plus one of just buffer were put aside. To each was added carefully with a syringe volumes of 0.4–1.2 ml homogeneously mixed polymer stock. Each was violently shaken and centrifuged to separate the phases. The partition coefficient, K_p^N, is the ratio of (OD_{260}–OD_{320}) in the upper phase to that in the lower, corrected by the buffer blank. The same procedure was followed for the denatured DNA, obtained by heating to 100° for 10 min and quick cooling in an ice bath. The gradual increase in K_p shown in Figure 3 for DNA of 2×10^6 and 2×10^5 daltons is in contrast to the precipitous rise encountered for larger DNA reported by the above authors. A satisfactory separation could nevertheless be made. It sometimes proved necessary to repeat the fractionation for material less than 10^6 daltons.

(b) This technique was used to separate cross-linked ma-

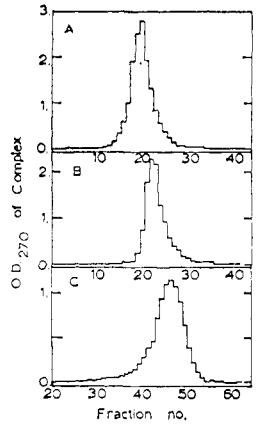

FIGURE 4: Cs_2SO_4 density gradient fraction patterns for Hg(II)–DNA.

	Mol Wt $\times 10^{-6}$ Daltons	Cross-Linked (?)	Fractionated for Size	Time on UC (hr)	Vol of Each Fraction (ml)
A	4.0	No	No	67	0.114
B	6.0	Yes	Yes	95	0.096
C	1.0	No	Yes	94	0.071

terial from uncross-linked. Volumes of 10 to 150 ml of 0.2–1.0 mg/ml of mitomycin-treated DNA were dialyzed at 0° into 0.04 M NaOH–0.179 mM NaCl–1 mM Na_2EDTA for 4 hr and allowed to come to room temperature for 10 min. They were then neutralized with 2 M sodium phosphate buffer and dialyzed into successively lower ionic strengths of phosphate and finally at least three times into HMP (pH 7.0).

An appropriate volume of homogeneous polymer stock was poured into a cylinder, DNA solution was added, the ground-glass stopper was replaced, and the cylinder was violently shaken. The mixture was centrifuged and the upper layer of polyethylene glycol containing cross-linked DNA was removed. The polyethylene glycol was salted out with 23% (w/w) 1:1 M K_2HPO_4 and KH_2PO_4. The aqueous

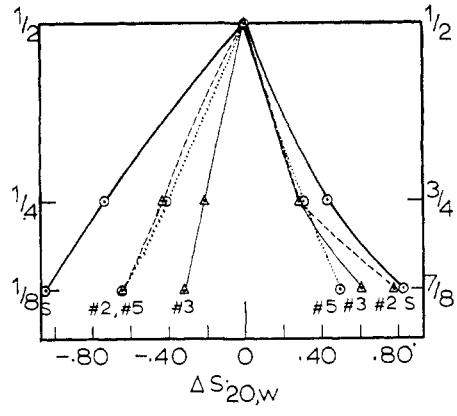

FIGURE 5: The spread in s after isopropyl alcohol fractionation. The vertical numbers are the fractional positions on the boundary at which s is measured, 1/8, 1/4, 1/2, 3/4, and 7/8 of the distance through the boundary. If x is this fraction, $\Delta s = s_{20,w}(x) - s_{20,w}(1/2)$. (—⊙—) Unfractionated calf thymus DNA, $s_{20,w}$ = 5.93 S; (--△---) fraction 2, $s_{20,w}$ = 4.93 S; (—⊙—) fraction 3, $s_{20,w}$ = 5.21 S; and (—⊙—) fraction 5, $s_{20,w}$ = 6.87 S.

TABLE I: Cross-Linking of Calf Thymus DNA by Mitomycin C.

DNA		Mitomycin C Concn	% Cross-Linked	
Mol Wt	Concn (μg/ml)	(μg/ml)	Calcd[b]	Found
50×10^6[a]	100–200	10		36
		20		49
		50		62
2.3×10^6	510	41	16	17
	550	53	20	17
	550	5.3	5	3
2.3×10^5	965	1900	28	25
	965	125	6	4
1.3×10^5	650	2500	14	14
	620	630	6	7

[a] From Szybalski and Iyer (1964a,b) *C. johnsonii* DNA. This DNA differs in G + C content from the calf thymus DNA reported here. Also the concentration is not specified. Calculations using the median concentration for these are only a few per cent off. [b] Using the semiempirical equation given in the text.

phase with the DNA was dialyzed into BPES for storage or in the case of the smallest DNA into BPES and then into the cold alkali to repeat the cycle.

G + C Fractionation. The Hg(II) complex with DNA was subjected to a Cs_2SO_4 density gradient equilibrium centrifugation as described previously (Nandi *et al.*, 1965). $HgCl_2$ was added to produce a ratio of 0.33 Hg^{2+} to DNA phosphate in a solution 0.05 M Na_2SO_4 and buffered by 5 mM $Na_2B_4O_7$ (pH 9.0). Enough Cs_2SO_4 (Harshaw Chemicals) was dissolved to produce a density of 1.57 g/cm^3 corresponding to the buoyant density expected of the complex at this binding ratio. Each sample was spun in a SW-50 swinging-bucket rotor in a Spinco Model L-2 ultracentrifuge for from 65 to 100 hr at 39,000 rpm and 18°. The time estimated from a relationship of Meselson (1957)[3] was checked by subjecting a portion of the material for one run to the analytical centrifuge (Spinco Model E) and observing that no further optical change occurred 24 hr after the estimated time. The bucket rotor was stopped and drops immediately collected into vials containing 0.10–0.20 ml of H_2O. The optical density of each fraction was read at 270 nm in a 1-mm path-length quartz cell using a Beckman DU-2 spectrophotometer. The distributions for three runs are displayed in Figure 4.

Results

The efficacy of the molecular weight fractionation can be demonstrated by a comparison of the boundary spread of sonicated DNA and some fractions of this material. Values of s were calculated at 1/8, 1/4, 1/2, 3/4, and 7/8 through the sedimentation boundary for a crude distribution analysis

[3] Vinograd and Hearst (1962).

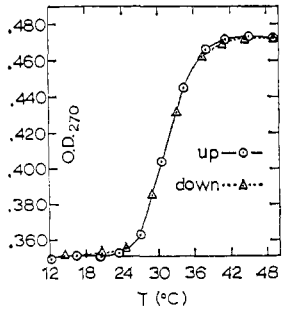

FIGURE 6: The melting and renaturation of cross-linked calf thymus DNA (6.0×10^5 daltons) showing complete reversibility. The experiment was conducted in medium salt buffer. The circles show increasing temperatures, the triangles decreasing temperatures. At least 10 min was allowed for equilibration before the optical density was read.

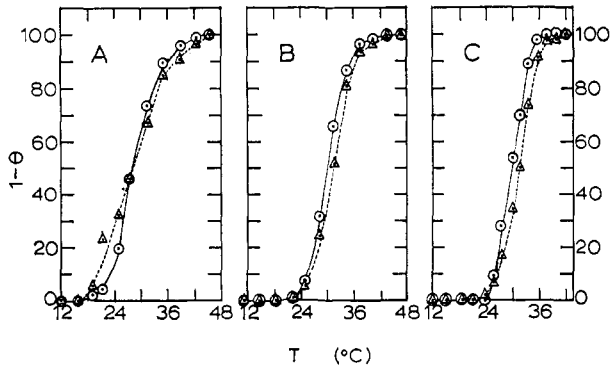

FIGURE 7: The helix–coil transition of some cross-linked and uncross-linked DNA. All melting was done in medium salt buffer. The uncross-linked material is represented by circles, the cross-linked by triangles. Those in the same box were done simultaneously. (A) 1.0×10^5 daltons, (B) 6.0×10^5 daltons, and (C) 2.3×10^6 daltons. All samples were previously fractionated for size.

(Figure 5). There is a significant gain in monodispersity. By applying the Einstein relationship, one can show that a major portion of the observed breadth of the fractionated samples is probably due to diffusional spreading.

From the mitomycin treatment, a semiempirical equation based on the Poisson distribution can be formulated to relate the degree of cross-linking to the concentration of DNA (c_D in micrograms per milliliter) and mitomycin C (c_{MC} in micrograms per milliliter) and the molecular weight of the former. This formula differs substantially from that

$$\text{fraction cross-linked} = 1 - \exp[-1.6 \times 10^{-11} c_D c_{MC}^{0.6} \times \text{mol wt}]$$

given by Summers and Szybalski (1967). Some predictions and experimental results are shown in Table I.

An example of the optical reversibility of cross-linked short DNA appears in Figure 6.

Discussion

We begin with a short digression into some qualitative aspects of the melting of low molecular weight DNA (Crothers *et al.*, 1965). There are two features of this melting; one is the transition measured by an optical density increase, arising from the decrease in the fraction of H-bonded bases from one to close to zero within a temperature interval of a few degrees. The second is the actual separation of strands; this occurs because of the increased entropy when two strands are no longer bonded together and can move separately. As the size of the molecule decreases, the energy per molecule for forming the helical state decreases but the entropy gain upon strand separation remains nearly the same. The combined effect is that T_m drops with lower molecular weight. Furthermore, the smallest DNA molecules tend to melt only from the ends, resulting in a broader helix–coil transition. Calculations based on this heterogeneous zipper model show that the breadth should depend on about the square root of the size for a random copolymer, excluding consideration of strand separation. This latter greatly sharpens the melting of individual molecules (Crothers *et al.*, 1965). Consequently, the denaturation of sheared DNA could at best show only qualitative agreement with the square root dependence.

Cross-linking overcomes this difficulty. Figure 7 reproduces the helix–coil transition curves from cross-linked and uncross-linked DNA of differing size melted in high pH medium salt buffer. Note that the breadth of the transition increases with decreased size, both for cross-linked and uncross-linked material. Comparing cross-linked DNA with uncross-linked material of the same molecular weight, the melting transition of the former is broader and this difference becomes more significant as the DNA gets smaller. It appears that strand separation does indeed sharpen the transition of normal small DNA ($\sim 2 \times 10^5$ daltons). Because this does not occur in cross-linked DNA, this material can furnish a stronger test for the predicted $M^{-1/2}$ dependence of the transition breadth. Our experiments (Figure 8) indicate an exponent of -0.3. That the DNA has not yet reached the size where melting from the ends completely dominates the melting (R. J. Cohen and D. M. Crothers, paper in preparation) may account for some of the discrepancy. Quite likely of greater importance are the moderate range fluctuations from random base sequence encountered in small fragments of calf thymus DNA. This is discussed after the next paragraph.

Note that the T_m of cross-linked DNA is somewhat higher than that of uncross-linked nucleic acid, again agreeing with the theory of Crothers *et al.* (1965). However the T_m of DNA of 1.0×10^5 daltons is abnormally low. Probably

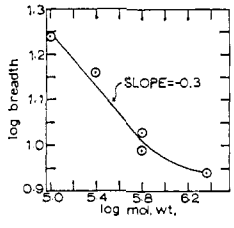

FIGURE 8: A log–log plot of the melting breadth *vs.* the molecular weight of cross-linked DNA. Melting breadth is defined as the temperature difference between 10 and 90% melted DNA. The sodium ion concentration is 0.41 M; the pH 11.4. The slope is -0.3 between 1.0×10^5 and 6.0×10^5 daltons.

TABLE II: Melting of (G + C)-Fractionated Calf Thymus DNA.

Mol Wt × 10^{-5} Dalton	DNA Cross-Linked	Fractionated for Size	Fraction No.	T_m (°C)	Breadth (°C)	1/Slope (°C)
6.0	Yes	Yes	u[a]		9.8	8.4
			u		9.7	9.3
			18	27.8	8.6	7.4
			22	29.2	8.8	6.9
			27	32.6	7.8	6.0
4.0	No	No	u	29.0	10.0	1.80
			u		9.0	9.8
			14		9.0	10.5
			15	28.4	7.7	8.0
			19		7.4	9.0
			24	30.3	9.0	7.6
			25	32.6	8.6	7.5
1.0	No	Yes	u		11.9	11.9
			40		12.6	13.0
			46		12.3	11.4
			50		10.2	10.7

[a] u = unfractionated; T_m is given only for those of each molecular weight that can be directly compared; the breadth is the usual definition, the temperature interval between 10 and 90% melted; and 1/slope is measured at the midpoint. The G + C content increases with fraction number. All melting was done in medium salt high pH buffer.

in this case the helix is somewhat disrupted by the mitomycin cross-link.

Lastly, we would like to comment on the base composition heterogeneity. Fragments of calf thymus DNA show only one defined peak per run (Figure 4) unlike the well-separated density classes in sheared lambda phage (Skalka *et al.*, 1968). To further characterize the density separation, Hg^{2+} was removed from several fractions by extensively dialyzing them against 1 M NaCl and then into the medium salt buffer for melting. Two fractions and untreated DNA or three fractions were melted simultaneously. Table II indicates that the T_m's differed by as much as 6° between extremes of a peak. It appears that fractionation according to G + C content has taken place. However, the breadth of the transition was decreased only by 10–20%. Comparison of these data with the results of Crothers *et al.* (1965) on melting of fragments of T2 DNA shows that the slope of the melting transition of the fractionated calf thymus DNA fragments is smaller by roughly a factor of two.

These results supply evidence that helps distinguish between extreme models for the distribution of bases in calf thymus DNA. The large breadth of the melting transition of high molecular weight calf thymus DNA shows that there is considerable segregation of bases into (A + T)- and (G + C)-rich sections (Crothers, 1968). A primary question is the distance scale of this segregation. It would be possible, for example, that DNA of special function, and hence special base composition, exists in blocks of many million molecular weight, corresponding to large-scale separation. An illustration of this kind of behavior is the finding of large fragments of lambda phage that are separable by banding density (Skalka *et al.*, 1968). Long-range fluctuations in T2 DNA are much smaller (Thomas and Pinkerton, 1962), and the melting transition is considerably sharper for this latter than for intact lambda DNA. An alternative model for base segregation is that it occurs on a much smaller distance scale. It is possible, for example, that the base sequence in regions of 10^5 molecular weight is far from random with local clustering of G + C and A + T pairs to an extent greater than expected for a random sequence. Our results favor this latter interpretation for calf thymus DNA. We find that samples of a few hundred-thousand molecular weight, fractionated for G + C content, show a much broader melting curve than expected for a random sequence, or than found for T2 DNA samples of similar size. Thus these short molecules of a given base composition do not closely approximate a set of molecules of random sequence. We cannot, however, eliminate the possibility that additional sequence fluctuations, in excess of random expectation, occur on a large distance scale. The main point our experiments establish is that short range (less than 10^5 molecular weight) composition fluctuations are larger in calf thymus DNA than in T2 phage DNA.

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Studies on Analogs of Isosteric and Allosteric Ligands of Deoxycytidylate Aminohydrolase*

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ABSTRACT: Cytosine arabinoside 5′-monophosphate (araCMP) and cytidine 5′-monophosphate (CMP), analogs of deoxycytidine 5′-monophosphate (dCMP), were deaminated at a very low rate by deoxycytidylate aminohydrolase. The addition of deoxycytidine 5′-triphosphate-Mg (dCTP-Mg), allosteric activator of this enzyme, produced a marked increase in the rate of deamination of araCMP and CMP. The kinetics of deamination of araCMP and CMP are cooperative and deoxythymidine triphosphate enhances the cooperativity. In the presence of dCTP the kinetics obeys to the Michaelis–Menten equation. At very high CMP concentrations inhibition by excess of substrate occurs and this inhibition is released by dCTP. It seems that CMP competes with dCTP for regulatory sites. Studies with analogs of allosteric effectors demonstrate the very high specificity of the allosteric sites of deoxycytidylate aminohydrolase.

The kinetic data obtained with CMP suggest the occurrence of three conformational isomers of deoxycytidylate aminohydrolase.

The activity of deoxycytidylate aminohydrolase is regulated by highly specific allosteric effectors: dCTP-Mg is the allosteric activator, dTTP-Mg is the allosteric inhibitor (Geraci *et al.*, 1967; Scarano *et al.*, 1967a). At saturating concentrations of the allosteric effectors the enzyme occurs as the enzyme–dCTP-Mg complex or as the enzyme–dTTP-Mg complex (Scarano *et al.*, 1967b). Moreover, the allosteric effectors cause specific changes of the affinity of the enzyme for competitive inhibitors (Rossi *et al.*, 1967).

The striking changes of the affinity of the enzyme for both substrate and competitive inhibitors, caused by allosteric effectors, prompted us to investigate analogous changes of the substrate specificity of the enzyme.

The present paper reports experiments of the activity of deoxycytidylate aminohydrolase toward several analogs of the substrate. In addition, studies on the specificity of the allosteric effectors of the enzyme are reported.

Material and Methods

Homogeneous deoxycytidylate aminohydrolase was prepared as previously described (Geraci *et al.*, 1967). The activity of the enzyme toward the analogs was measured either by a spectrophotometric assay or by an isotope assay similar to those used to determine the activity toward deoxycytidylate (Scarano *et al.*, 1967a).

Samples of chemically synthesized araCMP[1] were kindly donated by G. A. Fisher of Yale University and by M. J. Wechter of Upjohn Co. AraCTP was prepared from araCMP

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[1] Abbreviations used are: araCMP, arabinosylcytosine monophosphate; araUMP, arabinosyluracil monophosphate; araCTP, arabinosylcytosine triphosphate; CH_3dCTP, 5-methyldeoxycytidine triphosphate.