Proc. Natl. Acad. Sci. USA
Vol. 74, No. 1, pp. 188–192, January 1977
Biochemistry

Mutagen-nucleic acid intercalative binding: Structure of a 9-aminoacridine: 5-iodocytidylyl(3′-5′)guanosine crystalline complex

(9-aminoacridine-DNA binding/drug intercalation/frameshift mutagenesis)

T. D. SAKORE*, SHRI C. JAIN, CHUN-CHE TSAI†, AND HENRY M. SOBELL

Department of Chemistry, The University of Rochester, Rochester, New York 14627; and Department of Radiation Biology and Biophysics, The University of Rochester School of Medicine and Dentistry, Rochester, New York 14620

Communicated by Alexander Rich, November 8, 1976

ABSTRACT **9-Aminoacridine forms a crystalline complex with the dinucleoside monophosphate, 5-iodocytidylyl(3′-5′)-guanosine. We have solved the three-dimensional structure of this complex by x-ray crystallography and have observed *two* distinct intercalative binding modes by this drug to miniature Watson-Crick double helical structures. The first of these involves a pseudosymmetric stacking interaction between 9-aminoacridine molecules and guanine-cytosine base-pairs. This configuration may be used by 9-aminoacridine when intercalating into DNA. The second configuration is an asymmetric interaction, largely governed by stacking forces between acridine and guanine rings. This type of association may play an important role in the mechanism of frameshift mutagenesis.**

9-Aminoacridine has long been known to be a potent frameshift mutagen in viruses and bacteria (ref. 1, see also Fig. 1). It is one in a general class of aminoacridine dyes (others include proflavin, acridine yellow, and acridine orange) that bind to DNA and possess mutagenic activity. The precise nature of 9-aminoacridine-DNA binding was clarified by Lerman over a decade ago, who introduced the stereochemical concept of drug intercalation to explain his spectroscopic and hydrodynamic DNA-dye binding data (2, 3). Since this time, a large number of synthetic drugs and antibiotics have been shown to utilize intercalation in their binding to DNA. These include anticancer drugs such as actinomycin and daunomycin (4–6), antimalarial drugs such as chloroquine and quinacrine (7), the antischistosomiasis drug hycanthone (a hydroxylated metabolite of Miracil D) (8), and the antitrypanosomal compounds ethidium bromide and propidium iodide (9). Although these drugs intercalate into DNA, they are not all mutagenic. For this reason, it is not clear how drug intercalation is related to the origin of frameshift mutagenesis.

This paper describes the structure of a crystalline complex containing 9-aminoacridine and the self-complementary dinucleoside monophosphate, 5-iodocytidylyl(3′-5′)guanosine (Fig. 2). The structure demonstrates *two* distinct intercalative binding modes by this drug to miniature Watson-Crick double helical structures. The first of these involves a pseudo-symmetric stacking interaction between 9-aminoacridine molecules and guanine-cytosine base-pairs. This configuration may be used by 9-aminoacridine when intercalating into DNA. The second configuration is an asymmetric interaction, largely governed by stacking forces between acridine and guanine rings. This type of association may play an important role in the mechanism of frameshift mutagenesis.

* Permanent address: Department of Chemistry, Indian Institute of Technology, Bombay, India.
† Present address: Department of Chemistry, Kent State University, Kent, Ohio 44242.

MATERIALS AND METHODS

9-Aminoacridine was purchased from K & K Laboratories, Inc., Plainview, N.Y. and used without further purification. The dinucleoside monophosphate, cytidylyl(3′-5′)guanosine, was obtained as the ammonium salt from Sigma Chemical Co. and used without further purification. Iodination of the dinucleoside monophosphate was accomplished with a synthesis devised by David C. Ward and his colleagues at Yale University, as described in a previous communication (10). Plate-like crystals were obtained by slow evaporation over several days of equimolar mixtures of 9-aminoacridine and 5-iodocytidylyl-(3′-5′)guanosine dissolved in a 50% water/methanol (vol/vol) solvent system. Preliminary characterizations of these crystals were done by comparing the ultraviolet absorption spectra of solutions obtained from washed single crystals with solutions containing known stoichiometric mixtures of these compounds. These spectral studies indicated a complex containing equimolar quantities of both compounds. Space group and unit cell dimensions were initially obtained from precession photographs with nickel-filtered CuKα radiation, and then refined by least squares from 12 independent reflections measured on a Picker FACS-1 automatic diffractometer. The crystals are monoclinic, space group $P2_1$, with a = 13.98 Å, b = 30.58 Å, c = 22.47 Å, β = 113.9°.

A single crystal of the aminoacridine: 5-iodocytidylyl(3′-5′)guanosine complex measuring approximately 0.3 mm × 0.2 mm × 0.5 mm was mounted in a 1.0 mm glass capillary with some mother liquor. Data were collected at room temperature with nickel filtered CuKα radiation using the theta-two theta scan method out to a maximum two-theta angle of 72°; 4418 reflections were measured, of which 2251 were significantly above background. The intensities were corrected for the Lorentz and polarization factors; however, no absorption corrections were used. The overall isotropic temperature factor and scale factor were derived by Wilson statistics (11), and normalized structure factors were then computed using the K-curve method (12). The positions of four iodine atoms were determined from the (E^2–1) Patterson map. With this information, a Patterson superposition function was calculated using a minimum function. This revealed the positions of two cytosine rings, three phosphorus atoms, and fragments of several ribose sugar rings. Phases calculated from this partial structure were then used in a sum-function Fourier synthesis (where amplitudes are $[|2F_o|-|F_c|]$ and phases are the calculated phases) (13) to generate additional structural information. The complete structure was developed after computing a large number of Fourier, difference Fourier and sum-function Fourier syntheses, often leaving out portions of the structure that were ei-

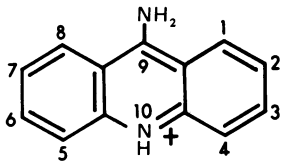

FIG. 1. Chemical structure of 9-aminoacridine.

ther unclear or in doubt and allowing them to reappear in later Fourier maps. This minimized bias in the structure analysis and assisted the Fourier refinement. The final structure contains 248 atoms in the asymmetric unit: *four* aminoacridines, *four* 5-iodocytidylyl(3′-5′)guanosine molecules, and 24 water molecules. The current residual (based on observed reflections) is 19.3%. Further Fourier and least squares refinement is needed.

RESULTS

Figs. 3 and 4 show a portion of the asymmetric unit of the 9-aminoacridine: 5-iodocytidylyl(3′-5′)guanosine (hereafter, denoted aminoacridine: ioC-G crystal structure as determined by this crystallographic study. The structure consists of *two* 2:2 aminoacridine: ioC-G complexes, each forming intercalated miniature Watson-Crick double-helical structures that stack to form infinite sandwich-like columns of acridine molecules and guanine-cytosine base-pairs along the *x* direction of the crystal lattice. In the first part of the asymmetric unit (shown in Fig. 3), the intercalated 9-aminoacridine molecule (C) is oriented such that its amino group points toward the *narrow* groove of the miniature double helix. The stacked 9-aminoacridine molecule (A), however, is oppositely oriented and lies above and below guanine-cytosine base-pairs of the intercalated dinucleoside monophosphate. A different situation exists in the second part of the asymmetric unit (shown in Fig. 4). Here, the intercalated 9-aminoacridine molecule (*C*) is oriented such that its amino-group lies in the *wide* groove of the double helix. The stacked 9-aminoacridine molecule (*A*) is similarly oriented, its amino-group again pointing towards the wide groove.

Important additional differences between these 2:2 complexes are evident by studying the stacking patterns between aminoacridine molecules and guanine-cytosine base-pairs. This is shown in Fig. 5. In the first 2:2 complex, the intercalated aminoacridine molecule is *pseudo-symmetric* with respect to the base-pairs (stacking pattern BCD), while in the second 2:2 complex it is distinctly *asymmetric* (stacking pattern *BCD*). Accompanying the asymmetric intercalative binding mode is a large shift in ring overlap between guanine-cytosine base-pairs. This shift gives rise to the enhanced separation observed between iodine atoms on cytosine residues (i.e., 14.6 Å for *BCD*, 11.0 Å for BCD), but not to a change in the interatomic phosphorous distances (i.e., about 17.3 Å for *BCD* and BCD). The conformational change can best be described as a "sliding" of base-pairs upon the intercalated acridine molecule, an electronic effect most probably related to the orientation of the acridine nucleus relative to the base-pairs. A similar situation exists with the stacked aminoacridine molecules. Aminoacridine molecule A in the first 2:2 complex display a stacking pattern with guanine-cytosine base-pairs (between neighboring intercalated dinucleoside monophosphates) that is virtually indistinguishable from the asymmetric intercalative binding mode observed in the second 2:2 complex (compare stacking patterns DAB with *BCD;* iodine-iodine distance for DAB, 14.6 Å). On the other hand, aminoacridine molecule *A* in the second 2:2 complex exhibits a pseudo-symmetric stacking pattern similar

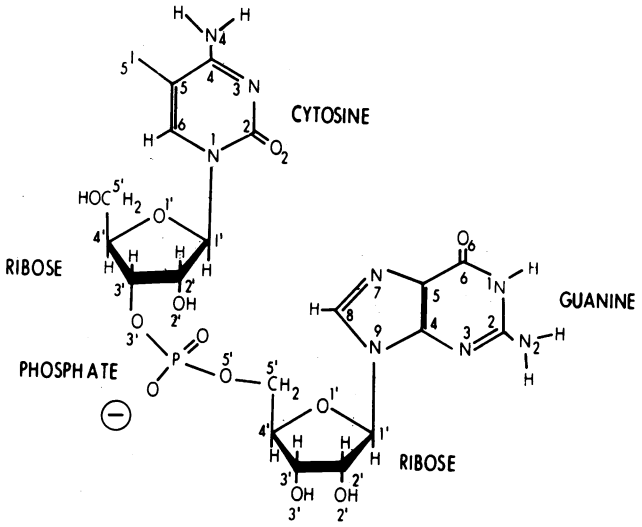

FIG. 2. Chemical structure of 5-iodocytidylyl(3′-5′)guanosine.

(although not identical) to that observed for the intercalative binding mode in the first 2:2 complex (compare stacking patterns *DAB* with BCD; iodine-iodine distance for *DAB*, 10.6 Å). The near identity of stacking patterns DAB and *BCD* suggests these to be particularly stable (and therefore highly stereospecific) stacking associations in this crystalline complex. We will discuss this point more completely in a later section.

In spite of these differences, however, both pseudo-symmetric and asymmetric intercalative complexes demonstrate similar stereochemical features with regard their sugar-phosphate conformations. These can best be described as *gauche-gauche* (a term used to describe the torsional angle around the C4′-C5′ bond) and the following pattern of ribose sugar ring puckering: C3′ *endo* (3′-5′) C2′ *endo* (14). These conformational changes, along with alterations in the glycosidic torsional angles, permit base-pairs to separate 6.8 Å and give rise to the twist angle observed between base-pairs above and below 9-aminoacridine molecules (estimated to be about 8° for the pseudo-symmetric intercalative structure and about 4° for the asymmetric intercalative complex), values estimated by projecting the interglycosidic carbon vectors on a plane midway between base-pairs and then measuring the angle between them. The sugar-phosphate conformations observed in these structures are very similar to the conformations observed in the ethidium: dinucleoside monophosphate crystalline complexes (10, 15–17).

The complex is heavily hydrated in the crystal lattice. Twenty-four water molecules have been located in the asymmetric unit, many of these forming hydrogen-bonded water-water tetrahedral-like structures and water-hydroxyl linkages to the sugar-phosphate chains. This leads us to believe that the associations of these compounds in the solid state are not significantly different from solution associations that may exist prior to cocrystallization.

DISCUSSION

The mechanism of aminoacridine induced frameshift mutagenesis is a subject that has attracted wide interest over many years (see ref. 18, for a recent review). Frameshift mutations consist of additions or deletions of varying numbers of base-pairs (these, however, must *not* be multiples of three). Although usually of the +1 or −1 type, larger additions and deletions occur with reasonable frequency (i.e., +2, or −2). The most

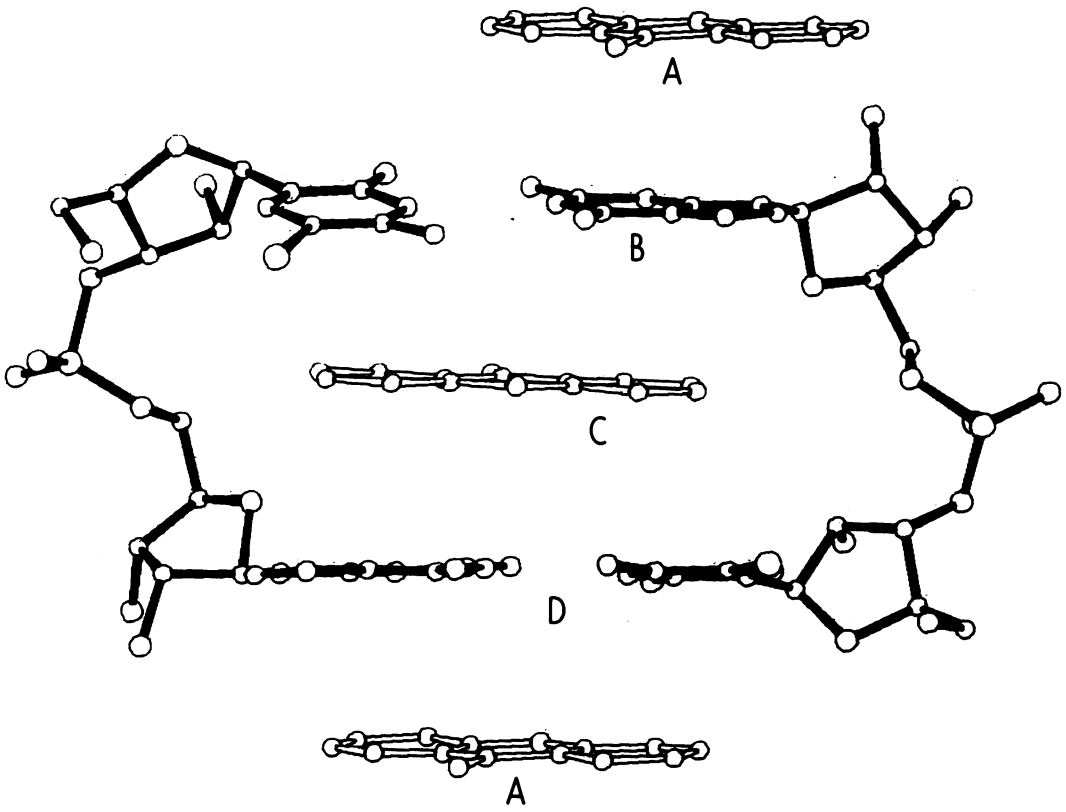

FIG. 3. A computer drawn illustration of the first part of the asymmetric unit of the 9-aminoacridine: ioC-G crystal structure viewed approximately parallel to the planes of the guanine-cytosine base-pairs and 9-aminoacridine molecules. ioC-G molecules are drawn with dark solid bonds; the intercalative and stacked 9-aminoacridine molecules are drawn with light open bonds. A *pseudosymmetric* intercalative binding mode is observed in this portion of the asymmetric unit.

plausible model to explain frameshift mutagenesis is a model proposed by Streisinger and his colleagues several years ago to explain the observed amino acid changes in a variety of plus-minus intragenically suppressed mutants of phage T4 lysozyme (19). In this model, DNA strand breakage with or without subsequent gap formation can lead to transient local melting and reannealing of DNA that can lead to mispaired configurations (looped-out single-stranded DNA regions) that are then stabilized by DNA repair. Subsequent DNA replication (or, perhaps, mismatch repair) can then give rise to addition- or deletion-type frameshift mutations. The model accounts for the general tendency for frameshift mutations to arise in the vicinity of strand discontinuities [for example, at the ends of chromosomes or near replication forks (20, 21)]. It also accounts for a general tendency of frameshift mutations to arise in areas of local base-sequence redundancy (19), and for frameshift mutational hot spots to occur in DNA regions that have short repetitive base sequences (22). According to this model, the mutagenicity of intercalating agents (such as 9-aminoacridine) could reflect an indirect effect of stabilizing looped-out base configurations through stacking interactions with individual bases, rather than by intercalation into double-helical DNA.

The current work is of interest in this regard for several reasons. In the first place, it has provided an opportunity to directly visualize a frameshift mutagen–nucleic acid interaction and this has provided evidence for *two* different intercalative binding modes in this 9-aminoacridine dinucleoside monophosphate model study. The pseudo-symmetric intercalative configuration can readily be utilized by 9-aminoacridine when it intercalates into double-helical DNA (T. D. Sakore and H. M. Sobell, manuscript in preparation). The asymmetric intercalative structure, on the other hand, *cannot* be utilized for drug intercalation into the double-helical DNA polymer. This reflects the magnitude of the helical screw axis dislocation that would have to accompany this binding mode (i.e., 2.7 Å for the asymmetric binding mode, compared with 0.5 Å for the symmetric binding mode; see ref. 23 for definitions) and the resulting difficulties in connecting neighboring sugar-phosphate chains with the intercalated dinucleotide structure. The asymmetric stacking mode could, however, be used by 9-aminoacridine in binding to single-stranded DNA regions (such as the looped-out structures described above or some other transient single-stranded DNA region present during replication or repair). We postulate that it is this type of interaction (rather than intercalation) which is responsible for acridine-induced frameshift mutagenesis.

It is of interest to compare the results of this study with another study of a similar kind in which 9-aminoacridine forms a crystalline complex with the self-complementary dinucleoside monophosphate, adenylyl(3′-5′)uridine (A-U) (24). Although this structure is fundamentally very different from the structure reported here [i.e., A-U dinucleoside monophosphates form a Hoogsteen-type base-pairing arrangement *without* double-helix formation (25)], there are several features in common. Both structures consist of alternate layers of 9-aminoacridine molecules and base-pairs arranged in columns along a crystallographic direction. These columns are held together through extensive hydrogen bonding with water molecules that provide well hydrated crystal lattice environments. Although it is not clear why one structure demonstrates drug intercalation into a miniature double-helical RNA fragment while the other does not, studies with ethidium complexed to several dinucleoside

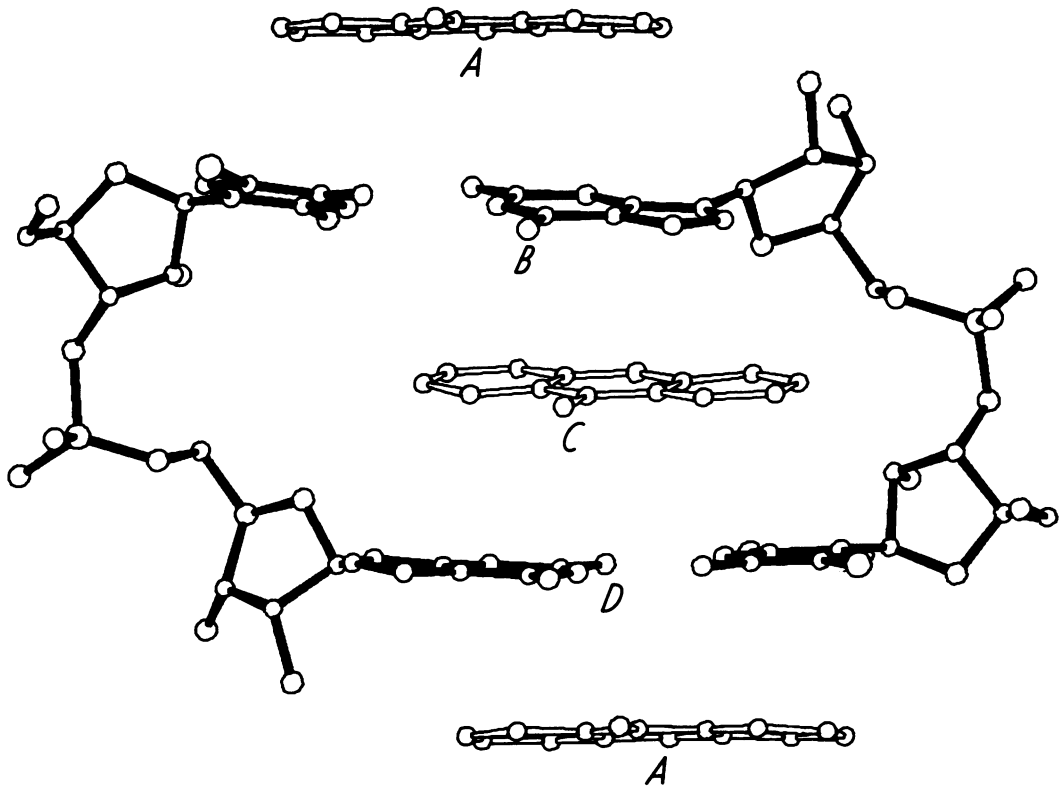

FIG. 4. The second part of the asymmetric unit of the 9-aminoacridine: ioC-G crystal structure viewed with a similar orientation to that shown in Fig. 3. ioC-G molecules are drawn with dark solid bonds; the intercalative and stacked 9-aminoacridine molecules are drawn with light open bonds. An *asymmetric* intercalative binding mode is observed in this portion of the asymmetric unit.

monophosphates in solution and in the solid state have shown the importance of sequence specificity (i.e., in particular, *pyrimidine-purine*) in stabilizing the intercalative complex through specific stacking interactions (15–17, 26). It is possible that similar considerations are important in understanding the 9-aminoacridine dinucleoside monophosphate interactions as well, and we will need to discuss this possibility in greater detail.

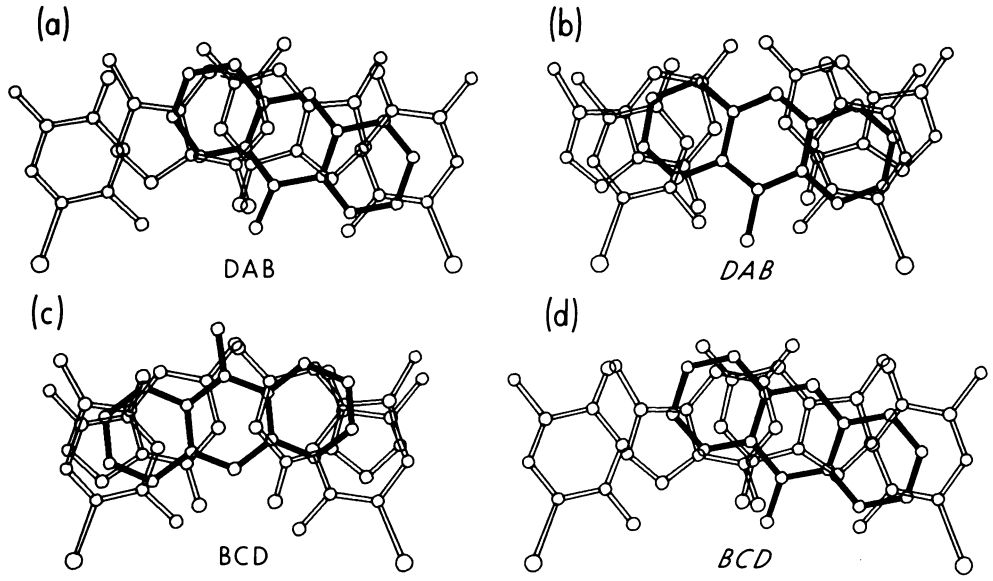

FIG. 5. Stacking patterns observed in the 9-aminoacridine: ioC-G crystalline complex. (a) DAB, stacked acridine molecule with guanine-cytosine base-pairs *between* neighboring intercalated ioC-G molecules in first part of asymmetric unit. (b) *DAB*, stacked acridine molecule with guanine-cytosine base-pairs *between* neighboring intercalated ioC-G molecules in second part of asymmetric unit. (c) BCD, intercalated acridine molecule with guanine-cytosine base-pairs in first part of asymmetric unit (pseudosymmetric intercalative binding mode). (d) *BCD*, intercalated acridine molecule with guanine-cytosine base-pairs in second part of asymmetric unit (asymmetric intercalative binding mode). In this figure, 9-aminoacridine molecules have been shown with dark solid bonds; guanine-cytosine base-pairs have been shown with light open bonds. See *text* for discussion.

This work has been supported in part by grants from the National Institutes of Health, the American Cancer Society, and the Energy Research and Development Administration. This paper has been assigned Report no. UR-3490-1051 at the Energy Research and Development Administration, the University of Rochester. T.D.S. is on leave from the Indian Institute of Technology, Bombay, India.

1. Crick, F. H. C., Barnett, L., Brenner, S. & Watts-Tobin, R. J. (1961) *Nature* **192,** 1227–1232.
2. Lerman, L. S. (1961) *J. Mol. Biol.* **3,** 18–30.
3. Lerman, L. S. (1963) *Proc. Natl. Acad. Sci. USA* **49,** 94–102.
4. Müller, W. & Crothers, D. M. (1968) *J. Mol. Biol.* **35,** 251–290.
5. Sobell, H. M. & Jain, S. C. (1972) *J. Mol. Biol.* **68,** 21–34.
6. Pigram, W. J., Fuller, W. & Hamilton, L. D. (1972) *Nature New Biol.* **235,** 17–19.
7. Nastasi, M., Morris, J. M., Rayner, D. M., Seligy, V. L., Szabo, A. G., Williams, D. F., Williams, R. F. & Yip, R. W. (1976) *J. Am. Chem. Soc.* **98,** 3979–3986.
8. Weinstein, I. B. & Hirschberg, E. (1971) in *Progress in Molecular and Subcellular Biology,* ed. Hahn, F. E. (Springer-Verlag, New York), Vol. 2, pp. 232–246.
9. Waring, M. J. (1970) *J. Mol. Biol.* **54,** 247–279.
10. Tsai, C. C., Jain, S. C. & Sobell, H. M. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 628–632.
11. Wilson, A. J. C. (1942) *Nature* **150,** 151–152.
12. Karle, J. & Hauptman, H. (1953) *Acta Crystallogr.* **6,** 473–476.
13. Sussman, J. L. & Kim, S. H. (1974) *American Crystallographic Association Abstracts, March 24–28* (University of California, Berkeley, J1).
14. Sundaralingam, M. (1969) *Biopolymers* **7,** 821–860.
15. Tsai, C. C., Jain, S. C. & Sobell, H. M. (1975) *Phil. Trans. R. Soc. London Ser. B.* **272,** 137–146.
16. Tsai, C. C., Jain, S. C. & Sobell, H. M. (1976) *J. Mol. Biol.,* in press.
17. Jain, S. C., Tsai, C. C. & Sobell, H. M. (1976) *J. Mol. Biol.,* in press.
18. Drake, J. W. & Maltz, R. H. (1976) *Annu. Rev. Biochem.* **45,** 11–37.
19. Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzaghi, E. & Inouye, M. (1966) *Cold Spring Harbor Symp. Quant. Biol.* **31,** 77–84.
20. Lindstrom, D. M. & Drake, J. W. (1970) *Proc. Natl. Acad. Sci. USA* **65,** 617–624.
21. Newton, A., Masys, D., Leonardi, E. & Wygal, D. (1972) *Nature New Biol.* **236,** 19–23.
22. Okada, Y., Streisinger, G., Owen, J., Newton, J., Tsugita, A. & Inouye, M. (1972) *Nature* **236,** 338–341.
23. Sobell, H. M., Tsai, C. C., Gilbert, S. G., Jain, S. C. & Sakore, T. D. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3068–3072.
24. Seeman, N. C., Day, R. O. & Rich, A. (1975) *Nature* **253,** 324–326.
25. Hoogsteen, K. (1959) *Acta Crystallogr.* **12,** 822–823.
26. Krugh, T. R. & Reinhardt, C. G. (1975) *J. Mol. Biol.* **97,** 133–162.