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Small-Angle Neutron Scattering Study of the Ternary Complex Formed between Bacterial Elongation Factor Tu, Guanosine 5′-Triphosphate, and Valyl-tRNA^{Val}

Bruno Antonsson,‡ Reuben Leberman,* and Bernard Jacrot
European Molecular Biology Laboratory, 156X, 38042 Grenoble Cedex, France
Giuseppe Zaccai
Institut Laue-Langevin, 156X, 38042 Grenoble Cedex, France
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ABSTRACT: The formation of the ternary complex between bacterial elongation factor Tu, GTP, and valyl-tRNA^{Val} has been studied by small-angle neutron scattering. Titrations of the protein with aminoacyl-tRNA solutions in both H_2O and 70% D_2O confirm the expected stoichiometry. The molecular weight obtained for the protein alone is significantly higher than expected and can be explained by postulating a monomer–dimer equilibrium. The titration data are then internally consistent with a dissociation of the dimer on ternary complex formation. The radius of gyration for the ternary complex and the calculation of the separation of the centers of mass of the protein and tRNA components suggest a compact model for the ternary complex.

In bacteria, one of the properties of the soluble polypeptide elongation factor Tu (EF-Tu) is to interact with elongator tRNA to produce the ternary complex EF-Tu·GTP·aminoacyl-tRNA. This complex is then used to promote the enzymatic binding of elongator-tRNA to the ribosome–mRNA complex. A number of chemical and biochemical studies have been described aimed at the determination of the mode of interaction of the two macromolecular components of the complex (Boutorin et al., 1981; Wikman et al., 1982; Antonsson & Leberman, 1984). These would indicate that the protein and nucleic acid moieties have fairly extensive areas of contact, consistent with the high binding constants found

* Author to whom correspondence should be addressed.
‡ Present address: Childrens Hospital of Los Angeles, Los Angeles, CA 90027.

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for these complexes (Pingoud et al., 1977; Louie et al., 1984; Abrahamson et al., 1985). In contrast to these results, the model proposed on the basis of low-angle X-ray scattering by Österberg et al. (1981) is a rather open structure with only the aminoacyl end of the tRNA interacting with the EF-Tu and the two macromolecules being inclined at about 45° to each other.

Small-angle neutron scattering (SANS) presents a complementary physical method for studying nucleic acid–protein complexes which has proved to be successful in studies of the interaction between tRNA and aminoacyl-tRNA synthetases (Dessen et al., 1978, 1982; Zaccai et al., 1979). By measurement of the scattering curves as the complex is formed and by use of contrast variation to separate protein from nucleic acid contributions, information is obtained on the radii of gyration and on the separation of the centers of mass of the macromolecular components and thereby suggests models of the complex structure. The study presented here leads to a model of the ternary complex different to that described by Österberg et al. (1981) but which is in accordance with chemical and biochemical results.

Materials and Methods

GTP, ATP, phosphoenolpyruvate (PEP), and pyruvate kinase (PK) were obtained from Boehringer, [^{14}C]valine and [^{3}H]GDP were from Amersham, Sepharose 4B and DEAE-Sepharose CL-6B were from Pharmacia, *N*-(2-hydroxyethyl)piperazine-*N*′-3-propanesulfonic acid (HEPPS) was from Sigma, and D_2O (99.96% D) was from Aldrich. *N*,*N*′-bis-(3-aminopropyl)piperazine-substituted Sepharose (BAPP-Sepharose) was prepared as previously described (Leberman et al., 1974). All other chemicals were of analytical quality.

The buffers used had the following compositions: buffer A, 100 mM sodium acetate and 1 mM magnesium acetate, pH 4.6; buffer B, 94 mM HEPPS, 20 mM NaOH, 10 mM $MgCl_2$, 80 mM KCl, and 0.5 mM DTE,[1] pH 7.3; buffer C, 64 mM Tris,[1] 50 mM HCl, 5 mM $MgCl_2$, 1 mM NaN_3, 0.5 mM DTE, and 0.01 mM PMSF,[1] pH 7.6. All pH values were measured at room temperature. The labeled valine mix used for charging the tRNA contained 0.5 μmol/mL [^{14}C]valine (10 mCi/mmol) and 2 mM ATP in buffer C.

EF-Tu·GDP was isolated from *Escherichia coli* (MRE 600) as previously described (Leberman et al., 1980). The sample used in this study exhibited a single band on SDS–PAGE[1] and had a GDP binding activity of 22 300 pmol/mg. To convert EF-Tu·GDP to EF-Tu·GTP, 0.46 mM protein was incubated with 14 mM GTP, 14 mM PEP, and 0.44 mg of PK/mL of buffer B for 15 min at 37 °C, followed by 30 min at room temperature and then overnight at 4 °C.

$tRNA^{Val}$ was isolated from bulk tRNA by successive chromatographic fractionation on BAPP-Sepharose, DEAE-Sepharose CL-6B, and Sepharose 4B in ammonium sulfate solution (Holmes et al., 1975). By the usual charging assay, the sample used in this study had a valine acceptance value of 1600 pmol/A_{260} and was stored in 1/20 buffer A at −80 °C.

The purified $tRNA^{Val}$ was charged preparatively by incubating 1000 A_{260} units in 25 mL of labeled valine mix containing 4 mg of unfractionated aminoacyl-tRNA synthetases from *E. coli* at room temperature for 45 min. Two volumes of ethanol was added, and after centrifugation, the precipitate was dissolved in buffer A containing 0.3 M KCl. The aminoacylated tRNA was adsorbed to a small (10-mL) column of DEAE-Sepharose CL-6B equilibrated in buffer A containing 0.3 M KCl. The column was washed with the equilibration buffer to remove protein, and the tRNA was recovered by application of a step of 1 M KCl in buffer A. After precipitation with 2 volumes of ethanol, the valyl-$tRNA^{Val}$ was dissolved in 1/20 buffer A at a concentration of 660 A_{260} units/mL (1.04 mM) and stored at −80 °C.

Protein concentration was measured by the method of Ehresmann et al. (1973) and verified by measurements at 280 nm with the value of 0.8 for the absorption of 1 mg/mL EF-Tu·GDP (Arai et al., 1973) and by amino acid analysis. The agreement between these measurements was within 5%.

The samples used for measurements were prepared from the stock solutions described above. Blank values, for correction of background and attenuation of solvent, were obtained from solutions containing the appropriate concentrations of GTP, PEP, PK, buffer, and D_2O. For the measurements in water, the starting concentration of EF-Tu·GTP was 7.3 mg/mL in buffer B, and a 150-μL sample was titrated with 0, 0.5, 0.75, 1.0, 1.5, and 2.0 molar equiv of valyl-$tRNA^{Val}$ in 1/20 buffer A. For the measurements in 70% D_2O, the starting concentration of protein was 5.6 mg/mL and a 300-μL sample was titrated with 0, 0.5, 0.75, 1.0, and 2.0 molar equiv of valyl-$tRNA^{Val}$ in 1/20 buffer A containing 70% D_2O. One sample was prepared in 40% D_2O containing 5.7 mg/mL EF-Tu·GTP and 1.0 equiv of valyl-$tRNA^{Val}$. One sample of EF-Tu·GDP at a concentration of 6.9 mg/mL was also measured.

The neutron scattering measurements were made on the D11 small-angle camera at the Institut Laue Langevin (Ibel, 1976). Samples were contained in quartz cells of 1.00- and 2.00-mm optical path length for H_2O and D_2O solutions, respectively (a shorter path length must be used for H_2O because of the higher attenuation by incoherent scattering). The measurement times were between 15 and 60 min during which the sample temperature was maintained at 6 °C. A neutron wavelength (λ) of 6.5 Å was used with an angular range corresponding to $0.026 < q < 0.071$ Å^{-1} [$q = 4\pi(\sin\theta)/\lambda$, where θ is half the scattering angle]. Each experiment was done at least twice, with different preparations; some, like the scattering from the enzyme in water, were done 5 times. Scattering intensities $I(q)$ were analyzed in the Guinier approximation (Guinier & Fournet, 1955):

$$\ln I(q) = \ln I(0) - \tfrac{1}{3}R_g^2q^2$$

When there is only one type of particle in solution, $I(0)$ is related to its molecular weight, scattering power, and concentration, and R_g is the radius of gyration of the scattering density contrast [reviewed in Zaccai & Jacrot (1983)]. The R_g value obeys the same laws as its mechanical counterpart, in particular the parallel axes theorem which permits the calculation of the R_g of an assembly from its component values (Zaccai & Jacrot, 1983). For a solution of different types of particles of comparable size, $I(0)$ and R_g are given by

$$I(0) = \sum_i n_iI_i(0) \quad (1)$$

$$R_G^2 = \sum_i n_iR_{gi}^2I_i(0)/\sum_i n_iI_i(0) \quad (2)$$

where n_i, $I_i(0)$, and R_{gi} are particle number concentration, forward scattering intensity, and the radius of gyration of the particle i, respectively. The methods of contrast variation take advantage of the fact that the contrast of the particles can be changed by changing the composition of the solvent. Thus,

[1] Abbreviations: DTE, dithioerythritol; Tris, tris(hydroxymethyl)-aminomethane; PMSF, phenylmethanesulfonyl fluoride; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

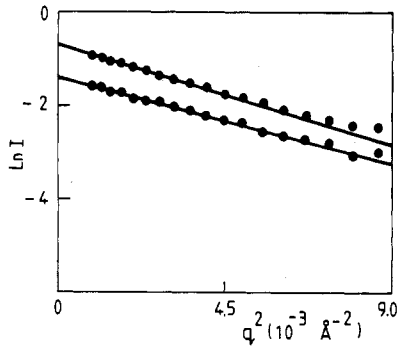

FIGURE 1: Guinier plots of (upper) EF-Tu·GTP at 7.3 mg/mL and (lower) EF-Tu·GDP at 6.9 mg/mL, both in H_2O buffer. The intensity scale is arbitrary. See text for definition of q.

the neutron scattering density of 70% D_2O is very close to that of tRNA (Li et al., 1983) so that the contrast of tRNA in buffers in 70% D_2O is negligible and only the protein is observed. In H_2O buffers, the contrasts of both protein and tRNA are high. The contrast of a given particle can be calculated from its chemical composition and the composition of the solvent. In H_2O, the calculated contrast is not very sensitive to the value of the partial specific volume (Jacrot & Zaccai, 1981).

Results and Discussion

The technical problems associated with this study are related to the intrinsic lability of GTP and aminoacyl-tRNA. To maintain the concentration of GTP at the desired level, the phosphoenolpyruvate and pyruvate kinase regenerating system was used. Preliminary experiments showed that, even at the low concentrations of enzyme, pyruvate kinase with its high molecular weight made a significant contribution to the neutron scattering. For this reason, the regenerating system was included in control solutions. Since we have used aminoacyl-tRNA radioactively labeled in the amino acid moiety, it was possible to monitor samples used for the scattering studies for the integrity of aminoacyl-tRNA. Within experimental errors, no differences were found between samples before and after titration. It soon became apparent that the conventional technique, used for studies on the interaction of tRNA and aminoacyl-tRNA synthetases [e.g., Dessen et al. (1982)], of extensive dialysis for equilibrating samples with different D_2O containing solvents was inappropriate in this instance, since considerable deacylation occurred during the dialysis time. An alternative procedure of careful addition of D_2O to the desired concentrations was adopted.

The Guinier [ln $I(q)$ vs. q^2] plots of both EF-Tu·GDP and EF-Tu·GTP, in H_2O solution, at protein concentrations of 6.9 and 7.3 mg/mL are shown in Figure 1. The values obtained for the radii of gyration for the two nucleotide complexes were essentially the same; 23.6 ± 0.3 and 24.0 ± 0.4 Å, respectively. However, the value of the molecular weight calculated from $I(0)$ and concentration values (Jacrot & Zaccai, 1981), taking account of bound nucleotide and magnesium ions (Antonsson et al., 1981), of 53 000 ± 3000 was significantly higher than the expected value of 43 600 (Jones et al., 1980).

Two titration series of EF-Tu·GTP with aminoacyl-tRNA were carried out: one in H_2O and the other in 70% D_2O. Under the former conditions, the scattering contributions of both macromolecules are observed, whereas under the latter the scattering due to the nucleic acid is matched out by the solvent. The concentration of EF-Tu varied from 7.3 to 3.7 mg/mL throughout the titration in H_2O and from 5.6 to 2.5 mg/mL in 70% D_2O. The individual Guinier plots obtained

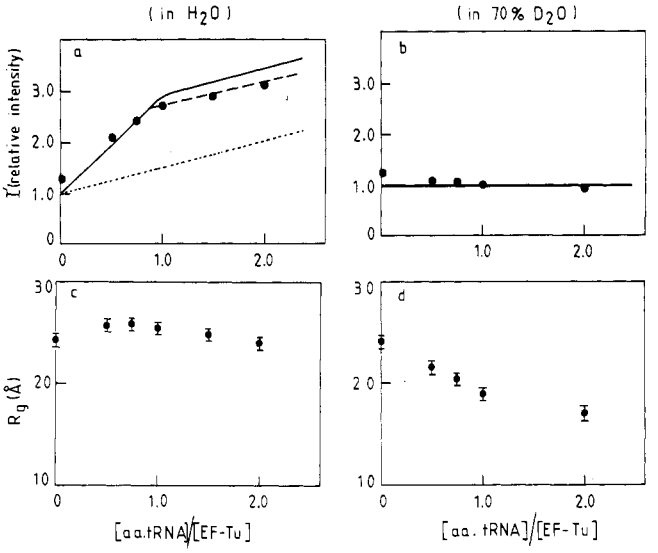

FIGURE 2: Parameters $I(0)$ and R_g as functions of aminoacyl-tRNA/EF-Tu·GTP molar ratio. The intensity scale I' is such that the value of 1.0 corresponds to the EF-Tu·GTP monomer at 1 mg/mL; the error bars correspond to the size of the data points. (a) $I(0)$ variation in H_2O. Dotted line was that calculated for no complex formation between aminoacyl-tRNA and EF-Tu·GTP. The full line was that calculated for 1:1 complex formation between monomer EF-Tu·GTP and aminoacyl-tRNA. (b) $I(0)$ variation in 70% D_2O where the scattering contribution of the aminoacyl-tRNA is negligible. (c) R_g variation in H_2O buffer. (d) R_g variation in 70% D_2O buffer.

for each titration value were of the same quality as those obtained with the protein alone (Figure 1). The titration curves obtained in H_2O and 70% D_2O are shown in Figure 2. The data in H_2O (panel a) show that as aminoacyl-tRNA is added to the EF-Tu·GTP solution the relative scattering intensity increases at a rate greater than that expected for the simple addition of aminoacyl-tRNA without complex formation. A break in the titration curve was observed corresponding to about 1 equiv of tRNA, and thereafter, the slope was the same as that predicted for the simple addition of tRNA (full and broken lines). The titration curve in 70%D_2O (panel b) shows a somewhat different phenomenon in that the relative scattering decreases on the addition of aminoacyl-tRNA and then flattens out after the addition of approximately 1 equiv. Two independent parameters are obtained from the Guinier plot, and the titration data also yield the change in apparent radius of gyration as a function of added aminoacyl-tRNA. These results are also shown in Figure 2 (panels c and d). In H_2O (panel c), we first observe a small increase in the radius of gyration as the tRNA is added followed by a small decrease at 1 equiv, which continues on further addition. In 70% D_2O (panel d) there is a sharp decrease in the radius of gyration to 1 equiv of RNA and a smaller decrease to 2 equiv.

SANS titration data can be interpreted quantitatively, by use of eq 2, with a model for the association of the different particles in solution (Dessen et al., 1978). The two sets of titration data present results that are not consistent with the accepted properties of EF-Tu. The titration end point agrees well with that expected for a 1:1 stoichiometry with possibly about 15% of the protein unable to complex with aminoacyl-tRNA. Thus, the broken line (Figure 2, panel a) through the experimental points lies below the line calculated for the entire protein sample being able to form a ternary complex. In 70% D_2O, where the contrast of tRNA is negligible, the relative intensity decreased with addition of aminoacyl-tRNA to 1 equivalent when it was expected to remain constant, and the sharp drop in the R_g over the same range was unexpected. Furthermore, the R_g of the protein alone, although in good

agreement with the value of 25 ± 1 Å found by Osterberg et al. (1981), is considerably higher than the value of 20 ± 1 Å that can be calculated from the low-resolution crystal structure (Kabsch et al., 1977). Since none of the samples show any evidence of aggregation and since in all crystal forms of EF-Tu the asymmetric unit is the dimer (Leberman et al., 1976), a likely explanation is that, at the concentrations of protein used in these studies, the EF-Tu is in monomer–dimer equilibrium. As the ternary complex is formed, this equilibrium is displaced, leading to dissociation of protein dimers. With this hypothesis, the titration data can be simulated quantitatively and the amount of dimer present calculated.

By use of the known molecular weight of EF-Tu, the value of $I(0)/c$ for monomer can be set to an arbitrary value of 1 (Figure 2). If c is the sum of c_1 (g/L monomer) and c_2 (g/L dimer), then the observed value of $I(0)/c$ will be given by Jacrot & Zaccai, 1981)

$$I(0)/c = c_1/c + 2c_2/c = c_1/c + 2(c - c_1)/c$$

Since the value observed for I(0)/c is 1.22 (Figure 2), c_1/c = 0.78, and 22% of the protein mass is present as dimers.

From the effects of contrast variation on exchangeable hydrogen atoms and on water of hydration, we can expect that the R_gs of the protein, tRNA, and complex in 70% D_2O to be lower than those in pure H_2O (Li et al., 1983). The titration data in Figure 2 show that, in 70% D_2O, we find an R_g of 19 Å in the presence of 1 equiv of aminoacyl-tRNA and 17 Å in the presence of excess aminoacyl-tRNA. What would be the effect of a hydration shell that has a higher density than bulk water (Li et al., 1983)? This shell, which has a density (relative to the solvent) of opposite sign to the protein, will reduce the apparent R_g of the complex. The correction to be applied can be estimated by placing a scattering mass at 28 Å (the separation of the centers of mass on the protein and the tRNA in the ternary complex given below) from the center of EF-Tu, equivalent to 85 water molecules, which is the excess density due to electrostriction found for tRNA in 0.1 M NaCl (Li et al., 1983). In 70% D_2O, the excess scattering length of 85 water molecules is 110×10^{-12} cm, and the excess scattering length of EF-Tu is -870×10^{-12} cm. Using the measured value for the R_g of EF-Tu in 70% D_2O in the ternary complex in 70% D_2O of 19 Å and applying the parallel axes theorem, we find a value of 20.8 Å for the corrected R_g. This agrees very well with the value of 21 Å estimated from the crystal structure.

The values for the amount of dimer and the R_g of EF-Tu·GTP monomer permit us to calculate a possible value for the R_g of the dimer. From eq 2, taking the limits of the observed R_g for EF-Tu·GTP and the limits calculated for the R_g of the monomer, we find that the R_g for the dimer is between 29.8 and 38.9 Å. These values are considerably higher than the value of 26 Å, which could be expected for a compact dimer of M_r 87 200. For an extended end-to-end dimer, the calculated value of R_g, assuming the maximum dimension of the monomer to be 75 Å (Kabsch et al., 1977), would be 43 Å.

The titration data in H_2O (Figure 2) show that between 1 and 2 equiv of added aminoacyl-tRNA the slope of the line is the same as the theoretical one obtained by assuming that the scattering power of aminoacyl-tRNA is the same as that of tRNA (Zaccai et al., 1979). This enables us to calculate an $I(0)$ value of 2.89 for the 1:1 complex whereas the observed value is 2.7, which means that 6.6% of the intensity is missing. We can then calculate the amount of EF-Tu unable to form the ternary complex; this is 13.6%, in agreement with the break in the titration curve. Using this value, it is now possible to use the observed R_g value (25.4 Å) of EF-Tu·GTP to deduce the R_g of the ternary complex. Since the binding constants are large, contributions due to free aminoacyl-tRNA and EF-Tu are small, and the R_g of the ternary complex is 25.7 Å.

As an internal check, we can now make an estimate of the R_g to be expected at an aminoacyl-tRNA:EF-Tu ratio of 2:1. Under these conditions, there is a mixture of EF-Tu·GTP (R_g = 21 Å), aminoacyl-tRNA (R_g = 23 Å), and ternary complex (R_g = 25.7 Å); taking a value of 13.6% for the inactive protein, we can calculate an R_g of 24.8 Å for the mixture. This values agrees well with 23.9 Å for the concentrated sample (c = 5.5 mg/mL) and 24.8 Å obtained on dilution (c = 3.7 mg/mL).

Assuming no large conformational changes in either the EF-Tu or the aminoacyl-tRNA on forming the ternary complex, consistent with the data obtained in D_2O, and then using the values of the radii of gyration for the protein, aminoacyl-tRNA, and ternary complex of 20, 23, and 25.7 Å, respectively, and applying the parallel axes theorem, we can calculate the distance between the centers of mass of the protein and tRNA moieties. The value we obtain is 28 ± 5 Å. Even with the rather large possible error, this value is small, much smaller than that of 36 Å reported by Osterberg et al. (1981).

Conclusions

The data presented above show the presence of a molecular weight species larger than the EF-Tu monomer in the initial protein solutions. This accounts for approximately the 20% higher intensity than could be expected for the monomer alone. The titration in 70% D_2O shows us the effect on the protein alone on addition of aminoacyl-tRNA. The decrease in intensity with increasing aminoacyl-tRNA concentration, which flattens out above an aminoacyl-tRNA:EF-Tu ratio of 1:1, suggests that the higher molecular weight component dissociates as ternary complex is formed. Since there is no evidence of extensive aggregation, a reasonable hypothesis was to assume the presence of EF-Tu dimer, which dissociates as monomer is removed by complex formation with aminoacyl-tRNA. From the intensity differences in both H_2O and 70% D_2O, we can calculate that the 20% higher intensity can be accounted for if there is 22% dimer in the initial EF-Tu·GDP or EF-Tu·GTP solutions.

The titration data in H_2O also indicated that not all the EF-Tu present could participate in ternary complex formation, and we estimate this fraction to be 13.6% of the total protein. This is in agreement with recent studies (Louie & Jurnak, 1985) on EF-Tu–aminoacyl-tRNA complexes.

Taking into account the presence of dimer in the initial protein solutions and a proportion of inactive EF-Tu molecules, we can calculate a set of structural parameters consistent with measured data of EF-Tu·GTP and the ternary complex. The observed value of R_g = 24.2 Å for the protein alone (monomer plus dimer) agrees well with the value of 25 Å found by Österberg et al. (1981) by low-angle X-ray scattering with solutions of about the same concentration. Equally, the value of R_g = 21 Å for the monomer, which we can calculate from our data, corresponds well with the value we can calculate from the crystal data. On the other hand, the R_g of 25.4 Å for the ternary complex is at variance with the value of 36 Å found by X-ray scattering (Österberg et al., 1981); the latter implies a rather extended complex, whereas our result indicates a compact model with the centers of mass of the EF-Tu and aminoacyl-tRNA being only 25 Å apart. We can find no simple explanation for the differences between our results and those of Österberg et al. (1981) and only note a difference in

the solvent compositions, where the X-ray scattering measurements were performed with solutions containing 100 mM NH_4Cl.

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500-MHz ^1H NMR Study of Poly(dG)·Poly(dC) in Solution Using One-Dimensional Nuclear Overhauser Effect†

Mukti H. Sarma, Goutam Gupta, and Ramaswamy H. Sarma*

Institute of Biomolecular Stereodynamics, State University of New York at Albany, Albany, New York 12222

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ABSTRACT: Secondary structures of poly(dG)·poly(dC) and poly(dG)·poly(dm^5C) in solution are determined by nuclear Overhauser effect (NOE) measurements on GH8-deuterated and -nondeuterated DNAs with low presaturation pulse lengths (10–25 ms) and low-power and prolonged accumulations in the range of 50 000–72 000 scans. Under these conditions, the NOE difference spectra were free from diffusion. Primary NOEs between base protons GH8/CH6 and sugar protons H1′, H2′/H2″, and H3′ suggest that in poly(dG)·poly(dC) both guanine and cytosine nucleotides adopt a C3′-endo, low anti χ = 200–220° conformation. Computer modeling of the NOE data enable identification for the first time, in terms of the geometry of the nucleotide repeat, handedness, and helix geometry, of the structure of poly(dG)·poly(dC) to be the A form, and the derived structure for the polymer duplex is very close to the single crystal structure of the double-helical d-GGGGCCCC [McCall, M., Brown, T., & Kennard, O. (1985) *J. Mol. Biol. 183*, 385–396]. Similar nuclear Overhauser effect data on poly(dG)·poly(dm^5C) revealed that G and m^5C adopt a C2′-endo, anti χ = 240–260° conformation, which indicates that this DNA exhibits the B form in solution. In summary, the results presented in this paper demonstrate that methylation of cytosines in poly(dG)·poly(dC) causes A → B transition in the molecule.

Single-crystal and fiber diffraction studies have clearly demonstrated that, depending upon base sequence and environmental conditions, DNA can assume the right-handed A and B forms or the left-handed Z form (Arnott et al., 1975, 1983; Connor et al., 1982; Drew et al., 1980; Fratini et al., 1982; Kennard, 1985; McCall et al., 1985; Shakked et al., 1983; Viswamitra et al., 1982; Wang et al., 1979, 1982; Wing et al., 1980). NMR studies on DNA polymer duplexes in solution have revealed the presence of B and Z forms (Dhingra et al., 1983; Mitra et al., 1981a,b; Patel et al., 1982). However, the presence of the A form for a DNA polymer duplex in ordinary solution conditions of salt and water has not been unequivocally established—this is not surprising because high humidity is expected to favor the classical B form, and hence, in ordinary solution conditions of salt and water one expects the B form.

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