Noncovalent Intercalative Complex Formation and Kinetic Flow Linear Dichroism of Racemic *syn*- and *anti*-Benzo[*a*]pyrenediol Epoxide–DNA Solutions†

Manouchehr Shahbaz and Nicholas E. Geacintov

Chemistry Department and Radiation and Solid State Laboratory, New York University, New York, New York 10003

Ronald G. Harvey*

Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Illinois 60637

Received October 16, 1985; Revised Manuscript Received January 17, 1986

ABSTRACT: In order to gain a better understanding of the molecular basis underlying the differences in biological activities of the diastereomeric syn and anti diol epoxides of benzo[*a*]pyrene (BPDE) (*trans*-7,8-dihydroxy-*syn*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene and *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, respectively), their interactions with DNA in aqueous solutions were studied and compared. Kinetic flow linear dichroism experiments indicate that both diastereomers (racemic mixtures) form intercalation complexes immediately after mixing; the association constant (23 °C, ionic strength ~0.005) is signficantly smaller (5200 M^{-1}) in the case of the syn than in the case of the anti diastereomer (12 200 M^{-1}). This difference is attributed to the greater bulkiness of the 7,8,9,10 ring of the syn stereoisomer, which is in the quasi-diaxial conformation as compared to the less bulky quasi-diequatorial conformation of the anti diastereomer. In contrast, the intercalative association constants of the tetraols derived from the hydrolysis of the two diol epoxides are similar in value. Upon formation of noncovalent *syn*-BPDE–DNA intercalation complexes, the reaction rate constant for the formation of tetraols (~98%) and covalent adducts (~2%) increases from 0.009 to 0.05 s^{-1} at pH 9.5 in 5 mM tris(hydroxymethyl)aminomethane buffer. The conformations of the aromatic chromophores of BPDE were followed by the kinetic flow dichroism technique as a function of reaction time; while the anti diastereomer changes conformation from an intercalative to an apparently external binding site, the syn diol epoxide molecules do not appear to undergo any measurable reorientation during or after the covalent binding reaction. These significant differences in the characteristics of the interactions of these two BPDE diasteromers with DNA, and the differences in the conformations of the adducts formed, are discussed in relation to the known differences in the mutagenic and tumorigenic activities of these compounds.

The mutagenic and carcinogenic activity of polycyclic aromatic hydrocarbons involves a series of complex factors, the first of which is the metabolic activation of the parent aromatic hydrocarbons to highly reactive electrophilic epoxide derivatives (Harvey, 1981; Conney, 1982; Dipple et al., 1984). Benzo[*a*]pyrene (BP) is one of the most extensively studied compounds of this important class of chemical carcinogens, and the binding of the metabolite benzo[*a*]pyrene-7,8-diol 9,10-oxide (BPDE) to cellular DNA is believed to be a critical event in the initiation of tumorigenesis and in mutagenesis (Brookes & Osborne, 1982; Yang et al., 1982; Ashurst et al., 1983; Stevens et al., 1985; Burgess et al., 1985). There are two diastereomeric forms of this molecule, *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*anti*-BPDE)[1] and *trans*-7,8-dihydroxy-*syn*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*syn*-BPDE); in the former, the 7-hydroxyl group and the epoxide ring are on opposite faces of the molecule, while in the syn diastereomer they are located on the same face of the molecule (Figure 1). Each of these diastereomers can be further resolved into a pair of enantiomers denoted by (+) and (−). In human, bovine, and rodent cells, (+)-*anti*-BPDE is found to be the principal metabolite of benzo[*a*]pyrene bound to DNA (Weinstein et al., 1976; Jeffrey et al., 1977).

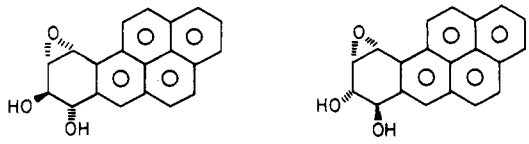
These stereoisomers display remarkable differences in biological activities. The (+)-anti enantiomer is by far the most carcinogenic stereoisomer in tumor initiation experiments in mice (Buening et al., 1978; Slaga et al., 1979) and as a mutagen in mammalian cells (Wood et al., 1977; Newbold et al., 1979; Brookes & Osborne, 1982). Tumor initiation experiments in mouse skin utilizing the more easily obtainable racemic mixtures, rather than the optically resolved enantiomers of the anti and syn isomers of BPDE, have demonstrated a high activity in the case of (±)-*anti*-BPDE, while (±)-*syn*-BPDE was essentially inactive as a skin tumor initiator (Pelling & Slaga, 1982). A similar pattern is observed in the inhibition of *φX174* and SV40 viral DNA replication, (±)-*anti*-BPDE being more effective than the syn diastereomer (Harvey et al., 1980). In some bacterial test systems, however, the syn stereoisomers are more mutagenic than the anti isomers (Wood et al., 1976, 1977; Fahl et al., 1981; Burgess et al., 1985; Stevens et al., 1985).

It is known that the anti and syn diastereomers are characterized by subtle differences in conformations and pronounced differences in chemical reactivities. In aqueous so-

† This work was supported by the Department of Energy (Contracts DE-AC02-76EV02386 and DE-AC02-78EV04959) and, in part, by the U.S. Public Health Service, Grant CA20851 awarded by the National Cancer Institute, Department of Health and Human Services, and by the American Cancer Society, Grant BC-1322.

[1] Abbreviations: *syn*-BPDE, *trans*-7,8-dihydroxy-*syn*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; *anti*-BPDE, *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; BPT, 7,8,9,10-tetrahydroxytetrahydrobenzo[*a*]pyrene.

0006-2960/86/0425-3290$01.50/0



(+) syn-BPDE (+) anti-BPDE

FIGURE 1: Structures of benzo[*a*]pyrene-7,8-diol 9,10-oxide stereoisomers. The (+) enantiomers of each diastereomer are shown.

lutions, these molecules are unstable and undergo specific and general acid catalyzed, as well as spontaneous, reactions to form mostly the tetraol 7,8,9,10-tetrahydroxytetrahydrobenzo[*a*]pyrene (BPT) (Keller et al., 1976; Yang et al., 1977; Yagi et al., 1977; Whalen et al., 1978, 1979). The reactivity of the syn diastereomer is about 30 times higher than that of the anti diastereomer at neutral pH or higher, and the reactivity is almost entirely attributed to the spontaneous hydrolysis mechanism at pH >7.0 (Whalen et al., 1977). These differences in reactivities are attributed to differences in the conformations of these stereoisomers. For each stereoisomer, there are two conformers that are in equilibrium with one another in aqueous solutions in which the hydroxyl groups are both either diaxial (ax,ax') or diequatorial (eq,eq') (Beland & Harvey, 1976; Yagi et al., 1977). Theoretical calculations (Klopman et al., 1979; Kikuchi et al., 1979) and experimental NMR data (Yagi et al., 1975; Beland & Harvey, 1976; Sayer et al., 1982, 1984) indicate that in aqueous solutions the (±)-*anti*-BPDE isomers exist predominantly in the diequatorial conformation, while the syn isomers have a preference for the diaxial conformation.

The higher chemical reactivity of the *syn*-BPDE diastereomer (spontaneous hydrolysis) has been explained in terms of two rapidly interconvertible conformations in which the benzylic C–O bond is either "aligned" or "not aligned" with the orbitals of the aromatic ring system. When both diastereomers are in their preferred conformations (pseudodiaxial and pseudodiequatorial for *syn*- and *anti*-BPDE, respectively), the C–O bond is not aligned. However, the syn diastereomer can more easily assume the more reactive diequatorial conformation, which corresponds to the aligned orientation; in the case of *anti*-BPDE on the other hand, the aligned conformation corresponds to the quasi-diaxial orientation, which is not favored in the case of this diastereomer (Sayer et al., 1982, 1984).

The conformation of the cyclohexene ring appears to be an important factor not only in the chemical reactivity of BPDE but also in the biological activities of this and other PAH diol epoxides; generally, diol epoxides with a preferred pseudodiequatorial conformation are highly tumorigenic, while those with a diaxial orientation are inactive or less tumorigenic (Jerina et al., 1982). The lower tumorigenicity of PAH diol epoxides with a quasi-diaxial conformation has been associated with the greater bulkiness, as compared to the diequatorial conformation, of the cyclohexene ring system, which inhibits interaction of these diol epoxides with DNA (Jerina et al., 1982). Klopman et al. (1979), on the basis of theoretical considerations, concluded that the formation of intercalation complexes of BPDE and DNA, and thus the chemical reactivity, should be inhibited in the case of *syn*-BPDE relative to that of *anti*-BPDE; similar conclusions have more recently been reached by Miller et al. (1985).

It is known that BPDE (Geacintov et al., 1981, 1984a; Meehan et al., 1982; MacLeod & Selkirk, 1982; Lin et al., 1980) and other epoxide derivatives (Kim et al., 1984) form noncovalent complexes with DNA before binding covalently to one of the DNA bases (predominantly N2 of guanine; Weinstein et al., 1976; Koreeda et al., 1978). The major reaction pathway, however, is DNA-catalyzed hydrolysis to tetraols (Geacintov et al., 1980, 1982a; MacLeod & Zachary, 1985; Meehan & Bond, 1984). The goals of this work are to obtain insight into the differences in the interactions of (±)-*syn*- and (±)-*anti*-BPDE with double-stranded DNA, which might ultimately lead to a better understanding of the differences in the biological activities of these stereoisomers on a molecular level. We have investigated differences in (1) the intercalative noncovalent association constants and covalent binding to native calf thymus DNA, (2) the differences in the DNA-catalyzed reaction rates, and (3) the conformations of the complexes formed when (±)-*anti*-BPDE and (±)-*syn*-BPDE bind noncovalently and covalently to double-stranded DNA.

Experimental Procedures

Racemic *syn*- and *anti*-BPDE were prepared by methods previously published (Harvey & Fu, 1978). Native calf thymus DNA (Worthington Biochemicals, Freehold, NJ) was prepared as previously described (Geacintov et al., 1982a); the hyperchromicities of our preparations were in the range of 37–40%. The reactions were carried out in solutions containing various amounts of DNA in 5 mM Tris buffer [tris(hydroxymethyl)aminomethane]–and 5 mM NaCl (to maintain a constant ionic strength), pH 9.5, at 23 ± 1 °C, to which were added small aliquots of the BPDE–tetrahydrofuran stock solutions; the final concentrations of tetrahydrofuran did not exceed 0.2% by volume.

The noncovalent association of BPDE with DNA was studied by monitoring the absorbance of the diol epoxide molecules bound to DNA at 353 nm (Geacintov et al., 1981); this absorbance signal decreases as a function of time because the diol epoxide molecules are gradually converted to tetraols, which form noncovalent complexes with a lower association constant. Two methods were utilized in conducting these binding experiments:

(1) The full-absorbance spectra in the 300–380-nm range were scanned at a scan speed of 120 nm s^{-1}. The first scan was initiated 5 s after the addition of BPDE to the DNA solutions; subsequent scans were made continuously in order to determine the kinetics of the reaction and to extrapolate the absorbance signal at 353 nm to its zero-time (time of addition of BPDE) value.

(2) The spectrophotometer wavelength was fixed at 353 nm, the absorbance at that wavelength was recorded as a function of time after mixing, and the absorbance was extrapolated to zero-time.

These experiments were repeated at different DNA concentrations but at constant BPDE concentration (4.1×10^{-6} M) in order to obtain the binding curves. The kinetics of the reaction were also monitored by a fluorescence method as previously described (Geacintov et al., 1982a).

The levels of covalent binding were determined spectroscopically (Geacintov et al., 1984b). The covalent adducts derived from (±)-*syn*-BPDE were found to be unstable; therefore, the determinations of the levels of covalent binding were made within 30 min of the preparation of the adducts.

The kinetic linear dichroism measurements were made by utilizing a Couette flow dichroism cell as described previously (Geacintov et al., 1984a).

Results and Discussion

Noncovalent Equilibrium Association Constants. Absorption spectra of (±)-*syn*-BPDE in aqueous buffer solutions

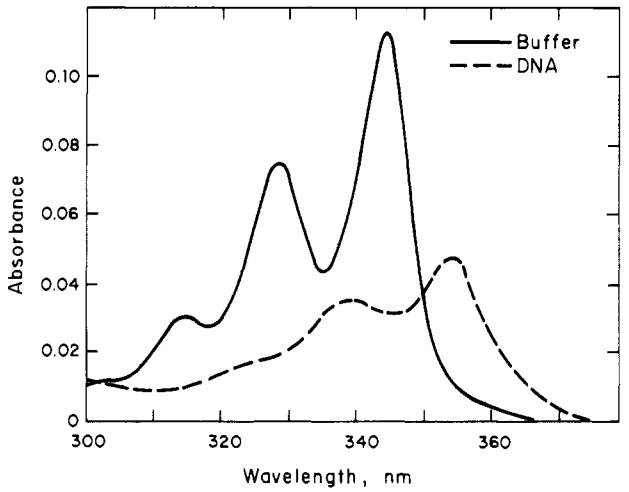


FIGURE 2: Absorption spectrum of 4.7×10^{-6} M (±)-*syn*-BPDE in 5 mM Tris buffer and 5 mM NaCl, pH 9.5 (—), and in the same buffer solution containing 1.5×10^{-3} M DNA (– –). Spectrum measured 5 s after mixing.

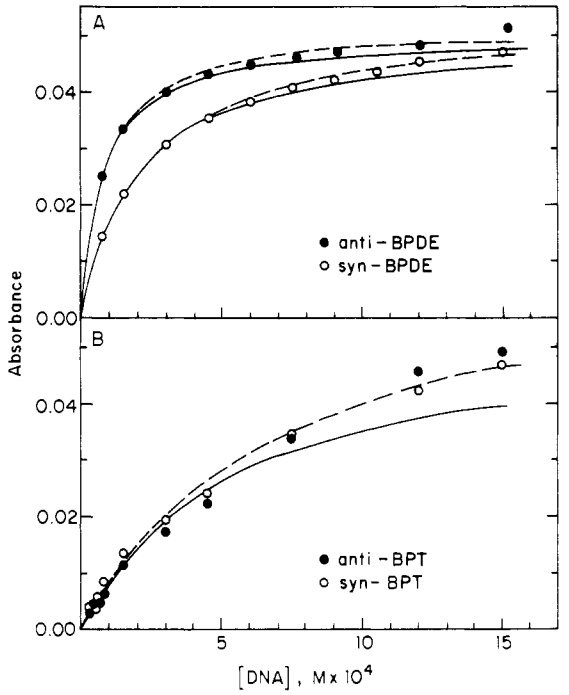


FIGURE 3: Absorbance of physical complexes measured at 353 nm as a function of DNA concentration at a constant concentration (4.7×10^{-6} M) of (A) (±)-*anti*-BaPDE (●) and (±)-*syn*-BaPDE (○) and (B) tetraols *anti*-BPT (●) and *syn*-BPT (○). (A) (– –) Best fits of eq 1 to the data with $K = 11\,700$ M^{-1} (anti) and 4600 M^{-1} (syn); (—) fits to the data (eq 1) utilizing the values of $K = 12\,200$ M^{-1} (anti) and 5200 M^{-1} (syn) obtained from the double-reciprocal plot (Benesi–Hildebrand) of Figure 4. (B) (– –) Best fit to the data according to eq 1 with $K = 1300$ M^{-1}; (—) fit of eq 1 utilizing the value of $K = 2000$ M^{-1} obtained from the double-reciprocal plot of Figure 5.

both in the absence and in the presence of an excess of DNA are shown in Figure 2. In buffer solution, the absorption maximum occurs at 344 nm but shifts to 353–354 nm in concentrated DNA solution; in the latter case there is a general broadening and a red shift in the spectrum, as well as a pronounced hypochromic effect. Similar behavior is observed in the case of (±)-*anti*-BPDE (Geacintov et al., 1981, 1983; Meehan et al., 1982; MacLeod & Selkirk, 1982).

At a constant BPDE concentration, the amplitude of the 353-nm band depends on the DNA concentration. Such behavior is compared for (±)-*anti*- and for (±)-*syn*-BPDE in Figure 3A and is significantly different for the two diastereomers. However, in the case of the two tetraols (derived from the hydrolysis of the syn and anti diol epoxides, respectively), there is no difference between *anti*-BPT and *syn*-BPT (Figure 3B), even though their stereochemistries are different (Yang et al., 1976; Yagi et al., 1977).

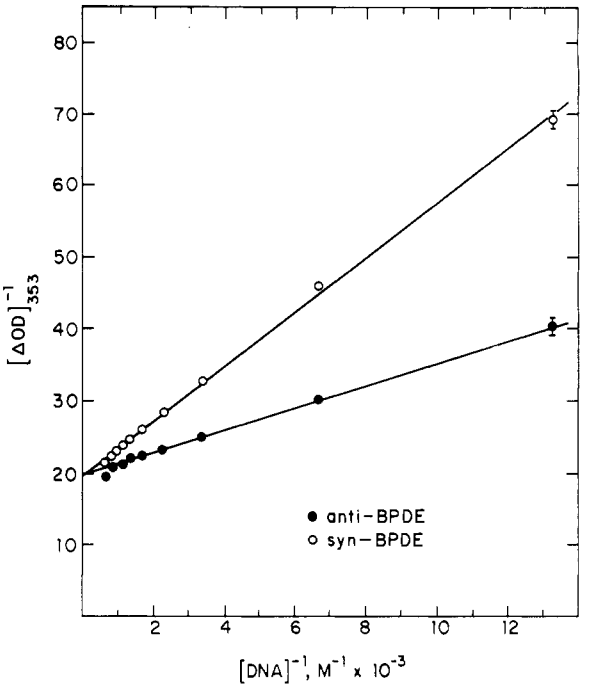


FIGURE 4: Double-reciprocal plots of the data of Figure 3A; (—) best fits of eq 2 utilizing $K = 12\,200$ M^{-1} for (±)-*anti*-BPDE and $K = 5200$ M^{-1} for (±)-*syn*-BPDE.

A crude estimate and comparison between the noncovalent binding constants can be made by utilizing a simple one-site binding model (Geacintov et al., 1981). In this approximation, the fraction of molecules X_b bound noncovalently to DNA is (assuming that there is an excess of DNA binding sites)

$$X_b = K[\text{DNA}]/(1 + K[\text{DNA}]) \quad (1)$$

where [DNA] is the DNA concentration and K is the equilibrium association constant. If there are multiple, independent binding sites with association constants K_1, K_2, K_3, ..., then K is the apparent association constant where $K = K_1 + K_2 + K_3 + \ldots$ (Klotz, 1974). If the absorbance of free diol epoxide molecules is neglected, then the absorbance at about 353 nm can be assumed to be proportional to X_b. The best computer fits of eq 1 to the experimental data are shown by the dashed lines in Figure 3. The values of K are 4600 and 11 700 M^{-1} for the syn and the anti diastereomers of BPDE, respectively. In the case of BPT, a single line gives the best fit for both types of tetraols ($K = 1300$ cm^{-1}).

Double-reciprocal plots (Benesi–Hildebrand) constitute still another method for estimating binding constants. On the basis of the approach outlined by Li and Crothers (1969) in which the absorbance of uncomplexed molecules is not neglected, the following equation can be derived for the change in absorbance or optical density at 353 nm (ΔOD_{353}, 1-cm optical path length):

$$\frac{1}{[\Delta OD]_{353}} = \frac{1}{\Delta\epsilon[C]} + \frac{1}{\Delta\epsilon[C]}\frac{1}{K}\frac{1}{[\text{DNA}]} \quad (2)$$

where [C] is the concentration of ligand, $[\Delta OD]_{353} = OD_{DNA} - OD_{buffer}$ measured at 353 nm at different DNA concentrations, and $\Delta\epsilon = \epsilon_b - \epsilon_f$, where ϵ_b and ϵ_f are the molar extinction coefficients of the bound and free ligands, respectively, measured at 353 nm.

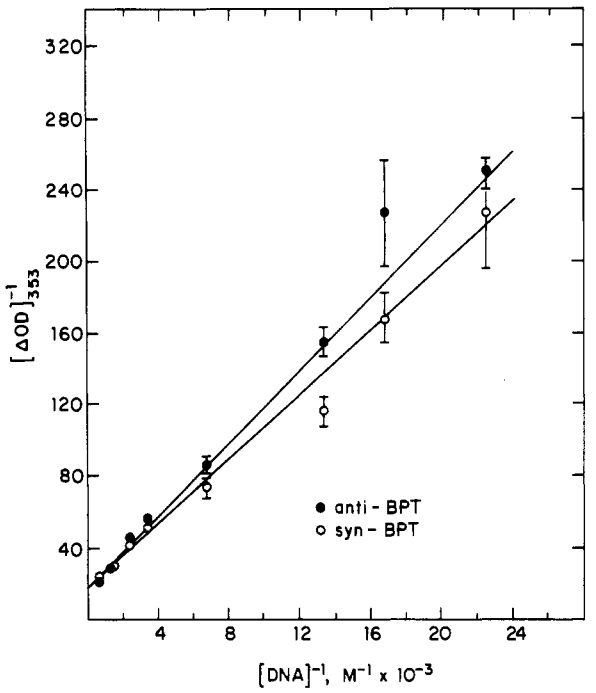


FIGURE 5: Double-reciprocal plots of the data of Figure 3B for the tetraols; (—) best fits of eq 2 to the data with $K = 1800\ M^{-1}$ for *anti*-BPT and $K = 2000\ M^{-1}$ for *syn*-BPT.

Typical double-reciprocal plots utilizing the data of Figure 3 are shown in Figures 4 and 5. The plots for the (±)-syn and (±)-anti diol epoxides are clearly different, and with utilization of eq 2, values of the apparent binding constants of $K = 5200$ and $12\,200\ M^{-1}$, respectively, are obtained. With these values, eq 1 was used to recalculate a best fit to the experimental data utilizing eq 1 (solid lines in Figure 3A). Considering the fact that the double-reciprocal plots favor the data at low DNA concentrations, while fits of eq 1 favor the data at high DNA concentrations, the agreement between the two methods is considered to be quite good. In the case of the tetraols, in contrast to the diol epoxides, analysis of the data in terms of either eq 1 (Figure 3B) or eq 2 (Figure 5) yields values of K in the range of $1700 \pm 400\ M^{-1}$.

Intercalative noncovalent complex formation between PAH diol epoxides and DNA is believed to be an important factor in determining the biological activities of these compounds (LeBreton, 1985). We find that the noncovalent association constant is about 2.5 times smaller in the case of (±)-*syn*-BPDE than in the case of the (±)-anti diastereomer. This result confirms the hypothesis of Klopman et al. (1979), who predicted that the greater apparent bulkiness of the quasi-diaxial (ax,ax′) conformation of *syn*-BPDE should result in a diminished ability, relative to *anti*-BPDE, to form noncovalent intercalation complexes. These results also lend support to the hypothesis of Jerina et al. (1982), who suggested that the apparent larger size of the 7,8,9,10 ring of *syn*-BPDE and other structurally related PAH diol epoxides may lead to a lower reactivity of this type of stereoisomer with DNA.

Kinetics of Reaction in the Presence of DNA. The pseudo-first-order reaction rate constant k for the disappearance of diol epoxides at different DNA concentrations in Tris buffer solutions at pH 9.5 is shown in Figure 6. More than 90% of the BPDE molecules are converted to tetraols in the case of (±)-*anti*-BPDE, and only a minor fraction undergoes covalent binding with the DNA bases (Geacintov et al., 1980; MacLeod et al., 1982).

In contrast to previous results in cacodylate buffer solutions at pH >9 in which there was no accelerating effect of DNA

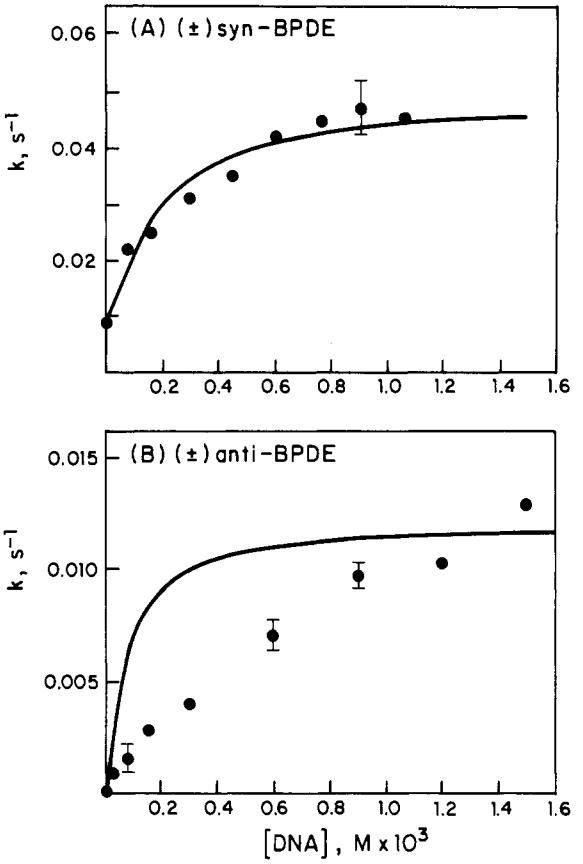


FIGURE 6: Pseudo-first-order reaction rate constant k (s^{-1}) as a function of DNA concentration. (A) (±)-*syn*-BPDE; (—) plots of eq 3 with $K = 5200\ M^{-1}$, $k_h = 0.091$, and $k_3 = 0.050\ s^{-1}$. (B) (±)-*anti*-BPDE; (—) DNA concentration dependence of k predicted according to eq 3 ($k \propto X_b$), with $K = 12\,200\ M^{-1}$.

on the reaction rate constant of (±)-*anti*-BPDE (Geacintov et al., 1984b), DNA catalysis of BPDE hydrolysis is observed in Tris buffer solution at pH 9.5 (Figure 6B). This effect can be attributed to the ability of Tris buffer to catalyze the hydrolysis of *anti*-BPDE noncovalently bound to DNA (Meehan & Bond, 1984). In 5 mM Tris buffer solutions at pH 9.5 and in the absence of DNA, the reaction rate constant for the formation of tetraols is about 15–20 times higher in the case of the syn diastereomer than in the case of *anti*-BPDE [these rate constants are $(9.0 \pm 0.5) \times 10^{-3}$ (syn) and $(5.5 \pm 0.5) \times 10^{-4}\ s^{-1}$ (anti)]. In spite of this already high reactivity of the syn diastereomer in the absence of DNA, a significant further acceleration of the reaction rate occurs upon noncovalent complex formation with DNA (Figure 6A).

We have shown previously (Geacintov et al., 1982a) that the dependence of k on the DNA concentration is well accounted for by the following equation:

$$k = k_h(1 - X_b) + k_3X_b \qquad (3)$$

where k_h is the solvolysis rate constant for free BPDE and k_3 is the rate constant of reaction of BPDE noncovalently complexed with DNA. A plot of eq 3 utilizing the previously determined value of $K = 5200\ M^{-1}$ (Figure 4) and $k_3 = 0.05\ s^{-1}$ provides an adequate fit of the dependence of k on the DNA concentration in the case of *syn*-BPDE (Figure 6A). Thus, k increases proportionately to the fraction of intercalated *syn*-BPDE molecules, and its dependence on the DNA concentration resembles the binding isotherm depicted in Figure 3A. In the case of *anti*-BPDE, however, where $K = 12\,200\ M^{-1}$, determined from the data in Figure 4, a much stronger dependence of k on the DNA concentration is predicted by

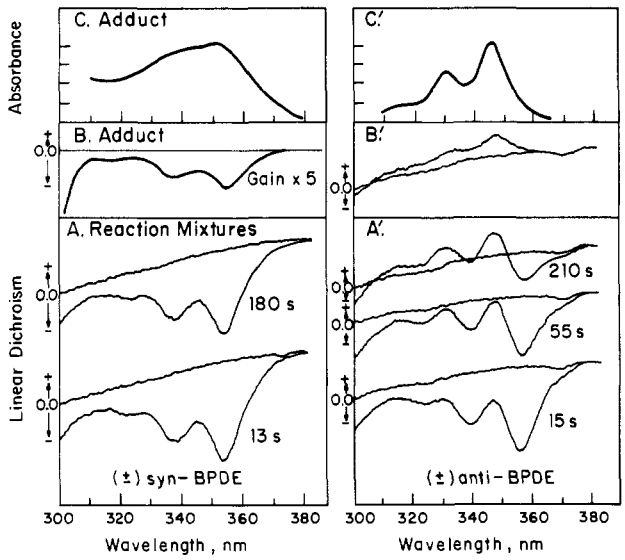


FIGURE 7: (A and A') Kinetic flow linear dichroism spectra of reaction mixtures containing 8.6×10^{-6} M BPDE, 7.6×10^{-5} M DNA, 5 mM Tris-buffer, and 5 mM NaCl, pH 9.5. Scans were initiated at the indicated times after adding BPDE; (B and B') Linear dichroism spectra of covalent adducts, obtained after extracting equilibrated reaction mixtures with ether to remove the tetraols. The absorption spectra of the covalent adducts are shown in panels C and C' for reference (the vertical scales are not comparable), but the percentage of diol epoxide molecules binding to DNA are ~2% (syn) and ~9–10% (anti). In the linear dichroism spectra the zero base lines are indicated; the signal gain was constant except in panel B, where the gain was increased by a factor of 5 and the strongly sloping base line was subtracted before reconstructing the linear dichroism spectrum.

eq 3 than is actually observed. This behavior is most likely due to a complex effect of Tris buffer molecules (Meehan & Bond, 1984), since there is no catalysis at this pH in another buffer solution (sodium cacodylate; Geacintov et al., 1984b). A more complete discussion of this effect is beyond the scope of this work and will be treated in more detail elsewhere (unpublished results).

Linear Dichroism Spectra of Noncovalent Complexes and Covalent Adducts. Kinetic flow linear dichroism spectra (Geacintov et al., 1984a) can provide important insight into the conformations of the noncovalent complexes that are formed immediately upon adding BPDE to DNA solutions and on changes in the orientations of the pyrenyl ring system as the physically bound BPDE molecules undergo covalent binding reactions with the DNA bases. Only those diol epoxide molecules that are bound to the DNA macromolecules contribute to the linear dichroism spectrum.

A typical set of experiments comparing the behavior of (±)-*syn*-BPDE to that of (±)-*anti*-BPDE are shown in Figure 7. In these experiments, a DNA concentration of only 7.6×10^{-5} M was utilized in order to minimize the extent of hydrolysis of diol epoxide molecules during the scanning of the spectrum. In the case of the syn diastereomer, the reaction rate constant k is equal to 0.0022 s^{-1} (Figure 6), and thus about 30–40% of the diol epoxide molecules were already hydrolyzed to tetraols within the 20 s required to scan the wavelength from 380 to 350 nm. The initial linear dichroism spectrum (labeled 13 s in Figure 7A), however, is mostly due to the complexed diol epoxides, since the binding constant is about 2.5 times lower for *syn*-BPT than for the diol epoxide. The contributions of tetraols bound to DNA to the absorbance and thus to the linear dichroism spectrum is 15% or less, 20 s after mixing. In the case of (±)-*anti*-BPDE, $k = 0.0017$ s^{-1}, the extent of hydrolysis of the diol epoxide molecules is negligible (~5%) during the time interval required to record the first linear dichroism spectrum (scan started 15 s after mixing, Figure 7A'). Therefore, the contribution of tetraols to the initial linear dichroism spectrum is even lower than in the case of (±)-*syn*-BPDE.

These initial (±)-*syn*- and (±)-*anti*-BPDE linear dichroism spectra are negative in sign and display characteristic minima at 337 and 354 nm and thus resemble the inverted and red-shifted absorption spectra of the pyrenyl chromophore (Ibanez et al., 1980). Taken together with the large (~10 nm) red shifts and the large hyperchromic effects that are characteristic of stacking interactions, the linear dichroism spectra support the conclusion that the noncovalent *syn*-BPDE-DNA complexes are of the intercalation type, as was previously demonstrated for (+)-*anti*-BPDE (Geacintov et al., 1984a).

The linear dichroism spectra of the diol epoxide–DNA reaction mixtures evolve as a function of time; these changes are compared in panels A and A' of Figures 7 for the two diastereomers within similar time intervals (~200 s after mixing). While there are no qualitative changes in the case of (±)-*syn*-BPDE, there is a small decrease in the overall amplitude of the signal. This decrease exhibits the same kinetics as the conversion of diol epoxide molecules to tetraols as measured by the fluorescence technique (data not shown). After ~180 s, only minor further additional changes in the magnitude of the A spectra were observed. The 180-s spectrum is mostly due to physically bound tetraol molecules. The contribution of the covalent adducts to the A spectrum of these equilibrated mixtures is minor; this is demonstrated in Figure 7B, which represents the dichroism spectrum of the covalent (±)-*syn*-BPDE–DNA adducts obtained after extraction of the noncovalently bound tetraols with ether. The absorption spectrum of the covalent adducts is shown in Figure 7C, and both are in good agreement with the results obtained by Undeman et al. (1983). Only ~2% of the (±)-*syn*-BPDE molecules initially added react by covalent binding to DNA [as compared to 9–10% in the case of the (±)-*anti*-diastereomer].

The evolution as a function of time of the linear dichroism spectra of racemic *anti*-BPDE is quite different (Figure 7'). There is a pronounced development of a positive linear dichroism signal with maxima at 329 and 346 nm due to covalently bound moieties (Figure 7B'). The characteristics of the covalent adducts derived from the covalent binding of (±)-*anti*-BPDE to DNA are discussed in more detail elsewhere (Geacintov et al., 1978, 1982b); the relatively small red shift in the absorption spectrum of the pyrenyl residue in the covalent adducts (Figure 7C') as compared to the spectrum in aqueous solution suggests a low degree of stacking interactions and possibly an external binding conformation of the adducts.

The major point of interest of the kinetic linear dichroism results shown in Figure 7 is that both the syn and anti diastereomers appear to form similar intercalative physical complexes with DNA. Upon covalent binding, there is a pronounced reorientation of the pyrene ring system in the case of (±)-*anti*-BPDE, while such reorientation does not seem to occur in the case of the syn diastereomer. Thus, the covalent adducts derived from the binding of the syn diastereomer appear to have a conformation similar to that of the noncovalent intercalation complexes. In the case of (±)-*anti*-BPDE [the observed behavior is mostly due to the (+) enantiomer; Geacintov et al., 1984a], the conformation of the covalent adducts is quite different from that of the noncovalent intercalation complex.

CONCLUSIONS

There is considerable evidence that differences in the nature of the adducts derived from the binding of the syn and anti

diastereomers of BPDE to DNA, rather than the level of covalent binding, may be of great importance in determining the biological activities of these compounds (Pelling & Slaga, 1982; Newbold et al., 1979; Brookes & Osborne, 1982; Stevens et al., 1985). The processing of the covalent adducts by the cells, e.g., recognition and repair by the appropriate enzymes and fidelity of replication, may depend on the conformations of these lesions. The conformations of the covalent adducts depend on the presence of the 7- and 8-hydroxyl groups (Geacintov et al., 1982b), as well as on the relative spatial configurations of these OH groups and the epoxide group.

Registry No. (±)-*syn*-BPDE, 58917-91-2; (±)-*anti*-BPDE, 58917-67-2; BPT, 59957-91-4.

References

Ashurst, S. W., Cohen, G. M., Nesnow, S., DiGiovanni, J., & Slaga, T. J. (1983) *Cancer Res. 43*, 1024–1029.

Beland, F. A., & Harvey, R. G. (1976) *J. Chem. Soc., Chem. Commun.*, 84–85.

Brookes, P., & Osborne, M. R. (1982) *Carcinogenesis 3*, 1223–1226.

Buening, M. K., Wislocki, P. G., Levin, W., Yagi, H., Thakker, D. R., Agaki, H., Koreeda, M., Jerina, D. M., & Conney, A. H. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5358–5361.

Burgess, J. A., Stevens, C. W., & Fahl, W. E. (1985) *Cancer Res. 45*, 4257–4262.

Conney, A. H. (1982) *Cancer Res. 42*, 4875–4917.

Dipple, A., Moschel, R. C., & Bigger, C. A. H. (1984) in *Chemical Carcinogens*, 2nd ed., ACS Monograph 182, Vol. 2, pp 41–163, American Chemical Society, Washington, DC.

Fahl, W. E., Scarpelli, D. G., & Gill, K. (1981) *Cancer Res. 41*, 3400–3406.

Geacintov, N. E., Gagliano, A. G., Ivanovic, V., & Weinstein, I. B. (1978) *Biochemistry 17*, 5256–5262.

Geacintov, N. E., Ibanez, V., Gagliano, A. G., Yoshida, H., & Harvey, R. G. (1980) *Biochem. Biophys. Res. Commun. 92*, 1335–1342.

Geacintov, N. E., Yoshida, H., Ibanez, V., & Harvey, R. G. (1981) *Biochem. Biophys. Res. Commun. 100*, 1567–1577.

Geacintov, N. E., Yoshida, H., Ibanez, V., & Harvey, R. G. (1982a) *Biochemistry 21*, 1864–1869.

Geacintov, N. E., Gagliano, A. G., Ibanez, V., & Harvey, R. G. (1982b) *Carcinogenesis 3*, 242–253.

Geacintov, N. E., Ibanez, V., Benjamin, M. J., Hibshoosh, H., & Harvey, R. G. (1983) in *Polynuclear Aromatic Hydrocarbons and Cancer: Formation, Metabolism and Measurement* (Cooke, M., & Dennis, A. J., Eds.) pp 554–570, Batelle Press, Columbus, OH.

Geacintov, N. E., Yoshida, H., Ibanez, V., Jacobs, S. A., & Harvey, R. G. (1984a) *Biochem. Biophys. Res. Commun. 122*, 33–39.

Geacintov, N. E., Hibshoosh, H., Ibanez, V., Benjamin, M. J., & Harvey, R. G. (1984b) *Biophys. Chem. 20*, 121–133.

Harvey, R. G. (1981) *Acc. Chem. Res. 14*, 218–226.

Harvey, R. G., & Fu, P. P. (1978) in *Polycyclic Hydrocarbons and Cancer* (Gelboin, H. V., & Ts'o, P. O. P., Eds.) Vol. 1, 133–165, Academic, New York.

Harvey, R. G., Hsu, W. T., Chang, G. T., & Weiss, S. B. (1980) in *Carcinogenesis: Fundamental Mechanisms and Environmental Effects* (Pullman, B., Ts'o, P. O. P., & Gelboin, H., Eds.) pp 13–31, Reidel, Dordrecht, Holland.

Ibanez, V., Geacintov, N. E., Gagliano, A. G., Brandimarte, S., & Harvey, R. G. (1980) *J. Am. Chem. Soc. 102*, 5661–5666.

Jeffrey, A. M., Weinstein, I. B., Jennette, K. W., Grzeskowiak, K., Nakanishi, K., Harvey, R. G., Autrup, H., & Harris, C. (1977) *Nature (London) 269*, 348–350.

Jerina, D. M., Sayer, J. M., Yagi, H., Croisy-Delcy, M., Ittah, Y., & Thakker, D. R. (1982) in *Advances in Experimental Medicine and Biology: Biological Reactive Intermediates IIA* (Snyder, R., Parke, D. V., Kocis, J. J., Jollow, D. J., Gibson, C. G., & Whittmer, C. M., Eds.) pp 501–523, Plenum, New York.

Keller, J. W., Heidelberger, C., Beland, F. A., & Harvey, R. G. (1976) *J. Am. Chem. Soc. 98*, 8276–8277.

Kikuchi, O., Hopfinger, A. J., & Klopman, G. (1979) *Cancer Biochem. Biophys. 4*, 1–8.

Kim, M. H., Geacintov, N. E., Pope, M., & Harvey, R. G. (1984) *Biochemistry 23*, 5433–5439.

Klopman, G., Grinberg, H., & Hopfinger, A. J. (1979) *J. Theor. Biol. 79*, 355–366.

Klotz, I. M. (1974) *Acc. Chem. Res. 7*, 162–168.

Koreeda, M., Moore, P. D., Wislocki, P. G., Levin, W., Conney, A. H., Yagi, H., & Jerina, D. M. (1978) *Science (Washington, D.C.) 199*, 778–781.

LeBreton, P. R. (1985) in *Polycyclic Hydrocarbons and Carcinogenesis* (Harvey, R. G., Ed.) American Chemical Society Symposium Series 283, pp 209–238, American Chemical Society, Washington, DC.

Li, H. J., & Crothers, D. M. (1969) *J. Mol. Biol. 39*, 461–477.

Lin, J. H., LeBreton, P. R., & Shipman, L. L. (1980) *J. Phys. Chem. 84*, 642–649.

MacLeod, M. C., & Selkirk, J. K. (1982) *Carcinogenesis 3*, 287–292.

MacLeod, M. C., & Zachary, K. L. (1985) *Carcinogenesis 6*, 147–149.

MacLeod, M. C., Mansfield, B. K., & Selkirk, J. K. (1982) *Carcinogenesis 3*, 1031–1037.

Meehan, T., & Bond, D. M. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 2635–2641.

Meehan, T., Gamper, H., & Becker, J. F. (1982) *J. Biol. Chem. 257*, 10479–10485.

Miller, K. J., Taylor, E. R., Dommen, J., & Burbaum, J. J. (1985) in *Molecular Basis of Cancer* (Rein, M., Ed.) Part A, pp 187–197, Liss, New York.

Newbold, R. F., Brookes, P., & Harvey, R. G. (1979) *Int. J. Cancer 24*, 203–209.

Pelling, J. C., & Slaga, T. J. (1982) *Carcinogenesis 3*, 1135–1141.

Sayer, J. M., Yagi, H., Silverton, J. V., Friedman, S. L., Whalen, D. L., & Jerina, D. M. (1982) *J. Am. Chem. Soc. 104*, 1972–1978.

Sayer, J. M., Whalen, D. L., Friedman, S. L., Paik, A., Yagi, H., Vyas, K. P., & Jerina, D. M. (1984) *J. Am. Chem. Soc. 106*, 226–233.

Slaga, T. J., Bracken, W. J., Gleason, G., Levin, W., Yagi, H., Jerina, D. M., & Conney, A. H. (1979) *Cancer Res. 39*, 67–71.

Stevens, C. W., Bouck, N., Burgess, J. A., & Fahl, W. E. (1985) *Mutat. Res. 152*, 5–14.

Undeman, O., Lycksell, P. O., Graslund, A., Astlund, T., Ehrenberg, A., Jernstrom, B., Tjerneld, F., & Norden, B. (1983) *Cancer Res. 43*, 1851–1860.

Weinstein, I. B., Jeffrey, A. M., Jennette, K. W., Blobstein, S. H., Harvey, R. G., Harris, C., Autrup, H., Kasai, H., & Nakanishi, K. (1976) *Science (Washington, D.C.) 193*, 592–595.

Whalen, D. L., Montemarano, J. A., Thakker, D. R., Yagi, H., & Jerina, D. M. (1977) *J. Am. Chem. Soc. 99*, 5522-5524.
Whalen, D. L., Ross, A. M., Yagi, H., Karle, J. M., & Jerina, D. M. (1978) *J. Am. Chem. Soc. 100*, 5218-5221.
Whalen, D. L., Ross, A. M., Montemarano, J. A., Thakker, D. R., Yagi, H., & Jerina, D. M. (1979) *J. Am. Chem. Soc. 101*, 5086-5088.
Wood, A. W., Wislocki, P. G., Chang, R. L., Levin, W., Lu, A. Y. H., Yagi, H., Hernandez, O., Jerina, D. M., & Conney, A. H. (1976) *Cancer Res. 36*, 3358-3366.
Wood, A. W., Chang, R. L., Levin, W., Yagi, H., Thakker, D. R., Jerina, D. M., & Conney, A. H. (1977) *Biochem. Biophys. Res. Commun. 77*, 1389-1396.
Yagi, H., Hernandez, O., & Jerina, D. M. (1975) *J. Am. Chem. Soc. 97*, 6881-6883.
Yagi, H., Thakker, D. R., Hernandez, O., Koreeda, M., & Jerina, D. M. (1977) *J. Am. Chem. Soc. 99*, 1604-1611.
Yang, L. L., Maher, V. M., & McCormick, J. J. (1982) *Mutat. Res. 94*, 435-447.
Yang, S. K., McCourt, D. W., Gelboin, H. V., Miller, J. R., & Roller, P. P. (1976) *J. Am. Chem. Soc. 99*, 1604-1611.
Yang, S. K., McCourt, D. W., & Gelboin, H. V. (1977) *J. Am. Chem. Soc. 99*, 5130-5134.

^{1}H NMR Studies on the Interaction between Distamycin A and a Symmetrical DNA Dodecamer†

Rachel E. Klevit,*,‡ David E. Wemmer,‡,§ and Brian R. Reid‡,∥
Departments of Chemistry and Biochemistry, University of Washington, Seattle, Washington 98195
Received November 6, 1985; Revised Manuscript Received January 21, 1986

ABSTRACT: High-resolution NMR techniques have been used to examine the structural and dynamical features of the interaction between distamycin A and the self-complementary DNA dodecamer duplex d-(CGCGAATTCGCG)$_2$. The proton resonances of d(CGCGAATTCGCG)$_2$ have been completely assigned by previous two-dimensional NMR studies [Hare, D. R., Wemmer, D. E., Chou, S. H., Drobny, G., & Reid, B. R. (1983) *J. Mol. Biol. 171*, 319-336]. Addition of the asymmetric drug molecule to the symmetric dodecamer leads to the formation of an asymmetric complex as evidenced by a doubling of DNA resonances over much of the spectrum. In two-dimensional exchange experiments, strong cross-peaks were observed between uncomplexed DNA and drug-bound DNA resonances, permitting direct assignment of many drug-bound DNA resonances from previously assigned free DNA resonances. Weaker exchange cross-peaks between formerly symmetry related DNA resonances indicate that the drug molecule flips head-to-tail on one duplex with half the frequency at which it leaves the DNA molecule completely. In experiments performed in H_2O, nuclear Overhauser effects (NOEs) were observed from each drug amide proton to an adenine C2H and a pyrrole H3 ring proton. In two-dimensional nuclear Overhauser experiments performed on D_2O solutions, strong intermolecular NOEs were observed between each of the three pyrrole H3 resonances of the drug and an adenine C2H resonance, with weaker NOEs observed between the drug H3 resonances and C1'H resonances. The combined NOE data allow us to position the distamycin A unambiguously on the DNA dodecamer, with the drug spanning the central AATT segment in the minor groove.

The sequence-specific recognition of DNA molecules by proteins and small molecules is an important component in the regulation of many biological processes. Two related antibiotics, netropsin and distamycin, have received much attention as models of sequence-specific, nonintercalative DNA-binding molecules. For the most part, physical studies to date have focused on the smaller netropsin molecule, and the results from such studies have been extended by analogy to the larger distamycin molecule. Studies involving exchangeable imino proton NOEs[1] (Patel, 1982) have been performed in solution on a complex of netropsin and the symmetric dodecamer d(CGCGAATTCGCG)$_2$; crystallographic studies on this complex have also been reported (Kopka et al., 1985). The recently published crystal structure of the netropsin-DNA complex confirms earlier proposals that the drug binds in the minor groove of B-form DNA and interacts specifically with AT base pairs.

We have used high-resolution ^{1}H NMR techniques to study the complex between distamycin and d(CGCGAATTCGCG)$_2$. The proton resonances of this DNA duplex have been completely assigned by previous two-dimensional NMR (2D NMR) experiments (Hare et al., 1983).

Materials and Methods

Sample Preparation. The DNA dodecamer was synthesized according to the solid-phase phosphite triester method as previously described (Hare et al., 1983). Distamycin A was obtained as the hydrochloride salt from Sigma. D_2O (99.996%

† This work was supported by NIH Grant GM-32681. The 500-MHz spectrometer is supported by instrumental grants from the Murdock Foundation, the National Science Foundation (PCM80-18053), and the National Institutes of Health (GM-28764-01S1).
‡ Department of Chemistry.
§ Present address: Department of Chemistry, University of California at Berkeley, Berkeley, CA 94720.
∥ Department of Biochemistry.

[1] Abbreviations: 2D NMR, two-dimensional nuclear magnetic resonance; NOE, nuclear Overhauser effect; NOESY, two-dimensional NOE spectroscopy; COSY, two-dimensional *J*-correlated spectroscopy; FID, free induction decay; 1D, one dimensional.

0006-2960/86/0425-3296$01.50/0