This algorithm has been run on a small size computer (a 9810 A Hewlett Packard calculator).

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Magnetic Circular Dichroism of Netropsin and Natural Circular Dichroism of the Netropsin–DNA Complex†

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ABSTRACT: We report the first measurement of the magnetic circular dichroism (MCD) of the basic polypeptide antibiotic netropsin (Nt). The MCD shows that the longest wavelength absorption band of Nt is the sum of more than one component and permits a radically new interpretation of the circular dichroism of the complex which Nt forms with DNA. We conclude that Nt has no major effect on the CD and thus the helical structure of the bases of the DNA to which it is bound. Thus the ability of Nt to inhibit the function of DNA polymerase, RNA polymerase, and the photoreactivating enzyme must be mediated by factors other than a distortion of the helical structure of the bases.

The basic oligopeptide antibiotic netropsin binds to regions of double-stranded DNA which are rich in adenine–thymine base pairs and inhibits both DNA polymerase and RNA polymerase activity (Zimmer, 1975, and references cited therein) and the photoreactivating enzyme (Sutherland, 1978). Netropsin and its complexes with various DNAs and copolymers have been studied extensively by a variety of spectroscopic and hydrodynamic methods (Zimmer et al., 1970, 1971a,b, 1972; Reinert, 1972; Wartell et al., 1974; Luck et al., 1974; Zasedatelev et al., 1974; Zimmer, 1975; and references cited therein).

In this paper we report the first measurement of the mag-

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netic circular dichroism (MCD)[1] spectrum of Nt.[2] Insights gained from the MCD permit a new interpretation of the natural circular dichroism (CD) spectrum of the Nt:DNA complex and lead to the conclusion that the attachment of Nt to DNA causes little if any change in the secondary structure of the majority of the bases of the DNA. This conclusion is consistent with the model proposed for the Nt:DNA complex in which Nt lies in the minor groove of the double helix (Wartell et al., 1974; Zimmer, 1975, and references cited therein). It follows that the ability of netropsin to inhibit enzymes such as DNA polymerase, RNA polymerase, and the photoreactivating enzyme is not due to a change in the helical conformation of the bases, but may reflect blockage of access to the minor groove of the DNA or stabilization of the double helix.

Methods and Materials

Circular dichroic spectra were measured with a spectrometer which has been described elsewhere (Sutherland et al., 1976). The CD and MCD spectra are reported in units of differential absorption or molar extinction and differential absorption or molar extinction per unit magnetic field (in tesla) (Sutherland et al., 1974). Absorption spectra were recorded with a Cary 118C spectrophotometer (Varian Associates, Palo Alto, Calif.). Both the spectrophotometer (Sutherland & Boles, 1978) and the CD/MCD spectrometer have been interfaced to a computer (Model 4051, Tektronix, Beverton, Ore.). Spectra were collected and stored in digital form. Absorption and CD difference spectra were generated by subtracting one spectrum from another using the computer. Original and deconvoluted spectra were plotted on a Versatec (Santa Clara, Calif.) Model 1100 printer-plotter.

The Nt used in these experiments was the gift of Dr. Robert Hosley, Eli Lilly Research Laboratories, Indianapolis, Ind. (Lot 209-653B-270-1). Prior to use, all Nt samples were purified by recrystallization from double-distilled H_2O. Crystals were then washed with cold ethanol, dried, and stored at 4 °C. Concentrations of Nt were determined assuming a molar extinction coefficient of 2.15×10^4 at 297 nm (Zasedatelev et al., 1974). Calf thymus DNA was purchased from Sigma Chemical Corp. Before use it was purified by phenol extraction and then exhaustively dialyzed to remove all traces of phenol.

Results and Discussion

Magnetic Circular Dichroism. The absorption and MCD spectra of free Nt are shown in Figure 1. The MCD has a trough near 305 nm, crosses zero near 280 nm and has a peak near 265 nm. Thus the MCD between 260 and 340 nm does not have the same shape as the absorption band, as it would if the absorption envelope contained only one component (Stephens, 1974). The additional structure of the MCD (i.e., a peak *and* a trough) within a single absorption band is conclusive evidence that the absorption band is an envelope which contains more than one component. Since the chromophores responsible for absorption in this region have less than threefold symmetry, the MCD bands must be of type B (Stephens, 1974). The MCD also suggests the existence of more than one component

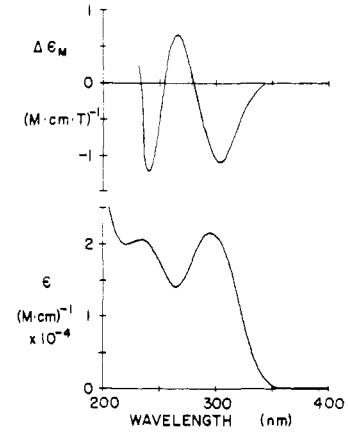

FIGURE 1: Absorption and MCD spectra of Nt in 100 mM sodium phosphate buffer, pH 7. The MCD spectrum has a trough near 305 nm and a peak near 265 nm, thus indicating the presence of at least two absorption bands with nonparallel transition dipole moments in the single absorption envelope centered at 297 nm.

in the second absorption band with a peak absorption at 235 nm.

Free in solution, Nt does not exhibit any natural circular dichroism (CD). The external magnetic field in the MCD experiment perturbs the molecule and reveals the existance of more than one component in the absorption envelope. Binding to a chiral substrate (such as DNA) is another type of perturbation capable of revealing multiple components in an absorption envelope. Thus, awareness of the existence of more than one band in the long wavelength absorption envelope of Nt is crucial for a proper interpretation of the natural CD of the Nt:DNA complex (infra vide).

The long wavelength absorption bands of Nt are due to its two 1-methylpyrrole-2-carboxamide residues. Thus the two transitions required to account for the MCD spectrum might result from the mixing of two transitions, each isolated on one of these residues. A second possibility is that each of the residues possesses more than one transition in the 260–350-nm region. Two lines of evidence favor the latter hypothesis. First, using MCD, Håkansson et al. (1977) showed that the longest wavelength transitions of five-membered heterocyclic ring compounds are degenerate, i.e., the absorption envelope contains more than one transition. Second, the shape of the absorption spectrum of the related antibiotic distamycin A, which contains three 1-methylpyrrole-2-carboxamide residues, is similar to that of Nt (peak at 303), while the molar extinction coefficient is approximately 50% larger (3.4×10^4) (Zimmer, 1975). Indeed, netropsin-like molecules with between one and five residues have qualitatively similar spectra (Zimmer, 1975; Zimmer et al., 1971). There is only a slight red shift with increasing number of residues. If there were strong interactions between 1-methylpyrrole rings (and "mixing" of their excited states) the molar extinction coefficient would not be additive and the shape of the absorption would be significantly perturbed. Since this is not the case, it follows that, to a first approximation, each of the 1-methylpyrrole-2-carboxamide residues can be considered as an independent chromophore. Thus the complex MCD spectrum results from the mixing by the magnetic field of transitions located on the same chromophore.

Natural Circular Dichroism. The CD spectrum of the Nt:DNA complex is strikingly different from the CD of DNA alone (Zimmer et al., 1970, 1972a; Luck et al., 1974; Zasedatelev et al., 1974). The CD spectrum of Nt mixed with calf thymus DNA is shown in Figure 2A, while the CD of an equal

[1] Abbreviations used: ADS, absorption difference spectrum; Nt, netropsin; CD, natural circular dichroism; MCD, magnetic circular dichroism; λ, wavelength.

[2] A preliminary account of this work was presented at the 22nd Annual Meeting of the Biophysical Society, Washington, D.C., March 27-30, 1978.

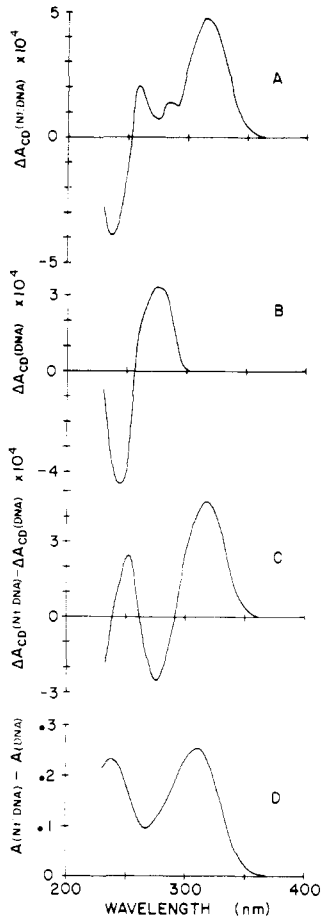

FIGURE 2: (A) CD of the Nt:DNA complex. The concentration of Nt in this solution was 1.2×10^{-5} M, while the concentration of calf thymus DNA (nucleotides) was 1.4×10^{-4} M. The solution contained 100 mM phosphate buffer, pH 7.0. (B) CD of the an equal concentration of DNA: (C) The putative CD spectrum of bound Nt computed by subtracting the spectrum shown in B from that of A. This spectrum shows a peak and a trough at the same relative positions as the trough and peak shown in the MCD of Nt (Figure 1), indicating that the transitions responsible for these features are associated with the Nt molecules and not to perturbations of the DNA. (D) The absorption of the same Nt:DNA complex whose CD spectrum is shown in A minus the absorption of an equal concentration of DNA.

concentration of the same DNA without Nt is shown in Figure 2B. The ratio of the concentrations of nucleotides to total Nt is 11.7. Under these conditions of DNA, Nt and salt concentrations, more than 80% of the Nt is bound and the saturation limit for Nt binding is 11 nucleotides per Nt (Sutherland, Duval, Farland, & Griffin, submitted). Thus more than 75% of the Nt sites on the calf thymus DNA are occupied. The complex shows a new CD band at wavelengths greater than 300 nm with a peak near 315 nm. In addition, there are gross differences for $\lambda < 300$ nm between the CD of the complex and that of DNA alone.

While it has been recognized that the induced CD for wavelengths greater than 300 nm is due to bound Nt, it was originally believed that the gross changes in the CD observed for wavelengths less than 300 nm indicated a substantial perturbation in the conformation of the DNA (Zimmer et al., 1970, 1972). However, new insights gained from MCD on the spectroscopic properties of Nt, specifically the existence of more than one transition in the absorption envelope centered at 297 nm, permit us to propose a deconvolution of the contributions of Nt and DNA to the CD spectrum of their complex.

In Figure 2C we show the CD spectrum obtained by subtracting the CD of DNA (Figure 2B) from the CD of the Nt:DNA complex (Figure 2A). The spectrum shown in Figure 2C represents the CD spectrum of bound Nt *only if* the CD spectrum of the DNA is unaffected by complex formation. Two lines of evidence support the hypothesis that spectrum 2C is, in fact, a reasonable representation of the CD spectrum of bound Nt.

First, we know from MCD that the long wavelength absorption band of Nt is an envelope which contains more than one component. The semiconservative CD spectrum plotted in Figure 2C is consistent with that expected for a molecule which has more than one independent transition in a single absorption envelope. Indeed, allowing for inversion of sign and a slight red shift (seen also in absorption), there is a striking resemblance between Figure 2C and the MCD of free Nt shown in Figure 1. In the case of MCD, the magnetic field removes the degeneracy between the absorption of left and right circularly polarized light while in the case of the complex, the degeneracy is removed by the chirality of the DNA to which the Nt binds. Second, Zasedatelev et al. (1974) showed that difference CD spectra similar to that shown in Figure 2C are obtained using DNA from several sources as well as several A-T containing copolymers even though the CD spectra of the various DNAs and copolymers differ greatly among themselves.[3] From these data, Zasedatelev et al. (1974) concluded that the presumed perturbation of the structure of DNA induced by Nt is independent of the base composition of the DNA. In view of the new MCD data, however, our interpretation is that the difference spectrum shown in Figure 2C is the CD of bound Nt. The invariance of this spectrum with respect to the base content and CD of the DNA reflects the fact that the conformation of the bound NT is approximately the same for all substrates.

The validity of the spectrum shown in Figure 2C as the CD spectrum of bound Nt implies that the CD spectrum of the DNA was unchanged by Nt binding. It has an important corollary regarding the structure of the Nt:DNA complex. Since the CD of DNA is sensitive to the conformation of the bases, it follows that interaction with Nt does not greatly perturb the geometry of the majority of the bases in the DNA helix. While we cannot exclude the possibility that the secondary structure of a small fraction of the bases is perturbed, we can exclude significant changes such as tilting (Zimmer et al., 1970, 1971b, 1972) of a significant fraction of the bases. The failure of Nt to perturb the secondary structure of the majority of the bases in DNA is extremely important in interpreting experiments in which Nt inhibits the action of enzymes which bind to DNA including DNA and RNA polymerase (Zimmer et al., 1971) and the photoreactivating enzyme (Sutherland, 1978). If Nt distorted the helical structure of the DNA, then inhibition of enzymic activity might reflect this change in structure; since Nt appears not to alter the helical structure, some other mechanism must be available to inhibit enzymatic activity. Since Nt binds in the minor groove, one possibility is that Nt blocks enzyme function by denying the enzymes access to that portion of the DNA. Another possibility is that Nt may act by stabilizing the double helical structure.

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[3] There are minor differences among the CD difference spectra for the various DNAs and copolymers both below and above 300 nm.

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T4 RNA Ligase Joins 2′-Deoxyribonucleoside 3′,5′-Bisphosphates to Oligodeoxyribonucleotides†

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ABSTRACT: T4 RNA ligase catalyzes the ATP-dependent addition of a single 2′-deoxyribonucleoside 3′,5′-bisphosphate to the 3′-hydroxyl of an oligodeoxyribonucleotide. The bisphosphate is joined to the deoxyoligomer by a 3′→5′ phosphodiester bond and the product, which is terminated by a 3′-phosphate, is one nucleotide longer than the substrate. Bisphosphates of dAdo, dCyd, dGuo, dThd, and dUrd are donors and oligodeoxyribonucleotides with dA, dC, dG, dT, or dU 3′ termini act as acceptors. The preferred residue for both donor and acceptor is dCyd. Deoxyoligomers from 3 to 12 residues in length are active as acceptors. To obtain good yields, high concentration of enzyme, long incubation time at low temperature, and manganous rather than Mg(II) ion are required. Under optimal conditions, yields calculated with respect to deoxyoligomer converted to product vary from 40 to greater than 95%. The turnover number of the enzyme for DNA joining is extremely low but, because the preparation is nearly free of DNases, there is less than 3% degradation of substrate or product after 6 days of reaction. We anticipate that this reaction will serve as the basis for a method for the stepwise enzymatic synthesis of DNA of defined sequence.

RNA ligase from bacteriophage T4-infected *Escherichia coli* catalyzes the ATP-dependent formation of a 3′→5′-phosphodiester bond between one oligoribonucleotide with a 3′-hydroxyl group (the acceptor molecule) and another with a 5′-phosphate (the donor molecule) (Silber et al., 1972). The reaction may be either intramolecular (Silber et al., 1972) or intermolecular (Walker et al., 1975; Kaufmann & Kallenbach, 1975). The intermolecular reaction has proved useful for the synthesis of oligoribonucleotides of defined sequence (Walker et al., 1975; Ohtsuka et al., 1976; Sninsky et al., 1976; Uhlenbeck & Cameron, 1977).

The enzyme also uses DNA as a substrate (Snopek et al., 1976) but the reported yields of the intermolecular reaction with oligodeoxyribonucleotides were too low to be useful for practical syntheses (Sugino et al., 1977). We have been developing RNA ligase as a DNA synthesis reagent and have found reaction conditions that allow high yields with single-strand deoxyoligomers (McCoy & Gumport, unpublished results) and, in addition, have demonstrated that the donor molecule can be a single nucleotide, if it is a 2′-deoxyribonucleoside 3′,5′-bisphosphate (pdNp).[1] It is the single nucleotide addition reaction of RNA ligase with DNA substrates that is the subject of the present report. A preliminary report of this work has appeared (Hinton et al., 1978). This reaction is the analogue of the single ribonucleoside bisphosphate addition to oligoribonucleotides (England & Uhlenbeck, 1978; Kikuchi et al., 1978) and to large RNAs (England & Uhlenbeck, personal communication) and is expected to be similarly useful

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[1] Abbreviations following the IUPAC-IUB Commission on Biochemical Nomenclature recommendations [*J. Mol. Biol. 55,* 299 (1971), and *Proc. Natl. Acad. Sci. U.S.A. 74,* 2222 (1977)] are used throughout. The one-letter abbreviations for simple nucleotides will be used. For example, pdAp represents 2′-deoxyadenosine 3′,5′-bisphosphate. Other abbreviations used: Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; BAP, bacterial alkaline phosphatase; DTT, dithiothreitol; BSA, bovine serum albumin.

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