Quinoxaline Antibiotics Enhance Peptide Nucleic Acid Binding to Double-Stranded DNA†

Niels Erik Møllegaard,‡ Christian Bailly,§ Michael J. Waring,|| and Peter E. Nielsen*,‡

Department of Medical Biochemistry and Genetics, The Panum Institute, Blegdamsvej 3c, DK-2200 Copenhagen N, Denmark, Laboratoire de Pharmacologie Antitumorale du Centre Oscar Lambret and INSERM U524, IRCL, Place de Verdun, 59045 Lille, France, and Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ, U.K.

Received February 3, 2000; Revised Manuscript Received May 11, 2000

ABSTRACT: The effects of a wide range of DNA binding drugs on peptide nucleic acid (PNA) binding to double-stranded DNA by strand displacement have been investigated using a gel retardation assay. The bis-PNA [H-(Lys)-TTJTTJTTTT-$(eg)_3$-TTTTCTTCTT-Lys-NH_2] was used together with a 248 bp DNA fragment containing an appropriate target for the PNA. Most of the ligands that were studied, including DNA minor groove binders as well as intercalators and bis-intercalators, either have no effect or strongly inhibit PNA binding to DNA. By contrast, quinoxaline antibiotics facilitate PNA−DNA complex formation. The "PNA-helper" effect of echinomycin was studied in more detail using time and temperature dependence experiments to elucidate the mechanism. PNA binding to DNA follows pseudo-first-order kinetics, but the initial rate of binding is accelerated more than 10-fold in the presence of 10 μM echinomycin. The activation energy for PNA binding to dsDNA is lowered 2-fold by the antibiotic (45 vs 90 kJ/mol in the control). The reasons why quinoxalines promote the binding of PNA to DNA are not entirely clear but may well include distortions (opening) of the double helix that facilitate PNA invasion. This study establishes that the efficacy of DNA-targeted PNA antigene molecules could potentially be enhanced by judiciously adding certain DNA-interactive ligands.

Peptide nucleic acids (PNA)[1] are DNA mimics with potential applications in molecular biology, diagnostics, and gene therapy. Targeting of double-stranded DNA by homopyrimidine PNA occurs by a process of invasion of the double helix whereby two PNAs bind to the complementary DNA strand while the noncomplementary polynucleotide is displaced as a single strand (*1*−*3*). PNA binding to double-stranded DNA selectively inhibits protein binding and consequently has potential in transcription arrest (*2*). Furthermore, the PNA-induced single-stranded loop can create artificial transcription promoters for RNA polymerases (*4*).

The binding of PNA is very sensitive to ionic strength, and therefore, a fundamental issue that needs to be addressed is the requirement for a relatively low salt concentration (<50 mM) to permit the efficient formation of strand displacement complexes. [However, once formed, the $(PNA)_2$−DNA triplex is remarkably stable (up to at least 500 mM salt).] Therefore, binding might be impeded under physiological conditions (*5*), and it would be of great interest to find procedures for promoting the binding of PNA to its target sequence under conditions likely to prevail in vivo. Previously, it has been shown that the superhelical density of DNA has a significant influence on PNA binding, suggesting that structural parameters in DNA can increase the level of binding of PNA (*6*).

Another potential approach to reinforcing the binding of PNA to double-stranded DNA consists of using a DNA-binding ligand capable of specifically promoting the strand invasion process. This could occur either through ligand interactions within the PNA−DNA complex or via DNA distortions which increase the potential for PNA invasion of the double-stranded DNA. Unlike the phosphate backbone of DNA, the polyamide backbone of PNA is uncharged. The usual electrostatic repulsion between complementary strands is thus absent, and as a consequence, the hybridization between a target DNA strand and a complementary PNA strand is in general more stable than with the equivalent oligodeoxynucleotide (*1*, *7*, *8*). However, the reduced number of negative charges could be detrimental to the binding of drugs which are usually positively charged. Wittung et al. have shown that DNA-intercalating drugs such as ethidium bromide and ruthenium complexes cannot intercalate into PNA−DNA duplexes (*9*). However, ethidium was reported to increase the rate of oligomer T PNA binding to poly(dA/dT) (*10*).

† This work was supported by research grants from the European Union and the Lundbeck Foundation (to P.E.N.), from the Novo Nordisk Foundation, Løvens Kemiske Fabriks Fond and The Danish Medical Research Council (to N.E.M.), and from the European Union, Cancer Research Campaign, Association for International Cancer Research, and Sir Halley Stewart Trust (to M.J.W.).

* To whom correspondence should be addressed. Phone: +45 35327762. Fax: +45 35396042. E-mail: pen@imbg.ku.dk.

‡ The Panum Institute.

§ IRCL.

|| University of Cambridge.

[1] Abbreviations: TBE buffer, 8.9 mM Tris base, 8.9 mM boric acid, and 2.5 mM Na_2EDTA (pH 8.3); PNA, peptide nucleic acids; eg, nine-atom ethylene glycol; J, pseudoisocytosine; dsDNA, double-stranded DNA.

10.1021/bi000254x CCC: $19.00
Published on Web 07/13/2000

These considerations prompted us to screen a large number of known DNA-binding drugs for their potential ability to modulate the binding of a bis-PNA to its double-stranded DNA target. More than 50 ligands, including groove binders as well as intercalators and bis-intercalators, were tested using a gel mobility shift assay. The large majority of drugs have no effect or inhibit PNA binding. However, the screening procedure has led to the unexpected finding that members of the quinoxaline family of antibiotics promote significantly the binding of PNA to a double-stranded DNA target. Here the "PNA helper" effect of the antibiotic echinomycin has been investigated quantitatively.

MATERIALS AND METHODS

Drugs and PNA. Antibiotics and drugs were obtained from commercial sources or generously provided by colleagues. In all cases, they were used as supplied without further purification. Most of the tested drugs exhibited good aqueous solubility except the quinoxaline antibiotics and a few other drugs such as BePI, coralyne, ellipticine, and amiloride which are sparingly soluble in water. In these cases, they were first dissolved in DMSO or methanol and then diluted to working concentrations with appropriate volumes of 10 mM Tris-HCl (pH 7.0) and 10 mM NaCl. Drug concentrations were determined spectrophotometrically in 10 mm path length quartz cuvettes using the molar extinction coefficients given in the literature. The bis-PNA, PNA 977 [H-(Lys)-TTJT-TJTTTT-$(eg)_3$-TTTTCTTCTT-Lys-NH_2], was synthesized as previously described (*11*, *12*). The PNA concentration was determined from absorbance measurements at 260 nm using an ϵ_{260} of 1.61×10^5.

DNA Preparation. The 248 bp DNA target was obtained by digestion of plasmid pA8G2 (*5*) with *Eco*RI and *Pvu*II in generating overhanging ends of unique sequence. It was 3′-end-labeled at the *Eco*RI site with [α-^{32}P]dATP using the Klenow fragment of DNA polymerase I (20 units, Boehringer) under standard conditions. The DNA fragments were then separated on a 6.5% preparative polyacrylamide gel by electrophoresis for 2 h at 200 V in TBE buffer. The DNA bands were identified by autoradiography, excised, and collected by elution in 500 mM ammonium acetate, 10 mM magnesium acetate buffer.

Gel Mobility Shift Analysis. For the screening of drugs, the 3′-end-labeled DNA fragment was incubated with the PNA and the test drug for 30 min at room temperature in 10 μL of a 10 mM Tris-HCl buffer (pH 7.6) containing 1 mM EDTA. Two microliters of a 5% glycerol solution containing the electrophoresis dyes were added prior to loading the samples directly onto a 6% polyacrylamide gel. The PNA concentration (about 1 μM) was adjusted to produce 50% binding in the control (no drug) under the conditions that were used. The drug concentration was varied from 0.1 to 100 μM depending upon its known affinity for double-stranded DNA. Checks were performed to verify that the presence of the drug itself was not sufficient to retard the mobility of the double-stranded DNA target. In general, this was found to be the case; one exception (ditercalinium) can be seen in the top panel of Figure 1. For the kinetic experiments, the labeled DNA was incubated with the PNA and the drug in a final volume of 50 μL and, at intervals, aliquots (5 μL) were taken out and immediately frozen in dry ice. After all reactions had been stopped, samples were treated and electrophoresed as described above.

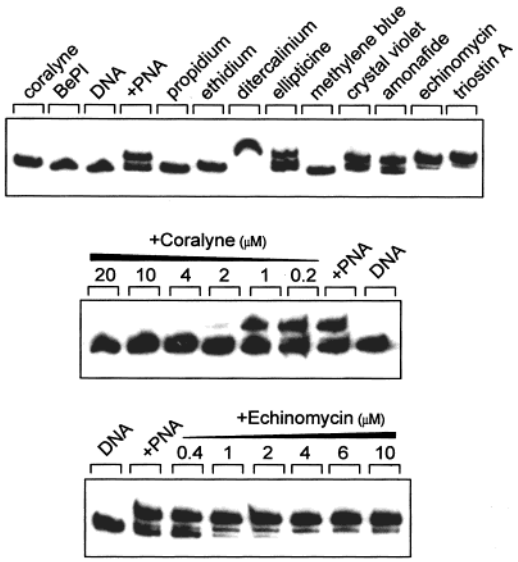

FIGURE 1: Effects of different types of drugs on the binding of PNA 977 to double-stranded DNA (top panel). The 248 bp DNA was ^{32}P-labeled at the 3′-end (lane DNA) and incubated with 1 μM PNA 977 in the absence (lane +PNA) or presence of the test drug at 10 μM. The middle and bottom panels show the concentration-dependent effects of coralyne and echinomycin which decrease and promote PNA binding to DNA, respectively. In all cases, samples were incubated for 30 min prior to loading onto a 6% nondenaturing polyacrylamide gel. We ascribe the weak band clearly seen in the bottom panel, migrating between free DNA and PNA-bound DNA, to PNA bound to single-stranded DNA.

Electrophoresis, Autoradiography, and Quantitation by Storage Phosphor Imaging. Unbound DNA and DNA−PNA complexes were resolved by polyacrylamide gel electrophoresis under nondenaturing conditions (0.3 mm thick, 6% acrylamide). After electrophoresis for about 90 min at 200 V in TBE buffer, the gel was transferred to Whatman 3 MM paper, dried under heat and vacuum, and subjected to autoradiography at −70 °C with an intensifying screen using X-ray films (Fuji R-X) or apposed overnight to a PhosphorImager screen. A Molecular Dynamics 425E Phosphor Imager was used to collect data from storage screens. Scans were analyzed by integrating all the densities between two selected boundaries using ImageQuant version 3.3 software.

RESULTS

The electrophoretic mobility of double-stranded DNA in polyacrylamide gels changes dramatically upon binding of PNA because of the formation of the $(PNA)_2$−DNA strand displacement complex (*5*, *13*). Therefore, a gel mobility shift assay provides a convenient means of assessing the effect of drugs on PNA binding, on the basis of the appearance of two bands in the gel corresponding to bound and unbound DNA at a chosen PNA concentration. For this purpose, PNA 977 [H-(Lys)-TTJTTJTTTT-$(eg)_3$-TTTTCTTCTT-Lys-NH_2] was incubated with a radioactively labeled *Eco*RI−*Pvu*II fragment from the plasmid pA8G2 containing a single 5′-$A_4GA_2GA_2$ PNA binding site. A predetermined concentration of PNA required to produce approximately 50% binding was employed for the control. The drug was added before the PNA. Any effects of drugs were manifest by an alteration in the intensity of the two bands corresponding to the free

Table 1: Effects of Drugs on PNA Binding to DNA[a]

Drugs That Inhibit PNA 977 Binding to DNA
minor groove binders
berenil, DAPI, distamycin, Hoechst 33258, netropsin, SN6999
intercalators
acridine orange, BePI, coralyne, daunomycin, ethidium bromide, dimidium, *N*-methyl-9-aminoacridine, methylene blue, mitoxantrone, nogalamycin, phenanthroline, proflavine, propidium, pyronaridine
bis-intercalators
ditercalinium, bisnaphthalimides
Drugs That Have No Effect on PNA 977 Binding to DNA
minor groove binders
chromomycin, mithramycin, olivomycin, pentamidine, propamidine
intercalators
actinomycin, amonafide, amsacrine, amsacrine-4-carboxamide, benzothiopyranoindazole, berberine, ellipticine, hycanthone, lucanthone, mitonafide, tilorone
others
amiloride, crystal violet, di-*tert*-butylproflavine, methyl green
Drugs That Increase PNA 977 Binding to DNA
quinoxaline drugs
echinomycin, triostin A, 2QN, TANDEM

DNA (faster band) and the PNA−DNA complex (slower band). An increase in the intensity of the complex band relates to a positive effect of the drug on binding of PNA, whereas a decrease in the intensity of the slower band reflects a negative effect of the drug on PNA binding.

A collection of more than 50 drugs was examined. Both AT-specific minor groove binders such as netropsin or berenil and GC-specific groove binders such as mithramycin were tested. A large variety of intercalating ligands were also used. These included structurally simple drugs such as acridine orange and dimidium as well as intercalators of more complex structure such as tilorone and amsacrine. A few of the intercalators prefer AT-rich sequences such as lucanthone and hycanthone (*14*), whereas others exhibit a pronounced selectivity for GC-rich sites such as amsacrine-4-carboxamide (*15*). In addition, we included intercalating drugs equipped with minor groove binding elements (e.g., actinomycin and daunomycin), DNA-threading intercalators (nogalamycin), and a DNA triple-helix-stabilizing intercalator (BePI). Therefore, the selected drugs differ markedly in terms of structure, sequence selectivity, and/or mode and kinetics of binding to double-stranded DNA. None of these drugs proved to be capable of enhancing PNA binding to dsDNA (Table 1) at concentrations in the range of 1−10 μM. Many had no effect on PNA binding, but another substantial group would significantly inhibit the formation of the PNA−DNA complex at concentrations of 1−10 μM. Similar effects were obtained with unconventional DNA ligands such as amiloride (*16*) and the triphenylmethane dyes crystal violet and methyl green (*17*). It is not easy to perceive any common features which distinguish PNA−DNA inhibitory compounds from those that had no detectable effect, except perhaps that the observed inhibitors include many characterized by high DNA binding affinity. Indeed, it is likely that many noninhibitory compounds would be found to impede PNA−DNA interaction if they were tested at much higher (though probably unrealistic) concentrations. Moreover, the influence of salt concentration and of target sequence has not been systematically examined, and it remains possible that certain compounds could inhibit the binding of PNA to a different target.

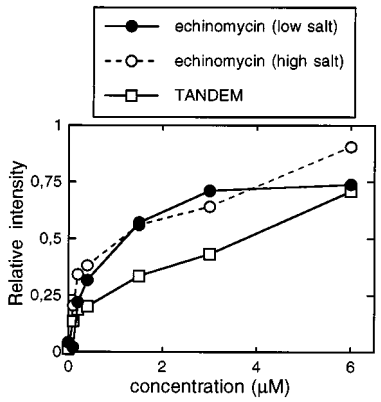

FIGURE 2: Concentration dependence of the effects of echinomycin in high salt (60 mM KCl, open symbols) and low salt (10 mm Tris-HCl, filled symbols) and the effect of TANDEM on PNA binding to DNA. The plots were obtained by densitometric analysis of autoradiograms from gel shift experiments such as those shown in the bottom two panels of Figure 1. We have observed that echinomycin inhibits PNA binding at higher concentrations. This is probably why the extent of relative binding is leveling off.

A few experiments were performed with different PNA−DNA target systems, but no dramatic differences in responses to drugs were found.

Next, we investigated the effect of bis-intercalating drugs such as ditercalinium (*18*), bis-naphthalimide derivatives (*19*), and the quinoxaline antibiotics (*20*). Surprisingly, while the former drugs completely inhibited the binding of PNA to DNA, the latter substances yielded enhanced PNA binding. Among all the compounds we have screened, only those belonging to the quinoxaline family were found to promote the binding of PNA to DNA (Table 1).

Typical gels resulting from these experiments are shown in Figure 1. In the presence of PNA alone (lane 4), two bands are resolved on the gel corresponding to the free double-stranded DNA (lower band) and the PNA−DNA complex (upper band). Drug effects appear as variations in intensities of the two bands in the presence of 10 μM ligand. As shown, coralyne, BePI, propidium, ethidium, and methylene blue decrease the level of PNA binding whereas ellipticine, crystal violet, and amonafide elicit no effect. In the case of ditercalinium, titration experiments (not shown) revealed that the single retarded band which appears corresponds to a drug−DNA complex formed because of strong binding of the drug to the DNA; it did not correspond to the PNA−DNA complex. Most notably, an increased level of interaction with PNA is exclusively found upon addition of the quinoxalines (echinomycin, triostin A, and its analogue 2QN) to the PNA−DNA incubation mixture. Beside these three antibiotics which bind preferentially in the minor groove of CpG sites in DNA, we found that the related synthetic quinoxaline analogue TANDEM, which is a TpA-specific bis-intercalating drug (*21*−*23*), increases the level of binding of PNA to DNA as well (Figure 2). The effect of echinomycin was not annulled by increasing the ionic strength. In the presence of 60 mM KCl, the antibiotic enhanced PNA binding to much the same relative extent as it did in the Tris buffer alone, showing that addition of salt had little or no effect on the capacity of echinomycin to promote $(PNA)_2$−DNA complex formation (lower panel of Figure 2).

For each compound shown in Table 1, we varied the drug concentration from approximately 0.2 to 20 μM. With most

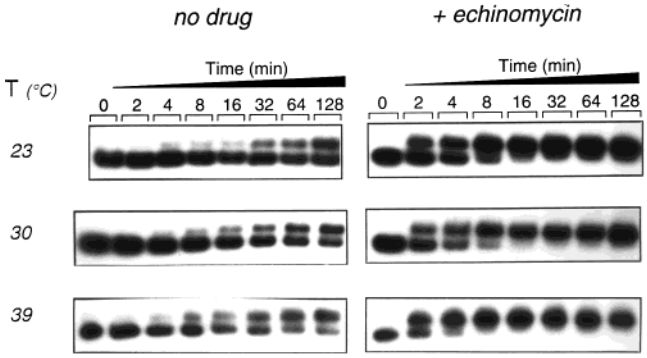

FIGURE 3: Kinetics of the binding of PNA 977 to DNA in the presence or absence of 10 μM echinomycin. DNA was incubated with the PNA with and without the antibiotic for various periods of time at 23, 30, and 39 °C. At intervals, aliquots were taken out of the main sample and reactions were stopped by transferring the tubes to dry ice. For other details, see the legend of Figure 1.

of them, an effect was observed at 1 μM. In the lower panels of Figure 1, two typical examples of drug concentration-dependent effects on PNA binding are presented. The alkaloid coralyne, which can stabilize DNA triplex formation (*24*), has a strong inhibitory effect on the formation of the $(PNA)_2$−DNA complex. At a concentration of 2 μM, coralyne is able to abolish the binding of the PNA completely. In contrast, echinomycin decisively facilitates PNA binding, and the effect is concentration-dependent. It is readily apparent at a drug concentration of 1 μM (Figure 1).

The positive effect on PNA binding observed with the quinoxaline antibiotics warranted closer investigation. Echinomycin, which is the lead compound in the series, was studied in more detail using both time and temperature dependence experiments to address the molecular mechanism of the effect of the quinoxaline drugs on the binding of the PNA to DNA. Figures 3 and 4 illustrate the kinetics of PNA binding to DNA in the presence or absence of 10 μM echinomycin.

Experiments were performed at 10, 23, 30, and 39 °C to measure the activation energy of the binding process. In each case, the DNA was preincubated with echinomycin and then mixed with the PNA at the specified temperature, and aliquots were removed and placed on dry ice to prevent further binding of PNA at time intervals ranging from 2 to 128 min. The results show that PNA binding is much faster in the presence of echinomycin. For instance, at 23 °C complete PNA binding is reached at around 30 min, whereas not even 50% binding is attained after 128 min in the absence of echinomycin. A similar effect of the drug is observed at 30 and 39 °C, but it is evident as expected that the PNA binds faster at the higher temperature both in the absence and in the presence of echinomycin (Figures 3 and 4).

It has previously been shown that PNA binding to double-stranded DNA obeys pseudo-first-order kinetics, $\ln(1 - \alpha) = -kt$, where α is the ratio of PNA−DNA complex to free DNA at time t and k is the pseudo-first-order rate constant (*25*). The time course of PNA binding to the DNA target allows us to estimate the initial rate of binding in the presence and absence of echinomycin. Plots of $-\ln(1 - \alpha)$ as a function of time (at 23 °C) are shown in Figure 5. The data show that the initial rate of binding in the control is 0.01 min^{-1} compared to 0.11 min^{-1} with echinomycin present. Thus, the rate is 11 times faster in the presence of the drug.

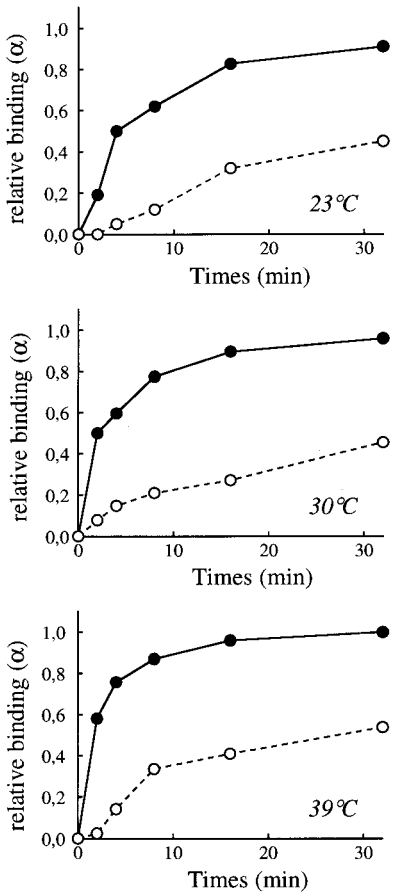

FIGURE 4: Kinetics of PNA binding to DNA in the absence (○) or presence (●) of echinomycin. The plots were determined by densitometric analysis of autoradiograms from three independent experiments, including the one illustrated in Figure 3. Relative binding refers to the fraction of $(PNA)_2$−dsDNA complexes formed (α = [bound]/[bound + free]).

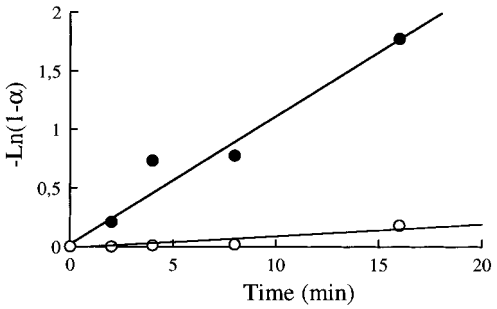

FIGURE 5: Kinetics of PNA strand invasion at 23 °C in the absence (○) and presence (●) of 10 μM echinomycin. Plots of this sort were used to calculate pseudo-first-order rate constants for PNA strand invasion at different temperatures, with and without echinomycin.

The activation energy for PNA binding to dsDNA under both conditions was estimated from the temperature dependence of the rate constants. The initial rates k of PNA binding at 10, 23, 30, and 39 °C were calculated from data sets collected at each temperature as in the example shown in Figure 5. The derived rate constants were used to construct an Arrhenius plot to quantify the energetics of the reaction (Figure 6). From such plots, we calculated activation energies of 90 kJ/mol for binding of PNA in the control and 45 kJ/mol in the presence of echinomycin. Although the accuracy in the absolute values was not determined, the results clearly

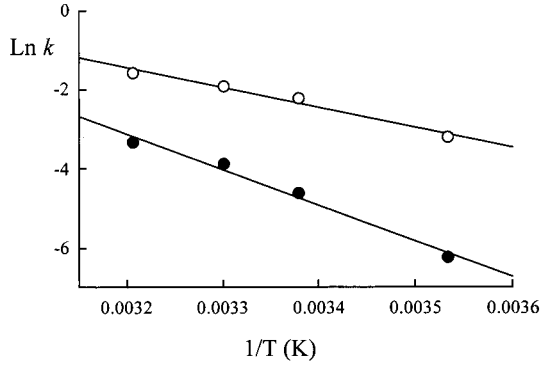

FIGURE 6: Arrhenius plots for determining the activation energy for PNA strand invasion in the absence (○) and presence of echinomycin (●). The activation energy was calculated from the (negative) slope of the plot of ln k vs $1/T$ (kelvin) multiplied by the gas constant ($R = 8.31$ J mol^{-1} K^{-1}). The lines are a result of the fit, from which the themodynamic parameters were calculated. The error of the fits is less than ±7%.

show that the activation energy is lowered 50% in the presence of echinomycin.

The catalytic effect of echinomycin on PNA binding was confirmed using a PNA with a different sequence [H-TTTTC-CTCTC-(eg)$_3$-JTJTJJTTTT-Lys-NH$_2$] binding to its cognate target 5′-GAGAGGAAAA.

DISCUSSION

The results presented here show quite clearly that the antibiotic echinomycin consistently promotes the binding of a bis-PNA to a target in double-stranded DNA. It is intriguing that the quinoxalines are the only antibiotics that have a facilitating effect on PNA binding, for the ternary nature of the system presents numerous opportunities for drugs to modify PNA−DNA interaction as a result of drug−DNA binding, drug−PNA binding, and drug−DNA−PNA binding. Much is known about the first of these processes and has already been mentioned. Far less is known about drug−PNA binding, save that it is generally dramatically reduced, nonspecific, or insignificant compared with DNA binding. But it is the third process that is most likely to promote PNA−DNA interaction through a selective stabilization of the effect upon the (PNA)$_2$−DNA triplex.

Previously, the interaction of DNA-binding ligands with PNA−DNA complexes has been examined by CD spectroscopy. Wittung et al. (*9*) reported that the AT-specific minor groove binders distamycin A and DAPI can both bind to PNA−DNA duplexes, presumably via minor groove interactions, but only the chromosome stain DAPI was found to bind to a (PNA)$_2$−DNA triplex. The considerably weaker affinity of DAPI for the double-stranded PNA−DNA target was attributed to the reduced number of negative charges in the minor groove of a PNA−DNA duplex compared to a normal DNA duplex. In the same study, the well-known intercalating drugs ethidium bromide (which is positively charged) and 8-methoxypsoralen (which is charge neutral) were tested, but in neither case was intercalation observed, irrespective of whether the target was a PNA−DNA duplex or a (PNA)$_2$−DNA poly(dA/dT) triplex. More recently, the effects of some DNA-binding ligands on the overall rate of PNA binding were also studied by CD spectroscopy (*10*). DAPI was found to decrease the rate of binding of PNA to DNA, whereas the intercalators ethidium and 9-aminoacridine increased the rate. The results presented here contrast with these earlier finding in that both 9-aminoacridine and ethidium bromide were found to inhibit the binding of PNA 977 to its target. It must be considered, however, that our study concerns the binding of a PNA to an isolated target whereas the target in the previous study was a poly(dA/dT) homopolymer duplex presenting multiple targets that presumably would support cooperative binding (*10*). Thus, the mechanism of binding may not be identical in the two situations which might in itself be sufficient to account for the opposite effects of the intercalators, without searching for more complicated explanations.

The unwinding of the double helix associated with bis-intercalation of echinomycin produces a large decrease in the base pair twist angle and consequently an expansion of the major groove of the helix, which could favor the access of the PNA. But DNA unwinding is not sufficient by itself to explain the effect of echinomycin since numerous mono- and bis-intercalating drugs tested here unwind the DNA decisively but have no effect on PNA binding to DNA. The antitumor agent bisnaphthalimide elinafide is a bis-intercalator which unwinds the DNA helix more than ethidium (though slightly less than echinomycin) and exhibits comparable sequence selectivity (*19*), but it does not promote PNA binding. There must be some other type of DNA structural feature that (perhaps together with the unwinding effect) allows the PNA easier access to its DNA target, though it is probably significant that both elinafide and ditercalinium are believed to bind in the major groove of the DNA duplex, as can PNA, whereas quinoxaline antibiotics bind in the minor groove where the nonintercalated portion of the ligand would be less likely to interfere sterically with the binding of PNA. We also consider that the positive effect of echinomycin on PNA binding cannot be attributed to the preferential binding of the antibiotic to CpG sequences in DNA (*26*, *27*). The main reason is that the synthetic quinoxaline peptide TANDEM also promotes PNA binding, but it exhibits a totally different preference for bis-intercalating around TpA steps (*23*, *28*).

We find that the activation energy of PNA binding is lowered in the presence of echinomycin. This is consistent with a mechanism whereby breathing of the double helix is facilitated, thereby making the Watson−Crick faces of the nucleobases statistically more accessible for PNA binding. It is also noteworthy that the quinoxaline antibiotics characterize themselves by forming drug−DNA complexes in which adenines are hyperreactive toward diethyl pyrocarbonate (*29*, *30*) and thymines are hyperreactive toward permanganate (*30*). Originally, the diethyl pyrocarbonate hyperreactivity was interpreted in terms of induced Hoogsteen base pairing at drug binding sites (*29*), but later studies concluded that increased base pair dynamic motion (breathing) furnishes a more likely explanation (*30*). Others have noted altered base pairing dynamics that follow binding of echinomycin to DNA (*31*). This peculiar property of the drug−DNA complexes may indeed explain the catalytic effect of quinoxalines on invasive binding of PNA to the double helix as it has previously been shown that helix-destabilizing events such as negative supercoiling (*6*), active transcription (*32*), or the presence of an adjacent occupied PNA target (*33*) will dramatically catalyze the binding.

In all events, this study suggests that the efficacy of DNA-targeted PNA antigene molecules could potentially be increased by augmentation with a suitable DNA-binding ligand. It therefore supplies a novel twist to the development of the PNA strategy.

ACKNOWLEDGMENT

We thank Mrs. Neel Louv Jansen, Miss Louise Vilsbøll, and Miss Julie Morgan for skilled technical assistance.

REFERENCES

1. Nielsen, P. E., Egholm, M., Berg, R. H., and Buchardt, O. (1991) *Science 254*, 1497−1500.
2. Nielsen, P. E., Egholm, M., and Buchardt, O. (1994) *Gene 149*, 139−145.
3. Cherny, D. Y., Belosterkovskii, B. P., Frank-Kametnetskii, M. D., Egholm, M., Burchardt, O., Berg, R. H., and Nielsen, P. E. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 1667−1670.
4. Møllegaard, N. E., Buchardt, O., Egholm, M., and Nielsen, P. E. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 3892−3895.
5. Nielsen, P. E., Egholm, M., Berg, R. H., and Buchardt, O. (1993) *Nucleic Acids Res. 21*, 197−200.
6. Bentin, T., and Nielsen, P. E. (1996) *Biochemistry 35*, 8863−8869.
7. Egholm, M., Buchardt, O., Christensen, L., Behrens, C., Freier, S. M., Driver, D. A., Berg, R. H., Kim, S. K., Nordèn, B., and Nielsen, P. E. (1993) *Nature 365*, 566−568.
8. Giesen, U., Kleider, W., Berding, C., Geiger, A., Orum, H., and Nielsen, P. E. (1998) *Nucleic Acids Res. 26*, 5004−5006.
9. Wittung, P., Kim, S. K., Buchardt, O., Nielsen, P., and Nordén, B. (1994) *Nucleic Acids Res. 22*, 5371−5377.
10. Wittung, P., Nielsen, P. E., and Nordén, B. (1996) *J. Am. Chem. Soc. 118*, 7049−7054.
11. Christensen, L., Fitzpatrick, R., Gildea, B., Petersen, K. H., Hansen, H. F., Koch, T., Egholm, M., Buchardt, O., Nielsen, P. E., Coull, J., and Berg, R. H. (1995) *J. Pept. Sci. 3*, 175−183.
12. Egholm, M., Christensen, L., Dueholm, K. L., Buchardt, O., Coull, J., and Nielsen, P. E. (1995) *Nucleic Acids Res. 23*, 217−222.
13. Peffer, N. J., Hanvey, J. C., Thomson, S. A., Hassman, C. F., Noble, S. A., and Babiss, L. E. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 10648−10652.
14. Bailly, C., and Waring, M. J. (1993) *Biochemistry 32*, 5985−5993.
15. Bailly, C., Denny, W. A., Mellor, L., Wakelin, L. P. G., and Waring, M. J. (1992) *Biochemistry 31*, 3514−3524.
16. Bailly, C., Cuthbert, A. W., Gentle, D., Knowles, M. R., and Waring, M. J. (1993) *Biochemistry 32*, 2514−2524.
17. Wakelin, L. P. G., Adams, A., Hunter, C., and Waring, M. J. (1981) *Biochemistry 20*, 5779−5787.
18. Delbarre, A., Delepierre, M., Garbay, C., Igolen, J., Le Pecq, J. B., and Roques, B. P. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 2155−2159.
19. Bailly, C., Braña, M., and Waring, M. J. (1996) *Eur. J. Biochem. 240*, 195−208
20. Waring, M. J. (1993) in *Molecular Aspects of Anticancer Drug−DNA Interactions* (Neidle, S., and Waring, M. J., Eds.) Vol. 1, pp 213−242, Macmillan, Basingstoke and London.
21. Lee, J. S., and Waring, M. J. (1978) *Biochem. J. 173*, 129−144.
22. Fox, K. R., Olsen, R. K., and Waring, M. J. (1978) *Biochim. Biophys. Acta 696*, 315−322.
23. Addess, K. J., and Feigon, J. (1994) *Biochemistry 33*, 12386−12396.
24. Lee, J. S., Latimer, L. J. P., and Hampel, K. J. (1993) *Biochemistry 32*, 5591−5597.
25. Demidov, V. V., Yavnilovich, M. V., Belotserkovskii, B. P., Frank-Kamenetskii, M. D., and Nielsen, P. E. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 2637−2641.
26. Low, C. M. L., Drew, H. R., and Waring, M. J. (1984) *Nucleic Acids Res. 12*, 4865−4879.
27. Van Dyke, M. M., and Dervan, P. B. (1984) *Science 225*, 1122−1127.
28. Low, C. M. L., Fox, K. R., Olsen, R. K., and Waring, M. J. (1986) *Nucleic Acids Res. 14*, 2015−2033.
29. Mendel, D., and Dervan, P. B. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 910−914.
30. Jeppesen, C., and Nielsen, P. E. (1988) *FEBS Lett. 231*, 172−176.
31. Leroy, J. L., Gao, X., Misra, V., Guéron, M., and Patel, D. J. (1992) *Biochemistry 31*, 1407−1415.
32. Larsen, H. J., and Nielsen P. E. (1996) *Nucleic Acids Res. 24*, 458−463.
33. Kurakin, A., Larsen, H. J., and Nielsen, P. E. (1998) *Chem. Biol. 5*, 81−89.

BI000254X