Sequence and Structural Selectivity of Nucleic Acid Binding Ligands†

Jinsong Ren and Jonathan B. Chaires*
Department of Biochemistry, University of Mississippi Medical Center, 2500 North State Street, Jackson, Mississippi 39216-4505

Received September 3, 1999; Revised Manuscript Received October 11, 1999

ABSTRACT: The sequence and structural selectivity of 15 different DNA binding agents was explored using a novel, thermodynamically rigorous, competition dialysis procedure. In the competition dialysis method, 13 different nucleic acid structures were dialyzed against a common ligand solution. More ligand accumulated in the dialysis tube containing the structural form with the highest ligand binding affinity. DNA structural forms included in the assay ranged from single-stranded forms, through a variety of duplex forms, to multistranded triplex and tetraplex forms. Left-handed Z-DNA, RNA, and a DNA−RNA hybrid were also represented. Standard intercalators (ethidium, daunorubicin, and actinomycin D) served as control compounds and were found to show structural binding preferences fully consistent with their previously published behavior. Standard groove binding agents (DAPI, distamycin, and netropsin) showed a strong preference for AT-rich duplex DNA forms, along with apparently strong binding to the poly(dA)−[poly-(dT)]₃ triplex. Thermal denaturation studies revealed the apparent triplex binding to be complex, and perhaps to result from displacement of the third strand. Putative triplex (BePI, coralyne, and berberine) and tetraplex [H₂TmPyP, 5,10,15,20-tetakis[4-(trimethylammonio)phenyl]-21H,23H-porphine, and N-methyl mesoporphyrin IX] selective agents showed in many cases less dramatic binding selectivity than anticipated from published reports that compared their binding to only a few structural forms. Coralyne was found to bind strongly to single-stranded poly(dA), a novel and previously unreported interaction. Finally, three compounds (berenil, chromomycin A, and pyrenemethylamine) whose structural preferences are largely unknown were examined. Pyrenemethylamine exhibited an unexpected and unprecedented preference for duplex poly(dAdT).

Intense interest exists in the design and synthesis of small molecules that might selectively bind to defined sites in DNA or RNA (1). Targeting particular sequences within right-handed, B-form DNA is one approach to producing the desired selectivity (2). Sequence selectivity might exploit the unique, sequence-dependent patterns of hydrogen bond donors and acceptors within the major and minor grooves of DNA. The design of ligands capable of sequence-specific DNA binding was recently realized by the Dervan group with the development of the hairpin polyamides (3−6). The recognition code for the hairpin polyamides was elucidated (4), and the effectiveness of these molecules as selective inhibitors of gene expression in vivo was demonstrated (7).

Another possible approach to selective nucleic acid binding is to target unique nucleic acid structures. In this strategy, unique molecular shapes would be targeted. DNA and RNA are both polymorphic, and exist in a variety of structural forms that might provide unique binding sites for small molecules (8). Although DNA exists predominantly in a right-handed duplex form in the genome, specific regions of the genome can exist in single-stranded form, or can adopt multistranded structures such as triplexes or tetraplexes. In addition, duplex DNA can adopt a variety of secondary structures depending on its sequence and solution environ-

† Supported by Grant CA35635 from the National Cancer Institute.
* Corresponding author. Telephone: (601) 984-1523. Fax: (601) 984-1501. E-mail: jchaires@biochem.umsmed.edu.

10.1021/bi992070s CCC: $18.00 © 1999 American Chemical Society
Published on Web 11/16/1999
ligand structural preference is described and utilized here, a competition dialysis method that provides a rigorous,
thermodynamically sound indication of structural selectivity.

Figure 1 shows a schematic diagram of the competition
dialysis experiment. The method evolved from a technique
first used by Muller and Crothers to explore the base
intercalation reactions (first used by Muller and Crothers to explore the base
dialysis experiment. The method evolved from a technique
modynamically sound indication of structural selectivity.

1 Abbreviations: BePl, 7H-8-methylenbenzo[c]pyrido[4,3-b]indole; H2-
TMPyP, meso-tetakis(N-methyl-4-pyridyl)porphine; NMM, N-methyl
mesoporphyrin IX; EDTA, ethylenediaminetetraacetic acid; DAPI, 4,6-
diamidino-2-phenylindole.

Materials and Methods

Nucleic Acids. Clostridium perfringens (lot 86H4010),
Micrococcus lysodeikticus (lot 108H4017), and calf thymus
DNA samples were purchased from Sigma Chemical Co. (St.
Louis, MO) and were sonicated, phenol extracted, and purified as previously described (13). Poly(dA) (lot 7067836021), poly(dT) (lot 8017834021),
poly(dA)−poly(dT) (lot 8097860021), poly(dAdT) (lot 8067870021), and poly(dGdC) (lot 8107910021) were purchased from Pharmacia Biotech, Inc. (Piscataway, NJ). Poly-
(rA) (lot 97F-4070) and poly(rA)−poly(U) (lot 10H4005) were purchased from Sigma Chemical Co. Synthetic poly-
nucleotides, base pairs, triplets, and tetrads.

Table 1: Nucleic Acid Conformation and Samples Used in
Competition Dialysis Experiments

<table>
<thead>
<tr>
<th>conformation</th>
<th>DNA or oligonucleotide</th>
<th>λ (nm)</th>
<th>ε</th>
<th>Tm (°C)</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>single-stranded</td>
<td>poly(dA)</td>
<td>257</td>
<td>8600</td>
<td>--</td>
<td>1.24</td>
</tr>
<tr>
<td>single-stranded</td>
<td>poly(dT)</td>
<td>264</td>
<td>8520</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>duplex DNA</td>
<td>C. perfringens (31% GC)</td>
<td>260</td>
<td>12476</td>
<td>82.5</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>calf thymus (42% GC)</td>
<td>260</td>
<td>12824</td>
<td>85.5</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td>M. lysodeikticus (72% GC)</td>
<td>260</td>
<td>13846</td>
<td>&gt;100</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>poly(dA)−poly(dT)</td>
<td>260</td>
<td>12000</td>
<td>75.2</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>[poly(dAdT)]2</td>
<td>262</td>
<td>13200</td>
<td>67.8</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>[poly(dGdC)]2</td>
<td>254</td>
<td>16800</td>
<td>&gt;100</td>
<td>--</td>
</tr>
<tr>
<td>DNA−RNA hybrid</td>
<td>poly(rA)−poly(dT)</td>
<td>260</td>
<td>12460</td>
<td>70.9</td>
<td>1.49</td>
</tr>
<tr>
<td>duplex RNA</td>
<td>poly(rA)−poly(U)</td>
<td>260</td>
<td>14280</td>
<td>62.5</td>
<td>1.54</td>
</tr>
<tr>
<td>Z-DNA</td>
<td>Br[poly(dGdC)]2</td>
<td>254</td>
<td>16060</td>
<td>&gt;100</td>
<td>--</td>
</tr>
<tr>
<td>triplex DNA</td>
<td>poly(dA)−[poly(dT)]2</td>
<td>260</td>
<td>17200</td>
<td>42.5</td>
<td>1.59</td>
</tr>
<tr>
<td>tetraplex DNA</td>
<td>(5′-T2G20T2)4</td>
<td>260</td>
<td>39267</td>
<td>89.0</td>
<td>1.06</td>
</tr>
</tbody>
</table>

poly(dA)−poly(dT) (lot 8097860021), poly(dAdT) (lot 8067870021), and poly(dGdC) (lot 8107910021) were purchased from Pharmacia Biotech, Inc. (Piscataway, NJ). Poly-
(rA) (lot 97F-4070) and poly(rA)−poly(U) (lot 10H4005) were purchased from Sigma Chemical Co. Synthetic poly-
nucleotides were used without further purification. Solutions containing the poly(rA)−poly(dT) DNA−RNA hybrid and the poly(dA)−[poly(dT)]2 triplex were prepared by mixing poly(rA) or poly(dA)−poly(dT) with poly dT in a 1:1 molar ratio, heating to 90 °C, and slowly cooling to room temperature. Tetraplex DNA [(5′-T2G20T2)4] was prepared by heating 5′-T2G20T2 (from Research Genetics, Huntsville, AL) to 90 °C for 2 min, slowly cooling to room temperature, and then equilibrating for 48 h at 4 °C before use. Left-
headed, Z-DNA was prepared by bromination of poly(dGdC) as previously described (14).

Ligand Molecules. Daunorubicin (lot 116H06752), ethidium
bromide (lot 75F0228), coralyne chloride (lot 106C0362), chromomycin (lot 106C0362), distamycin (lot 93F0641), actinomycin D (lot 96H4005), berberine (lot 68H1028), berenil (lot 122F00161), and BePl (lot 37H09494) were purchased from Sigma Chemical Co. and were used without further purification. 1-Pyrenemethylamine hydrochloride (lot 02724KS) and 5,10,15,20-tetrakis[4-(trimethylammonio)-
phenyl]-21H,23H-porphine (lot 06631MR) were purchased from Aldrich Chemical Co. (Milwaukee, WI). DAPI (lot 8A) was obtained from Molecular Probes, Inc. (Eugene, OR). H2TMPyP [meso-tetakis(N-methyl-4-pyridyl)porphine] (lot 071498) and NMM (N-methyl mesoporphyrin IX) (lot 080797) were purchased from Porphyrin Products, Inc. (Logan, UT). Netropsin was purchased from Serva Feinbio-
chemica (Heidelberg, Germany).
Concentration Determinations. Concentrations of nucleic acid samples were determined by UV absorbance measurements using the extinction coefficients and absorbance maxima listed in Table 1. Ligand concentrations were determined by visible absorbance measurements using extinction coefficients listed in the Supporting Information.

Quality Control of Nucleic Acid Samples. The quality of each nucleic acid sample was evaluated by recording their UV absorbance spectrum, their CD spectrum, and their thermal denaturation profile. Data from these measurements are shown in the Supporting Information for each nucleic acid structure included in the assay.

Competition Dialysis Assay. A buffer consisting of 6 mM Na₂HPO₄, 2 mM NaH₂PO₄, 1 mM Na₂EDTA, and 185 mM NaCl (pH 7.0) was used for all experiments. For each competition dialysis assay, 200 mL of the dialysate solution containing 1 μM ligand was placed into a beaker. A volume of 0.5 mL (at 75 μM monomeric unit) of each of the DNA samples listed in Table 1 was pipeted into a separate 0.5 mL Spectro/Por DispoDialyzer unit (Spectrum, Laguna Hills, CA). All 13 dialysis units were then placed in the beaker containing the dialysate solution. The beaker was covered with Parafilm and wrapped in foil, and its contents were allowed to equilibrate with continuous stirring for 24 h at room temperature (20–22 °C). At the end of the equilibration period, DNA samples were carefully removed to microfuge tubes, and were taken to a final concentration of 1% (w/v) sodium dodecyl sulfate (SDS) by the addition of appropriate volumes of a 10% (w/v) stock solution. The total concentration of drug (C_t) within each dialysis unit was then determined spectrophotometrically using wavelengths and extinction coefficients appropriate for each ligand (see Table S1 in the Supporting Information). An appropriate correction for the slight dilution of the sample resulting from the addition of the stock SDS solution was made. The free ligand concentration (C_f) was determined spectrophotometrically using an aliquot of the dialysate solution, although its concentration usually did not vary appreciably from the initial concentration of 1 μM. The amount of bound drug was determined by difference (C_b = C_t - C_f). Data were plotted as a bar graph using Origin software (version 5.1, Microcal, Inc., Northampton, MA).

UV Melting Studies. A buffer consisting of 6 mM Na₂HPO₄, 2 mM NaH₂PO₄, 1 mM Na₂EDTA, and 185 mM NaCl (pH 7.0) was used for all melting experiments. Ultraviolet DNA melting curves were determined using a Cary 3E UV/visible spectrophotometer (Varian, Inc., Palo Alto, CA), equipped with a thermostatic temperature controller. Solutions of DNA (final concentration of 2.0 × 10⁻⁵ M) were prepared by direct mixing with aliquots from a ligand stock solution, followed by incubation for 12 h at 24 °C to ensure equilibration. Samples were heated at a rate of 1 °C/min, while the absorbance was being continuously monitored at 260 nm. Primary data were transferred to the graphics program Origin (Microcal, Inc.) for plotting and analysis.

Fluorescence Titration Experiments. Fluorescence titrations were conducted and analyzed as previously described (15).

RESULTS AND DISCUSSION

We have utilized the competition dialysis assay to evaluate the structural selectivity of 15 ligands representing a variety of chemical classes. The results demonstrate the validity, utility, and value of the method as a rapid screening tool for structural selectivity. More importantly, the range and variety of small molecule structural selectivity will be clearly illustrated. Results obtained with common intercalators and groove binding agents with known binding preferences will first be described. Studies using compounds reported to be triplex selective will then be presented, followed by studies using several porphyrin compounds reported to be selective for tetraplex DNA. Finally, results from several compounds with previously unknown structural selectivities will be described.

Figure 2 shows results obtained for the common intercalating agents ethidium, daunorubicin, and actinomycin D.
Ethidium (Figure 2, left) is the prototypical intercalator, whose interactions with nucleic acids have been extensively investigated. Competition dialysis results obtained with ethidium will be described in detail, to introduce the method, the presentation of data, and data interpretation.

Competition dialysis results for ethidium are shown (Figure 2, left) as a bar graph in which the amount of ethidium bound is shown for each DNA structure included in the assay. These results were obtained after equilibration for 24 h, using 1 µM ethidium in the dialysate solution and a nucleic acid concentration of 75 µM in each sample dialysis tube. Nucleic acid concentrations are expressed in terms of the monomeric unit that comprises the polymer. This means nucleotide (nt) concentration for single-stranded forms, base pair (bp) concentrations for duplex forms, triplet concentrations for the triplex, and tetrad concentrations for the tetraplex. The data (Figure 2, left) show that there was no appreciable binding of ethidium to single-stranded forms [i.e., poly(dT) or poly(dA)]. Ethidium binds well to most duplex DNA forms, from both natural origin and synthetic deoxy-polyribonucleotide duplexes. The exception is poly(dA)−poly(dT), to which ethidium binds poorly as do most intercalators, an effect that is well-known and which is due to the unusual structural selectivity of each compound was unique and provided a thermodynamically sound screening procedure as intended.

Figure 2 shows results obtained for three common groove-binding agents, distamycin, netropsin, and DAPI. The selectivity patterns for these three compounds are similar, but not identical. The selectivity patterns found for these groove-binding agents are clearly distinct from those found for the intercalators (Figure 2). In general, all of these agents prefer right-handed duplex DNA forms, with a strong preference for AT base pairs, and virtually no binding to GC base pairs, as judged by its increased level of binding to M. lysodeikticus (71% GC) DNA relative to that to C. perfringens (31% GC) DNA. This result is fully consistent with its known sequence selectivity (12, 20, 21).

In contrast to ethidium, daunorubicin binds poorly, if at all, to RNA and to the DNA–RNA hybrid. Daunorubicin interacts to some extent with triplex and tetraplex forms. Its apparent Z-DNA binding will be discussed below.

Actinomycin D (Figure 2, right) exhibits a selectivity pattern distinct from those of both ethidium and daunorubicin. It prefers binding to right-handed duplex forms, but shows a strong preference for GC base pairs, with no binding at all to duplex deoxyribonucleotides containing only AT base pairs. Actinomycin D shows some binding to poly(dA) and to tetraplex DNA. Actinomycin D shows appreciable binding to Z-DNA, but further investigation showed that this behavior is somewhat illusory. Ethidium, daunorubicin, and actinomycin D all exhibited some apparent binding to Z-DNA. Circular dichroism studies (not shown) revealed, however, that these compounds were in fact allosterically converting the left-handed form of the polymer to a right-handed form, with stable complexes formed only with the latter. Such behavior was fully investigated previously by this laboratory for daunorubicin (22) and by the Krugh laboratory for ethidium and actinomycin D (23, 24).

The striking general features of the data presented in Figure 2 deserve emphasis at this point. The competition dialysis method allowed 13 structures and three different compounds to be examined rapidly, within a 24 h period. The pattern of structural selectivity of each compound was unique and characteristic. The method accurately reproduced the known binding preferences for these intercalators, and provided a therapeutically meaningful sort screening procedure as intended.

Figure 3 shows results obtained for three common groove-binding agents, distamycin, netropsin, and DAPI. The selectivity patterns for these three compounds are similar, but not identical. The selectivity patterns found for these groove-binding agents are clearly distinct from those found for the intercalators (Figure 2). In general, all of these agents prefer right-handed duplex DNA forms, with a strong preference for AT base pairs, and virtually no binding to poly(dGdC). There is apparent binding to the triplex poly(dA)−[poly(dT)]₂, but this will be examined in more detail in the next paragraph. None of these compounds appears to bind to the RNA sample, but DAPI shows slight binding to the DNA–RNA hybrid. Slight binding of netropsin and DAPI to the tetraplex is observed.

The interaction of the groove-binding agents with poly(dA)−[poly(dT)]₂, triplex DNA was examined more fully by melting experiments, with the results shown in Figure 4. In the absence of ligand, melting of poly(dA)−[poly(dT)]₂ is biphasic, with the third strand dissociating at a T_m of 42.5 °C and the remaining duplex melting at a T_m of 75.2 °C. The melting curves shown in panels A and B of Figure 4 show that the addition of distamycin and netropsin results in a destabilization and eventual displacement of the third strand. These compounds bind to and stabilize the free duplex. DAPI (Figure 4C) apparently does not displace the third strand, but stabilizes only the duplex form. We conclude from these ancillary studies that the apparent binding of these agents to poly(dA)−[poly(dT)]₂, triplex DNA seen in Figure 3 may be illusory, and may result from displacement of the
third strand and binding to the duplex form. Literature reports are generally consistent with this conclusion (25–28).

Figure 5 shows results obtained for agents reported to be selective for triplexes. BePI and coralyne were both reported to be triplex selective binding agents (29–31). Competition dialysis reveals that BePI does indeed show a preference for poly(dA)–[poly(dT)]₂ triplex DNA, but that it also interacts with almost all other structures in the assay except for single-stranded forms (Figure 5, left). Coralyne shows a more dramatic preference for poly(dA)–[poly(dT)]₂ triplex DNA relative to BePI under our solution conditions (Figure 5, middle). Surprisingly, coralyne also shows strong binding to single-stranded poly(dA), a characteristic unique among all of the compounds studied by the competition dialysis assay thus far. In contrast to BePI, coralyne shows more variety in its binding to duplex forms, and appears to interact more strongly with RNA and the DNA–RNA hybrid.

The binding of coralyne to poly(dA) was examined by absorbance titration experiments (not shown). These studies showed that the binding constant for the association of coralyne with poly(dA) was \( (1.05 \pm 0.1) \times 10^5 \text{ M}^{-1} \). For comparison, coralyne binding to calf thymus DNA was determined to be 1 order of magnitude weaker, with a \( K \) of \( (1.25 \pm 0.1) \times 10^4 \text{ M}^{-1} \). These independent binding studies verified the most surprising result to emerge from the competition dialysis experiment with coralyne.

Figure 5 (right) shows results obtained with berberine, a compound with heretofore poorly characterized structural selectivity. Berberine was chosen for study by inspection of its structure, which appeared to be similar in shape to BePI and coralyne. Berberine clearly prefers poly(dA)–[poly(dT)]₂ triplex DNA, and seems to bind to that structure as well as BePI and coralyne. In contrast to those compounds, the level of berberine binding to all other structural forms is greatly
reduced or absent. Competition dialysis reveals that berberine preferentially binds to poly(dA)-poly(dT)\textsubscript{2} triplex DNA with apparently greater selectivity than BePI or coralyne. Figure 6 shows the results of triplex DNA melting experiments using BePI, coralyne, and berberine. In contrast to the results shown in Figure 4, these agents all selectively stabilize the triplex form, and elevate the \( T_m \) for the melting of the third strand. The behavior shown in panels A and B of Figure 6 is consistent with published reports about the effects of BePI and coralyne on triplex melting (29, 30). The results in Figure 6C show that berberine, in contrast to BePI and coralyne, appears not to stabilize duplex DNA at all under these reaction conditions, but selectively stabilizes poly(dA)-poly(dT)\textsubscript{2} triplex DNA. That result is consistent with the pronounced selectivity shown in the competition dialysis study (Figure 5, right). We note that Lee et al. (29) concluded that berberine bound more weakly to triplex DNA than did coralyne, on the basis of the results of melting experiments carried out under different ionic conditions. Our results indicate that such is not the case, and we ascribe the different conclusions in part to the difficulty of properly interpreting multiphasic melting curves. Such difficulties in fact motivated us to design the more direct and less ambiguous competition dialysis system that more clearly shows structural preferences.

Figure 7 shows the results obtained for three porphyrin compounds. The large planar surface of porphyrins might result in favorable interactions with tetraplex DNA, and for this reason, the interaction of these compounds with tetraplex DNA has been studied by a variety of experimental...
approaches (32–35). H$_2$TMPyP (Figure 7, left) and 5,10,15,20-tetrakis[4-(trimethylammonio)phenyl]-21H,23H-porphine (Figure 7, middle) bind to all DNA structures except poly(dA). These two compounds are the first that we encountered in our assay that bind to an appreciable extent to poly(dT). While both of these compounds indeed bind to multistranded triplex and tetraplex structures, the preference over the other structures in the assay is only marginal at best.

In contrast, NMM (Figure 7, right) appears to bind only to tetraplex DNA. Note that its level of absolute binding is low, but that it does not bind to any form other than tetraplex in amounts that can be detected by our assay. Binding affinity in this case was apparently sacrificed for selectivity. Bolton’s laboratory found by fluorescence spectroscopy that NMM bound to tetraplex DNA but not to duplex forms (32). Our assay confirms their observation, and shows in addition that other nucleic acid conformations apparently do not bind NMM either.

Figure 8 shows results obtained for a miscellaneous but interesting series of compounds. Berenil (Figure 8, left) is a known groove binding agent (36). Its behavior is similar to that shown by the compounds in Figure 4, except that its absolute binding is somewhat weaker. In contrast to the groove binders shown in Figure 3, berenil is reported to
stabilize triplex DNA (37–39). The apparent binding to poly-
(dA)–[poly(dT)] 2 triplex evident in the left panel of Figure
8 is consistent with that report.

Chromomycin (Figure 8, middle) is thought to bind in the
minor groove as a dimer to runs of GC base pairs (40–42).
Competition dialysis shows that it indeed has a strong
preference for GC rich duplex DNA forms. Chromomycin
does not bind at all to duplexes containing all AT base pairs,
to RNA, to the DNA–RNA hybrid, to single-stranded forms,
or to tetraplex DNA.

Finally, results for pyrene methylamine are shown (Figure
8, right). This compound was selected for study because we
thought that its planar aromatic ring structure might render
it selective for either triplex or tetraplex forms. Instead, we
found a unique and unprecedented selectivity for duplex
poly(dAdT). The origin of this strong preference is by no
means clear, but shows that the competition dialysis method
can reveal unexpected examples of selectivity on which to
base more detailed explorations. The preference of pyrene
methylamine for poly(dAdT) was verified by independent
fluorescence titration experiments (data not shown). Binding
constants of (6.4 ± 0.4) × 105, (1.3 ± 0.1) × 105, and (0.7
± 0.1) × 105 M−1 were determined for pyrene methylamine
binding to poly(dAdT), to calf thymus DNA, and to poly-
(dGdC), respectively.

In summary, the competition dialysis method allowed the
structural selectivity of these 15 compounds to be examined
quickly and efficiently. The methods provided results that
were fully consistent with the known structural preferences
of test intercalators and groove binding agents. Each
compound that was studied appeared to have a unique,
characteristic pattern of structural selectivity, with distinct
differences evident among compounds. Novel, previously
unknown structural preferences were revealed by the assay,
most notably the strong triplex selectivity shown by ber-
berine, poly(dA) binding by coraline, and a striking prefer-
ence for duplex poly(dAdT) shown by pyrene methylamine.

The competition dialysis method clearly provides a reli-
able, thermodynamically sound assay for the rapid screening
of structurally selective compounds. While at present we have
included only 13 structures in the assay, there is no reason
a greater variety of structures could not be included. The
only limitations are that all included structures must be stable
under the solution conditions of the assay, and must be of
an appropriate size to be retained by the dialysis tubing
selected for use. While the current version of the assay is
relatively rapid, its efficiency could be further improved by
adapting it to a microplate format to reduce the manual
manipulations of the samples and to automate the spectro-
photometric quantitation of bound ligand.

While the competition dialysis method offers a powerful
new approach for the study of ligand structural selectivity,
we freely acknowledge several possible limitations of the
assay. First, the current assay uses relatively high salt (0.185
M NaCl) which tends to decrease the binding affinity of
charged ligands by decreasing the polyelectrolyte contribu-
tion to the binding free energy. Second, the ionic conditions
for the assay were selected to be appropriate to maintain the
stability of the particular nucleic acid structures chosen
for study. These ionic conditions may not be optimal for all
types of structures of interest, certain triplex forms, for example.
Different ionic conditions could most certainly be used for

the assay, but the structure and stability of all nucleic acids
forms used would need to be reestablished under the new
conditions. Third, different sizes of nucleic acids are used
in the assay (of necessity), which might result in a dispro-
portionately higher concentration of ends in some samples,
like the tetraplex form. One must be aware of a potential
bias that could result if a ligand preferentially bound to end
residues for some reason. Finally, one must be aware of what
might be considered “false positive” results. For example,
the assay registered apparently significant intercalator binding
to “Z-DNA”, but further studies revealed the allosteric
conversion of the polymer to the preferred right-handed form.
In addition, groove binders were observed to bind to “triplex"
DNA, but further melting studies revealed complex under-
lying interactions. The competition dialysis method was
designed to provide a rapid screening procedure that would
reveal interesting ligand binding behavior and which would
guide more detailed physical or biological studies. The
apparent false positives just described and the followup
studies that were carried out to investigate the behavior
illustrate how the competition dialysis method was in fact
intended to function, and emphasize the need to exercise
appropriate caution in the interpretation of initial results.

SUPPLEMENT INFORMATION AVAILABLE

Graphs of the CD and UV spectra and the thermal
denaturation curve of each nucleic acid structure used in the
competition dialysis assay and a table of extinction coeffi-
cients for all of the ligands used in this study. This material
is available free of charge via the Internet at http://
pubs.acs.org.

REFERENCES

4. White, S., Szewczyk, J. W., Turner, J. M., Baird, E. E., and
4, 569–78.
382, 559–61.
Press, San Diego, CA.
54, 267–77.
Greenwich, CT.
Biochemistry 21, 3933–40.
14. Moller, A., Nordheim, A., Kozlowski, S. A., Patel, D. J., and
(Johnson, M. L., and Brand, L., Eds.) Academic Press, San
Diego, CA.
3547–53.
Selectivity of Nucleic Acid Binding Ligands