

## ROLE OF MICROENVIRONMENT IN RECOGNITION OF DNA BY NONINTERCALATING LIGANDS

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### ABSTRACT

We have studied here the influence of cationic environment on the binding of antitumour antibiotics (netropsin and distamycin analogs) to pentanucleotide duplexes  $d(A)_5.d(T)_5$  and  $d(C)_5.d(G)_5$ . The geometries of the complexes of these ligands with oligonucleotides are optimized on the basis of minimization of total conformational plus interaction energy calculated using empirical potential energy functions. We have considered only the atmospheric effect of counterion distribution by incorporating a Debye Hückel screening coefficient for electrostatic terms. Computations are reported for mono- as well as divalent ions for concentrations 0.001 M, 0.01 M, 0.1 M and 1 M. Dielectric permeability value  $\epsilon = 4$  is used for all the calculations. DNA molecule is assumed to be in B-form. Our results show reduction in the binding energy as well as specificity with the increase in salt concentration. Difference in the interaction energy of different ligands also reduces at high salt concentrations.

### INTRODUCTION

**B**INDING of several antitumour antibiotics to natural and synthetic DNAs, RNAs, DNA-RNA hybrids has been studied in the past under various ionic and solvent conditions<sup>1-11</sup> to elucidate the role of environmental factors in DNA recognition. These studies, no doubt, showed the complex nature of the interaction and brought forth the fact that it depends upon: DNA base-sequence, DNA conformation, binding site on DNA, ligand conformation and nature of the functional groups on the ligands. However, detailed quantitative information regarding contributions due to individual functional groups and their variation due to environmental changes is not yet available in the literature.

Computer-based geometry optimization is a technique that has become exceedingly popular in the recent past<sup>12-18</sup> as it can simultaneously yield information regarding the geometry of the complexes as well as energetics. It is our desire to use this technique to study the role of microenvironment in recognition of DNA by the nonintercalating ligands.

The effect of environment on ligand binding to DNA can in principle be studied using various quantum chemical (*ab initio*, CNDO, EHT etc) techniques, using supramolecular framework and considering the discrete nature of the solvent molecules<sup>19-21</sup>. It can also be studied by a continuum type approach using Guntelberg Müller or Debye Hückel method<sup>22,23</sup> and atom-atom type potential. Recently, a combination of discrete and continuum approach was proposed by Claverie *et al*<sup>22</sup>. There

are some reports using statistical mechanical methods such as Monte Carlo<sup>24,25</sup> and a cluster expansion technique<sup>26</sup>, as also molecular mechanical treatment<sup>27</sup>. However, all these techniques suffer from the basic drawback of large computational time. Despite these limitations Climenti's group did large scale computations<sup>28</sup> using Monte Carlo technique, whereas Kollman *et al*<sup>29</sup> introduced a potential with distance dependent dielectric constant to optimize conformation of hexanucleotide fragment by molecular mechanics technique.

Incorporation of Debye Hückel screening factor for electrostatic potential is an old technique. It is computationally quite simple. Detailed electronic structure of the molecules can also be used with success to study counterion condensation on polymeric DNA<sup>30</sup>. We have used here this method to study binding of nonintercalating antitumour antibiotics, netropsin, distamycin-2 and an analog of distamycin with formamide group replaced by nitro and propyl amidinium tail replaced by  $-CH_2-CH_2-C\equiv N$  (distamycin A.An). Binding of these ligands is reported here with two pentameric sequences of DNA:  $d(A)_5.d(T)_5$  and  $d(C)_5.d(G)_5$ .

### METHOD

Computation of conformation as well as interaction energies is done on the basis of empirical potential energy function consisting of Lennard Jones' attractive repulsive or 6-12 ( $E_{\text{non}}$ ), electrostatic (monopole-monopole and dipole-induced dipole-  $E_{\text{ele}}$ ) and hydrogen bonding ( $E_{\text{hydro}}$ ) contributions. These are calculated using parameters described earlier<sup>31,32</sup>.

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The atmospheric effect of counterion distribution is calculated by incorporating the Debye Hückel screening coefficient  $\exp(-Kr)/\epsilon$  for the electrostatic term.

$$K^2 = 8\pi e^2 L M^* / 1000 D k T$$

Here  $e$  and  $L$  are electronic charge and Avagadro numbers respectively,  $M^*$  the effective molarity of the cation,  $D$  the dielectric constant of water,  $k$  the Boltzman constant,  $T$  the temperature, and  $\epsilon$  the dielectric permeability taken as 4.

The details of our modelling procedure were described earlier<sup>17,18</sup>. These three drugs were modelled at four different concentrations 0.001 M, 0.01 M, 0.1 M, 1 M of mono as well as divalent ions. Once the final models are obtained, the total interaction energy of the ligand with the DNA molecule is expressed as the sum of contributions between various functional groups (specific example given in table 1). Components  $E_{\text{non}}$ ,  $E_{\text{ele}}$ ,  $E_{\text{hyd}}$  are analysed. Some typical curves are depicted in figures 1-5.

## RESULTS AND DISCUSSION

### Relative affinity of different ligands with DNA :

The binding energy (EB) of the ligands with DNA gives a direct estimate of the relative affinity of the

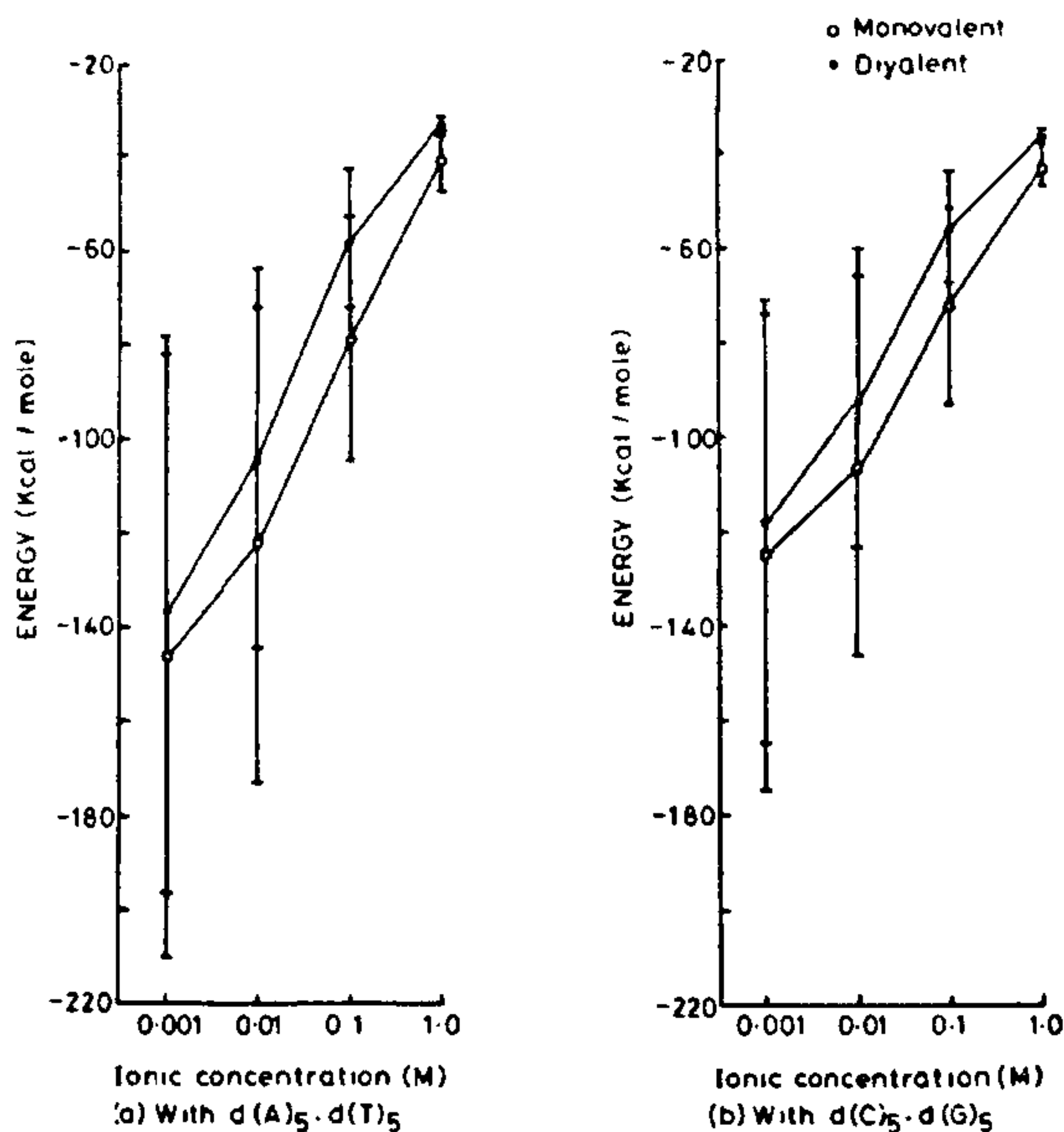
ligand with DNA. In ion-free medium EB was equal to -253.36, -167.58 and -73.07 kcal/mol for interaction of netropsin, distamycin-2 and distamycin-A.An respectively with  $d(A)_5.d(T)_5$ . In the presence of ionic environment EB showed a rise. For monovalent ion at 0.001 M concentration, the rise in EB was equal to 23.17, 13.11 and 3.07 kcal/mol for these three drugs respectively. Total EB followed the order: netropsin < distamycin-2 < distamycin A.An. It agreed with the experimental binding constant<sup>1-3</sup>. With the increase in the concentration of both mono-as well as divalent ions there was rise in EB. At 1 M concentration for monovalent ions the binding energies with  $d(A)_5.d(T)_5$  were -49.07, -55.17 and -46.61 kcal/mol. The same for divalent ion -36.51, -47.808 and -45.66 kcal/mol and much higher. There was change in the relative binding order at high ion concentration (1 M) and distamycin analogs had stronger binding to  $d(A)_5.d(T)_5$  compared to netropsin. This result explains why at 7 M urea netropsin DNA complex can be dissociated but not distamycin-DNA<sup>8-11</sup>.

The present calculations also showed that the differences in the binding energies for different ligands reduce considerably at high ion concentration. Thus, for example, at 0.001 M, EB for three drugs were -230.19, -154.47 and -70.0 kcal/mol. These values came closer at 1 M ion concentration.

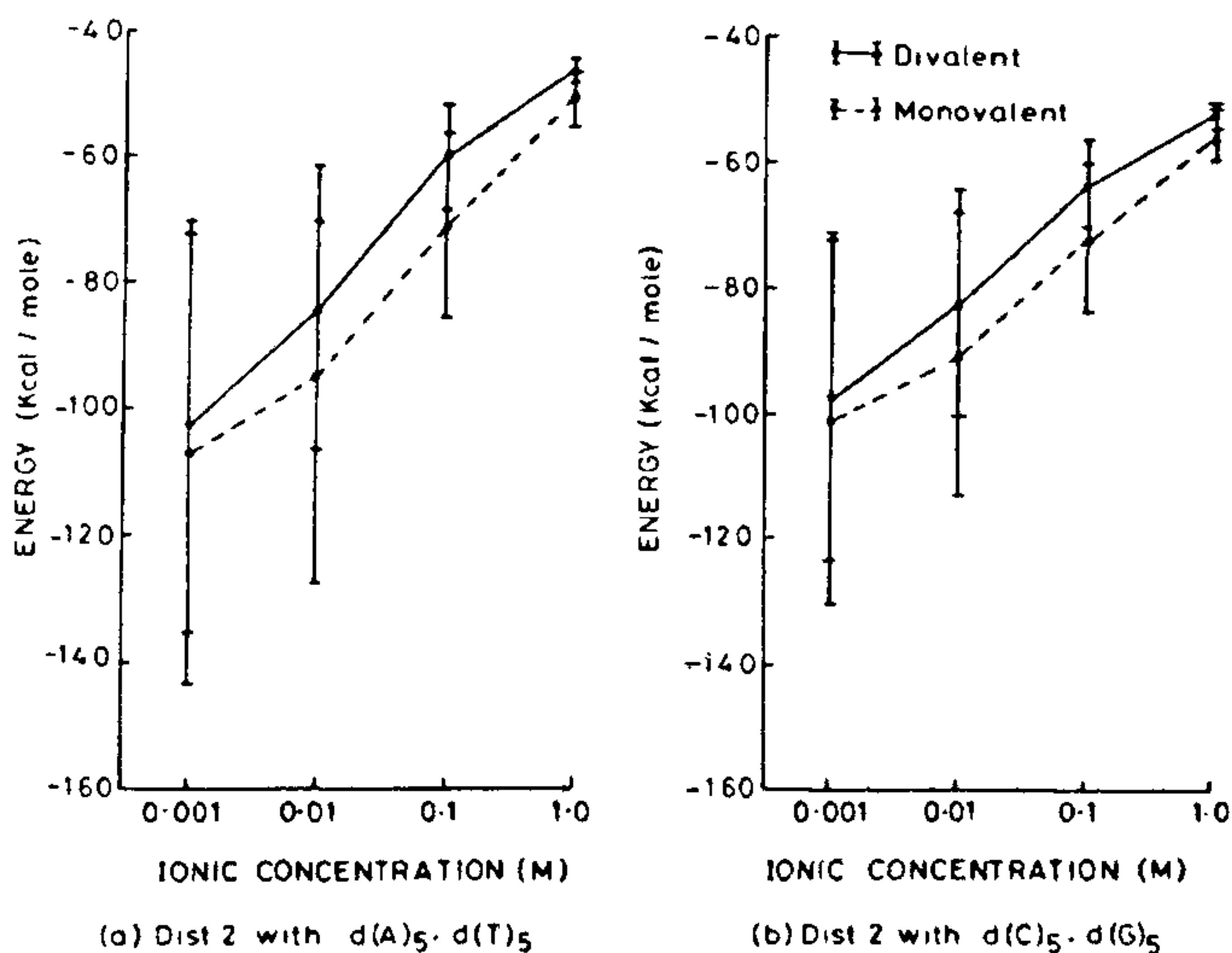
**Table 1** Contribution of electrostatic (monopole-monopole + dipole-induced dipole) interaction in total interaction energy of distamycin-2 with  $d(A)_5.d(T)_5$  and  $d(C)_5.d(G)_5$ . Interaction energy is calculated at dielectric permeability value  $\epsilon = 4.0$ . Salt concentrations are noted at the top of each column

Base sequence	$d(A)_5.d(T)_5$					$d(C)_5.d(G)_5$				
Ion concentration (M)	0.0	0.001	0.01	0.1	1.0	0.0	0.001	0.01	0.1	1.0
<b>Energies</b>										
Bases-amides	17.973	16.659	14.195	8.840	2.510	5.776	5.374	4.615	2.934	0.811
Bases-rings	-24.614	-22.466	-18.537	-10.549	-2.485	-6.327	-5.747	-4.687	-2.539	-0.404
Bases-tails	-22.658	-20.146	-15.698	-7.436	-1.025	-5.802	-5.127	-3.953	-1.871	-0.372
Back-amides	30.582	27.437	21.885	11.490	0.862	30.537	27.392	21.845	11.466	2.492
Back-rings	-51.430	-45.614	-35.494	-17.228	-2.980	-51.411	-45.596	-35.481	-17.232	-2.978
Back-tails	-68.888	-62.033	-50.170	-28.675	-9.127	-68.083	-61.234	-49.418	-28.142	-8.936
$E_{\text{ele}}$	-119.035	-106.163	-83.819	-43.558	-12.245	-95.310	-84.938	-67.079	-35.384	-9.387
$E_{\text{hyd}}$	-3.605	-3.452	-3.147	-2.365	-1.049	-2.130	-2.042	-1.864	-1.407	-0.621
$E_{\text{non}}$	-44.935	-44.935	-44.705	-44.28	-41.883	-51.852	-51.772	-51.809	-51.166	-50.441
Total	-167.576	-154.473	-131.667	-90.203	-55.177	-149.292	-138.752	-120.752	-87.957	-60.449

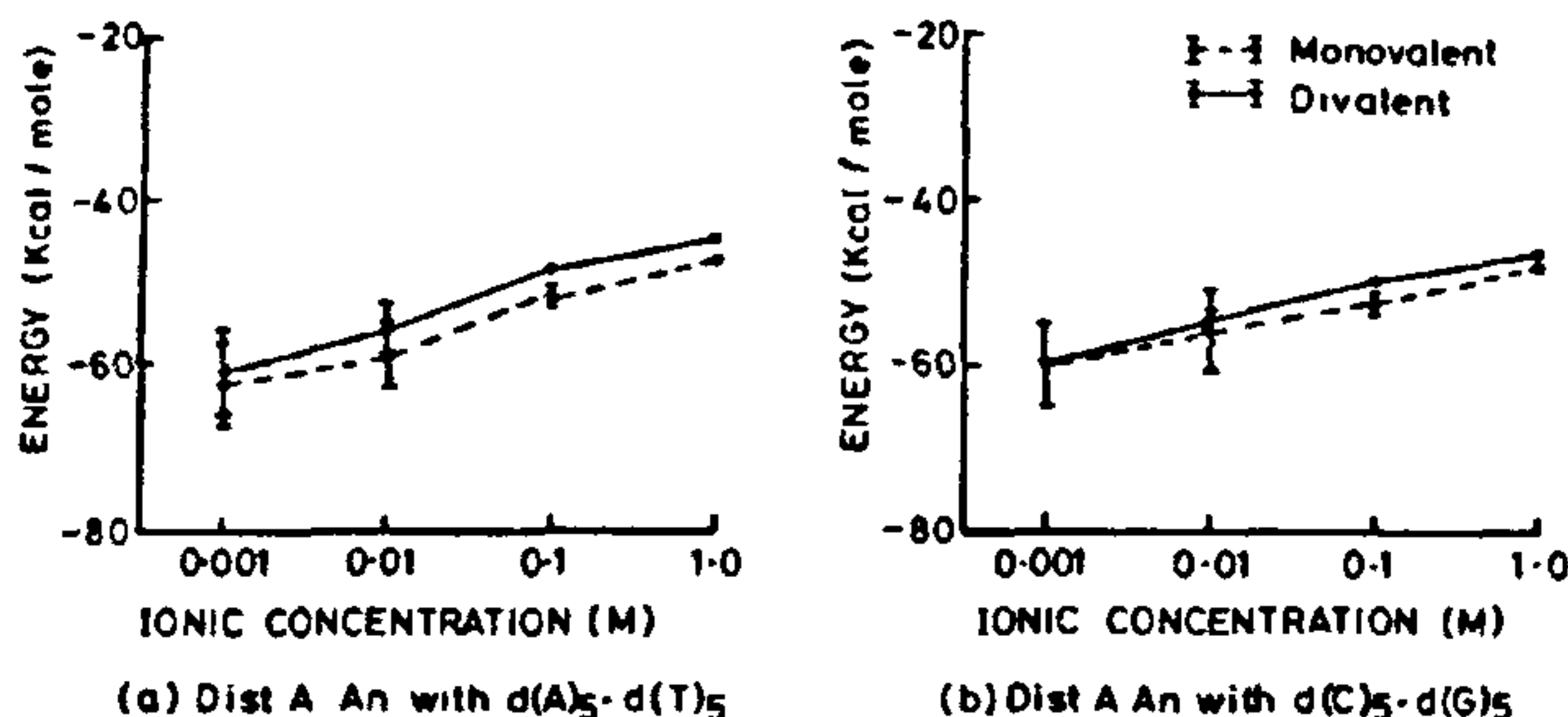
Note: All energies are in kcal/mol. Bases-amides, bases-rings, bases-tails, back-amides, back-rings and back-tails denote component of interaction energies of DNA bases and backbone with amides, rings and tail portions of the drug. Total binding energies are obtained by adding hydrogen bonding ( $E_{\text{hyd}}$ ) and Lennard-Jones attractive repulsive ( $E_{\text{non}}$ ) component to total electrostatic energy ( $E_{\text{ele}}$ ).



**Figure 1.** Effect of counterion environment on the total interaction energy of netropsin with (a)  $d(A)_5 \cdot d(T)_5$ ; (b)  $d(C)_5 \cdot d(G)_5$ . Abscissa denote concentration of monovalent or divalent ions. Ordinate gives the interaction energy in kcal/mol.



**Figure 2.** Effect of counterion environment on the total interaction energy of distamycin-2 with (a)  $d(A)_5 \cdot d(T)_5$  and (b)  $d(C)_5 \cdot d(G)_5$ . Ordinate and abscissa carry the same meaning as in figure 1.



**Figure 3.** Effect of counterion environment on the total interaction energy of distamycin-A.An with (a)  $d(A)_5 \cdot d(T)_5$ ; (b)  $d(C)_5 \cdot d(G)_5$ . Ordinate and abscissa carry the same meaning as in figure 1.

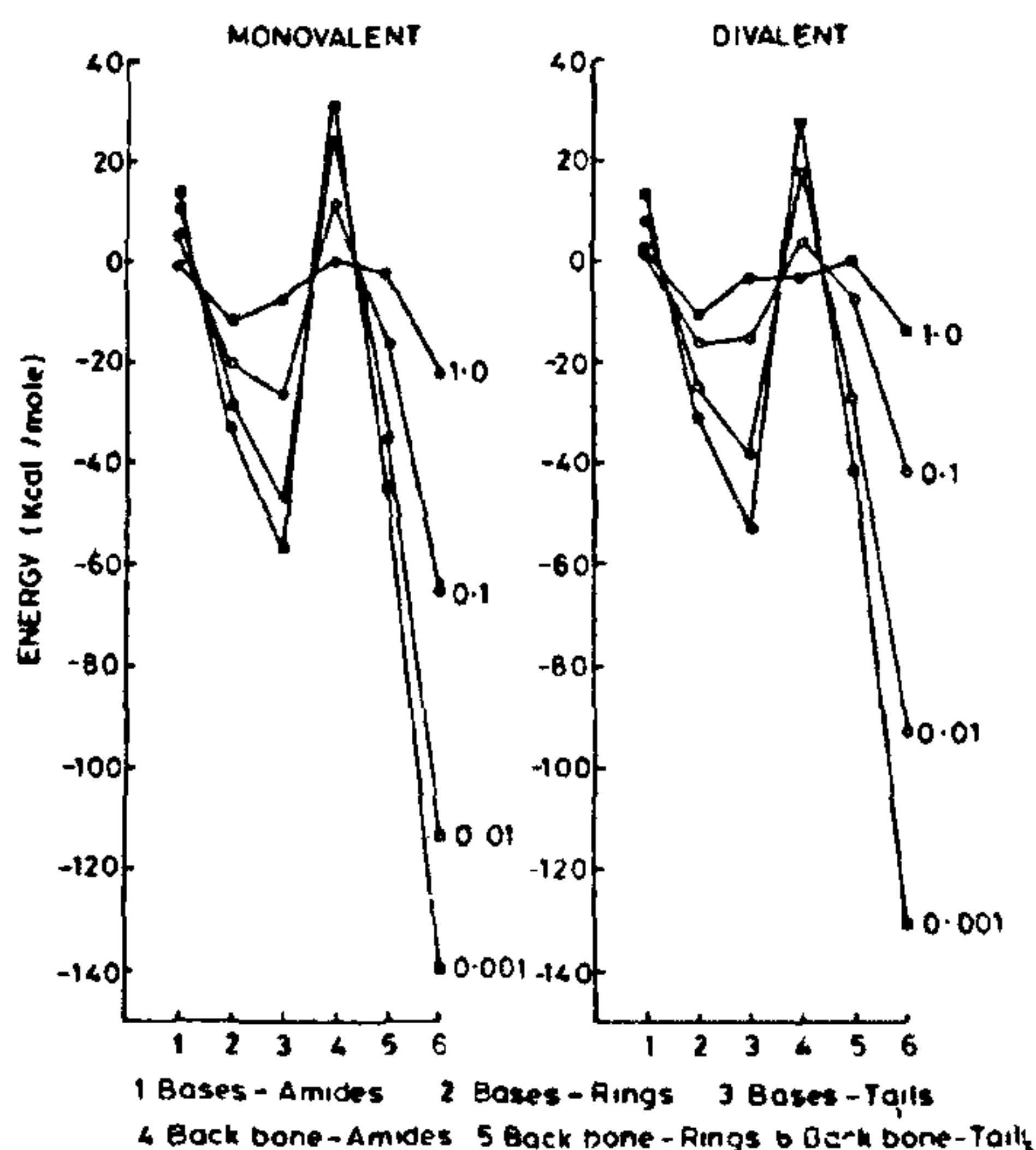
Thus there was a loss of ligand specificity at high ionic concentrations (figures 1-3).

#### Sequence specificity

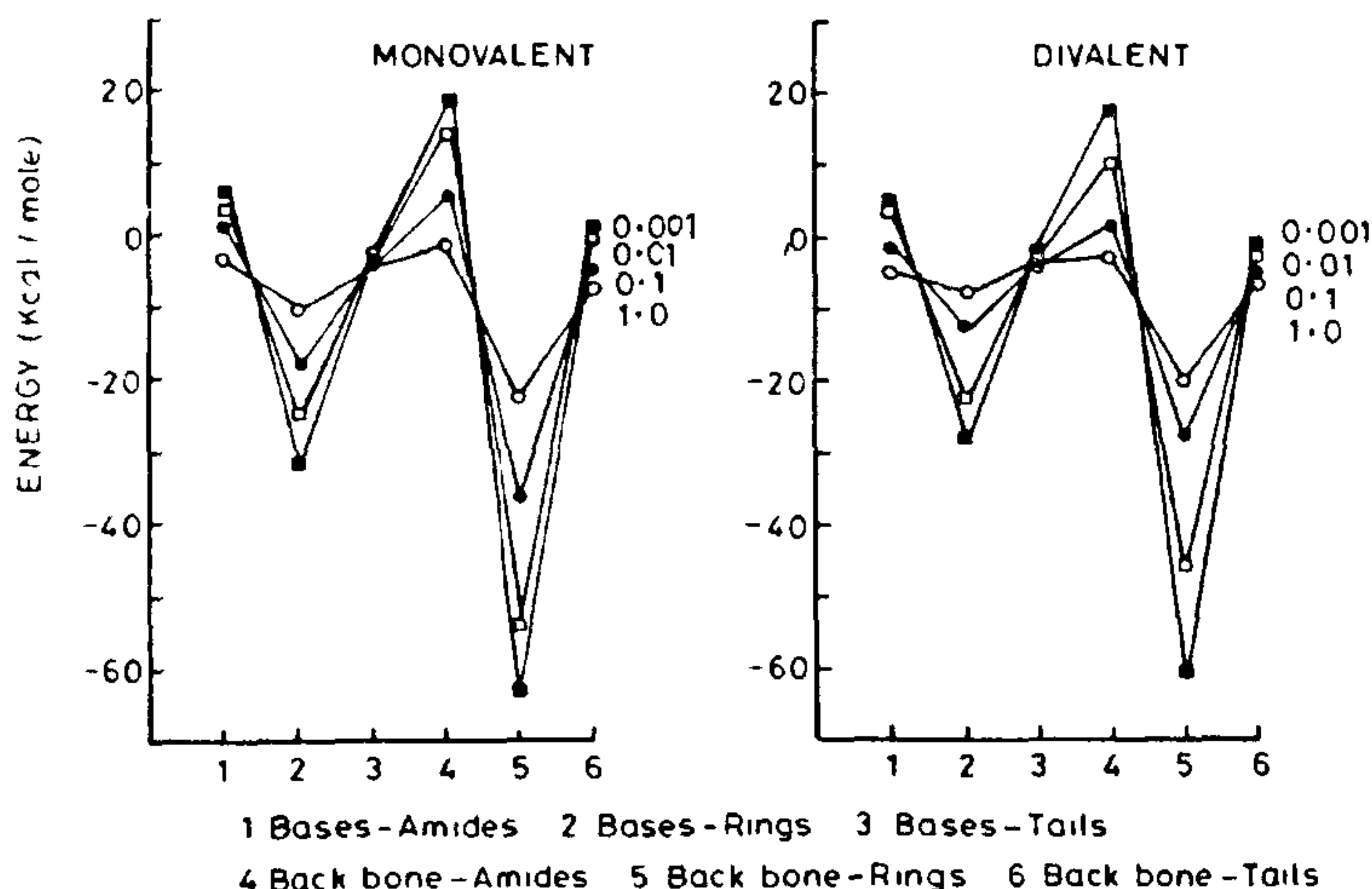
Interaction energy of the ligand with particular nucleotide sequence depends on both electrostatic ( $E_{ele}$ ) and non-electrostatic ( $E_{non} + E_{hydg}$ ) contributions. At 0.001 M monovalent ion concentration, the electrostatic participation is much larger compared to ( $E_{non} + E_{hydg}$ ) for netropsin and distamycin-2. ( $E_{ele}/E_{non} + E_{hydg} = 6.47, 2.34$ ), whereas for distamycin-A.An  $E_{ele}/E_{non} + E_{hydg} = 0.38$ . Moreover electrostatic contribution with  $d(A)_5 \cdot d(T)_5$  was always larger than with  $d(C)_5 \cdot d(G)_5$  (table 1 and figures 1-3) for the same environmental conditions. This leads to higher binding energies of the ligands with  $d(C)_5 \cdot d(G)_5$  for ion concentrations up to 0.1M.

With increase in the ion concentration the  $E_{non} + E_{hydg}$  contributions become more significant (table 1). A change in the binding order was observed between two sequences. The binding energy of netropsin and distamycin-2 with  $d(C)_5 \cdot d(G)_5$  is lower by 0.19 and 6.3 kcal/mol respectively for monovalent ion. However, for distamycin-A.An, it was  $-0.653$  kcal/mol for two sequences. In the case of divalent ions the same differences increased to 2.6, 6.38 and 1.647 kcal/mol for the three drugs and GC sequence had a slightly greater preference over AT sequence. However, the overall sequence specificity reduced substantially. The relative binding energy of netropsin with  $d(C)_5 \cdot d(G)_5$  at high ion concentration showed some difference with the experimentally measured affinity<sup>8</sup>. No binding was noticed for poly  $d(G)$ .poly  $d(C)$  by these authors. The reason is that we have assumed DNA conformation in B-form. Much of

the experimental data can be justified by change in the DNA conformation and the capacity of the ligands to bring it back to B-form since these ligands are highly stereospecific.



**Figure 4.** The total interaction energy of netropsin with fragments of  $d(A)_5 \cdot d(T)_5$ . for (a) monovalent (b) divalent counterion environment. Numbers 1-6 on the abscissa denote respectively contributions due to interaction of: 1-DNA bases with amides; 2-DNA bases with rings of the drugs; 3-DNA bases and drug tails; 4-DNA backbone with amide groups of the drugs; 5-DNA backbone with rings; 6-DNA backbone with tail portion of the drugs. Ordinate gives the total energy contribution. All contributions are calculated at dielectric permeability value  $\epsilon = 4$ .



**Figure 5.** The total interaction energy of distamycin-A. An with the fragments of  $d(A)_5.d(T)_5$  for (a) monovalent, (b) divalent counterion environment. Ordinate and abscissa have the same meaning as in figure 4.

#### Groupwise energy partition

Partitioning of the interaction energy of different functional groups on ligands with  $d(A)_5.d(T)_5$  is depicted in figures 4 and 5 for netropsin and distamycin-A. An for mono and divalent ions. For distamycin-2 we have depicted these results in table 1 for  $d(A)_5.d(T)_5$  and  $d(C)_5.d(G)_5$ . In all the three cases a similar behaviour of the interaction is observed between DNA bases and drug rings. There is an increase in the electrostatic component due to this interaction by about 30 kcal/mol for netropsin and 22 kcal/mol for distamycin analogs, for ion concentration changes from 0.001 M to 1 M. This term is mainly responsible for one loss of sequence specificity of high salt conditions.

Interaction of DNA bases with the tail portion of the drug is also sequence-specific and shows variation of 48 kcal/mol for netropsin and 20–22 kcal/mol in the case of distamycin-2. In the case of distamycin-A. An., this term does not show any significant change (5 kcal/mol).

Major contribution to stabilization of interaction of netropsin with  $d(A)_5.d(T)_5$  comes from DNA-backbone-drug-tail interaction. This term also shows maximum variation (about 120 kcal/mol) for ion concentration change from 0.001 M to 1 M. However, it does not contribute towards sequence-

specificity. The same change in the case of distamycin-2 was about 60 kcal/mol and negligible in the case of distamycin-A. An (5–10 kcal/mol) (figure 5). The nonspecific interaction between DNA backbone and the rings shows similar variation (50–40 kcal/mol for netropsin, distamycin-2 and distamycin A. An) (figures 5 and 6; table 1).

These results bring out several important facts regarding the environmental role of ligands in DNA recognition. They show that the presence of cationic tails is not an absolute requirement for DNA recognition. However, they significantly modify the relative affinity of the drug for DNA and the environmental effect on their binding. They can also enhance sequence-specificity and environmental effect on sequence-specific binding. We also observe that small changes in ligand conformation do not modify environmental effect significantly as electrostatic forces are long range forces. The environmental effect basically depends on the net electrostatic component in the interaction energy due to specific groups.

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