A Cyclic Sugar Unit Interacts More Strongly with DNA Than Do Open-Chain Polyol Counterparts in Sugar-Linked Synthetic DNA Intercalators

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We synthesized new DNA intercalators, polyol- or cyclic sugar-linked anthraquinone derivatives. The binding affinity of these DNA intercalators is suggested to be controlled by the configuration and steric structure of sugar chain with the cyclic sugar chain interacting more strongly than the open-chain polyol unit with double stranded DNA.

It is well known that some naturally occurring drugs increase their pharmacological activity by binding to DNA. For example, daunomycin,[1] a well-studied class of the anthracycline antitumor antibiotics, is a representative compound in such drugs. Its pharmacological activity expresses when a quinone-containing chromophore of daunomycin intercalates into base pairs in the minor groove of duplex DNA. This compound is composed of two structural parts, i.e., a DNA intercalation moiety and a saccharide moiety. In common with anthracyclines, saccharide chain groups are contained in their structure.[2] Furthermore, calicheamycin[3] and bleomycin,[4] DNA binding antitumor antibiotics, also contain sugar side chains.

Recently, the roles of these sugar moieties have been studied by the comparison among these compounds as well as with their analogues.[5] As the result of such studies, it is now considered that the sugar moiety strongly relates to its biological activity and binding sequence specificity.

From this point of view, we newly designed and synthesized an artificial DNA intercalator carrying sugar chains in order to understand more deeply how the sugar moiety is involved in DNA recognition and binding.

We adopted an anthraquinone structure that is the fundamental skeleton of anthracycline as DNA intercalation moiety, and introduced one or two optical isomeric polyol units or cyclic sugar chain (Figure 1). In the case of **1** or **2**, when these enantiomeric pairs bind to DNA, the difference in binding affinity to DNA due to the configuration difference in the side chain may be expected. On the other hand, **3** involves a cyclic sugar unit similar to that found in anthracycline antitumor antibiotics. The nature of the interaction of sugar unit with DNA when brought about close to the grooves may be different between the cyclic and open chain analogues since the former is rigid and sterically restrained while the latter is flexible. In understanding the role of sugar moiety in naturally occurring drugs, it should be of interest to investigate the DNA binding behavior of these synthetic DNA ligands.

1-(2-Aminoethyl)aminoanthraquinone and 1-[2-[*N*,*N*-bis(2-aminoethyl)]aminoethylamino]anthraquinone were synthesized as a precursor of sugar-linked intercalators **1**, **2** and **3** by the method previous reported.[6]

The mixture of 1-(2-aminoethyl)aminoanthraquinone (0.20 g, 0.75 mmol) and 1,5-D/L-gluconolactone (0.27 g, 1.50 mmol) in EtOH was stirred at 80 °C for 24 h. After cooling, the precipitate was collected and washed with a small amount of water. **1-D** or **-L** was afforded as a red powder (yield: **1-D** 15%; **1-L** 30%). The similar procedure used for the preparation of **2-D** or **-L**, which was isolated by gel filtration on TSK gel CELLULOSE CW-35-c as a red powder (yield: **2-D** 60%; **2-L** 75%).

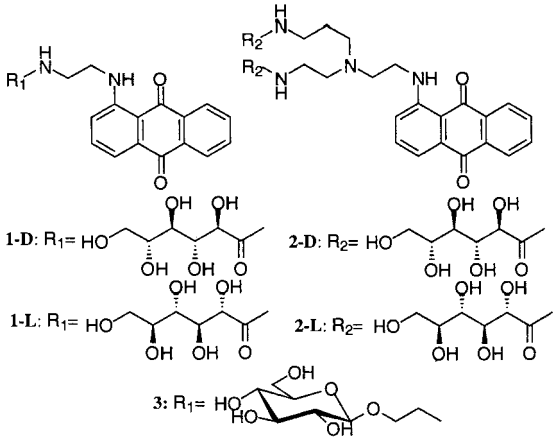

Figure 1. DNA ligands carrying polyol or cyclic sugar moiety.

In the synthesis of **3**, a mixture of 1-(2-aminoethyl)aminoanthraquinone (0.30 g, 1.13 mmol) and *O*-acetylated 2-iodoethyl D-glucopyranoside[7] (0.19 g, 1.35 mmol) in DMF was stirred at 40 °C for 24 h. After DMF was removed in vacuo, the crude oil of *O*-acetylated **3** was purified by silica gel chromatography (40:1 chloroform/MeOH). Then MeONa-MeOH solution was added to a solution of *O*-acetylated **3** in MeOH, and the mixture was stirred at r.t. for 5 h for deacetylation. The reaction was stopped by the addition of H^+ ion exchange cellulose (Whatman, CM52). The deacetylated compound **3** was then extracted from the ion exchange cellulose by the mixture of 4:1 MeOH/diethylamine. The compound **3** was obtained as a red solid (0.14 g, 26%) by concentrating the extract in vacuo. All the products were identified by ^{1}H NMR[8] and elemental analysis.

First we investigated the interaction of these sugar-linked intercalators with DNA by UV/vis spectroscopy (0.2 mM HEPES buffer pH 7.0, 10 mM NaCl). Figure 2 shows a spectral change of **3** during the addition of calf thymus DNA while keeping the concentration of **3** constant. The hypochromic and bathochromic shifts were observed that are typical to aminoanthraquinone derivatives on intercalation to DNA double strands. A similar spectral behavior was obtained for both **1** and **2** (Table 1). Thus the interaction of the present compounds with DNA is safely assigned to be intercalative as was with all the non-sugar aminoanthraquinone analogues studied in our previous work.[6,9]

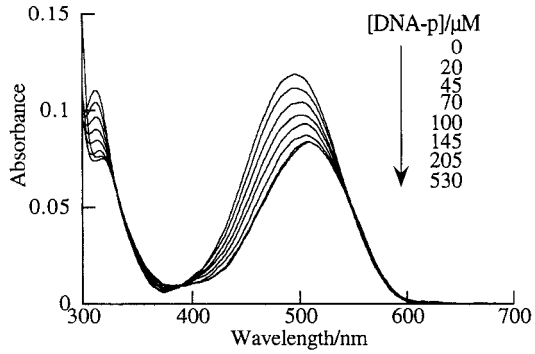

Figure 2. Spectral change of **3** on titration with calf thymus DNA. The titration was conducted for 20 μM **3** in 1 cm cuvette in 0.2 mM HEPES buffer (pH 7.0) with 0.01 M NaCl at 25 °C.

Table 1. Binding constants of polyol or cyclic sugar DNA intercalators with calf thymus DNA at 298 K

compound	Binding constants[a], $K \times 10^{-3}/dm^3mol^{-1}$	Hypochromicity/%
1-D	2.7	27
1-L	5.4	26
2-D	13	23
2-L	8.5	26
3	94	33

The constants were obtained for 20 μM of compounds in 0.2 mM HEPES buffer with 0.01 M NaCl (pH 7.0). [a]The average experimental error of the binding constants is less than 10%.

The binding constants (K) of these compounds to DNA were obtained from the spectral data according to the method described by McGhee and von Hippel.[10] The results are summarized in Table 1, which reflected the structural feature of the sugar moiety in the compounds. As mentioned above, **1-D** and **1-L** form an enantiomeric pair, and so do **2-D** and **2-L**. Therefore the differences in binding constants between D- and L-isomers reflect the diastereomerism in the complex formation between enantiomeric ligand and DNA. This effect seems only to amount to two-fold difference in the binding constant for both **1** and **2**.

The binding constant of **2** is greater than **1** by a factor of 2.7 in average. In this connection it should be pointed out that at pH 7.0, **2** exists as a mono cationic form due to the protonation of tertiary amine in the linker unit, while **1** remains electrically neutral. It is common that the affinity of the ligand toward DNA increases on increasing its cationic charge because of electrostatic stabilization of the complex. From this view, the greater binding constant of **2** over **1** is quite reasonable, but the extent of this stabilization effect seems relatively low (the factor of 2–3) though at present we do not know about the contribution from the interaction of open sugar chains with DNA.

The most interesting observation in Table 1 is that the binding affinity of **3** is much larger than **2**. Although the both compounds assume the same electric charge (mono cations) under the binding measurement conditions, the affinity of **3** is 7–11 times as large as **2**. It seems that this difference is primarily derived from the steric configuration effect concerning the sugar chain. We here point out some structural factors to be considered in this respect. The linker chains connecting the anthraquinone nucleus and the sugar are flexible and quite similar in effective length between **2** and **3**. However, the structure of the sugar chain is quite different for the two, i.e., **2** assumes an open-chain flexible configuration while **3** has a compact, cyclic structure. Another factor to be considered is that **2** carries two (branched) polyol units, which renders the side chain of **2** more bulky than that of **3**. Presumably, a sterically restrained, cyclic configuration of the sugar with its hydroxy groups sterically more strictly oriented makes the interaction with DNA favorable. It also deserves noting here in the passing that a simple elongation of sugar chain to di- or trisaccharide in a relevant sugar-linked intercalators did not result in a substantial increase in their DNA affinity (unpublished results). This means that the interaction between sugar moiety and DNA is not simply additive as is in the case of polyintercalators and multi-cationically charged ligands.

As to the detailed nature of the interaction between sugar and DNA there are yet much to be studied. However, certainly hydrogen bonding between hydroxy groups of sugar and nucleobases should be among the important contributing factors. The affinity between sugar groups and the phosphate groups in DNA as recently suggested[11] could also be an important factor. In addition, in the present aminosugar type ligands, the hydrogen bonding between the ammonium proton and nucleobase or anionic phosphate group can not be ruled out as are suggested in the nucleobase sequence-selective binding of aminoglycoside antibiotics[12] with DNA.

All of the above mentioned factors concerning hydrogen bonding are in fact too complex to be individually accessed to what extent they are in operation in each amino-sugar type ligand. However, we would like to conclude our present study by summarizing that if we want to design sugar-linked intercalators for nucleobase sequence recognition or site specific binding one should introduce a cyclic sugar moiety in close proximity to the intercalating polyaromatic unit.

References and Notes

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