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Interactions of calf thymus DNA with short chain oligoamides

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Abstract

In order to detect the biological activity of new synthetic short chain oligoamides, the present work gives the results of studies on the interaction between calf thymus DNA and short chain oligoamides: PyPyPy-γ-Dp (ligand 1) and PyPyPyPy-γ-Dp (ligand 2). In the circular dichroism (CD) spectra of the mixing solution of DNA with ligands a specific peak appears in the region of 300–380 nm wavelength, which indicates that both ligands 1 and 2 can bind calf thymus DNA specifically. ^{1}H NMR spectra show that aromatic ring protons of ligand 1 appear as an obvious down-field shift, while those protons in ligand 2 disappear since ligand 2 owns a higher binding affinity with DNA than that of ligand 1. Investigations of DNA transition by differential scanning calorimetry (DSC) reveal that DNA melting temperature rises due to binding with ligands, and that ligand 2 alters the DNA transition process more significantly. At the same time, the molar binding enthalpies of ligands with DNA are measured at 293 K. The difference between the binding affinity of ligand 1 and 2 with DNA implies that increasing the number of *N*-methylpyrrole increases both the binding site size and the binding affinity.

Keywords: Calf thymus DNA; Short chain oligoamide; Interaction; Binding affinity

1. Introduction

Numerous control mechanisms in the living cell are based on the recognition and interaction between biological activity molecules and biomacromolecules [1,2]. The design of synthetic ligands that read the information in the DNA duplex has been a central goal at the interface of chemistry and biology [3]. Syntheses of DNA-binding molecules, such as triplex-forming oligonucleotide [4], peptide nucleic acid [5], oligosaccharide [6] and oligopeptide [7,8], have been exploited. Distamycin, which contains *N*-methylpyrrole-carboxamides, belongs to a well known class of oligopeptide antibiotics called "Lexitropsins" [9,10]. The binding of some peptides in the minor groove of duplex DNA at specific AT-rich regions in a non-intercalating fashion has been demonstrated by X-ray crystallography, NMR, and physico-chemical investigations of the interaction between ligand and synthetic specific DNA sequences [11,12]. Some investigations have proved the binding of ligand with duplex DNA at CG region as well [9].

Our interests in the design and synthesis of DNA-binding molecules have led us to modify distamycin and introduce a γ-aminobutyric acid (γ) for the sake of increasing the binding size of the ligands. The structures of distamycin A and ligands 1 and 2 are shown in Fig. 1. Both the ligands 1 and 2 include various numbers of *N*-methylpyrrole amides, and a *N*,*N*-dimethylaminopropylamide is connected to the terminal of

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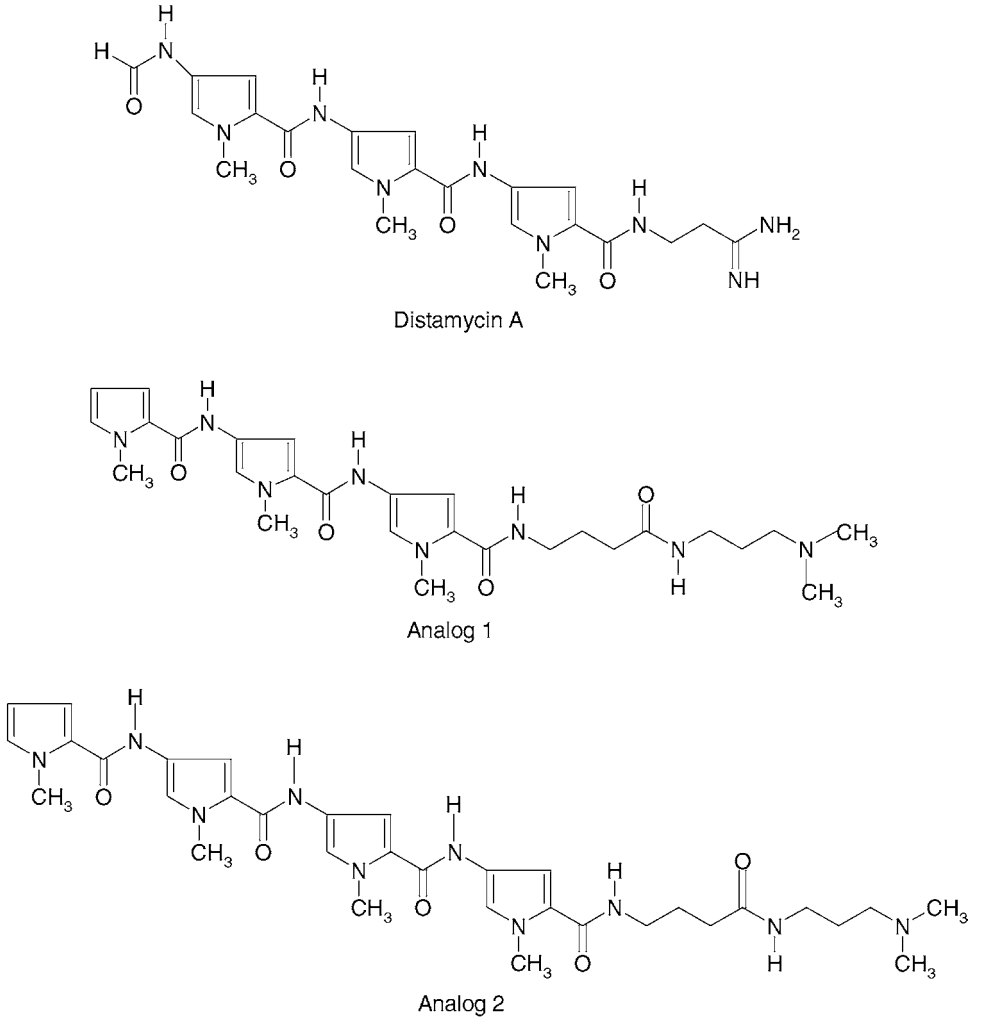

Fig. 1. The structures of distamycin A, ligand 1 and ligand 2.

γ-aminobutyric acid. The difference between ligand 1 and 2 is that there is one more *N*-methylpyrrole amide in ligand 2 than in ligand 1. Though synthetic oligonucleotides have been used to investigate the recognition of long chain distamycin analogs on specific DNA sequences [13,14], calf thymus DNA as an object of investigation is of significant meaning in the aspect of real macromolecule recognized by short chain oligoamides. Here, we give the results of studies on the interaction of calf thymus DNA with short chain oligoamides by circular dichroism (CD) spectropolarimetry, NMR, differential scanning calorimetry (DSC) and isothermal titration calorimeter (ITC), and compare the binding affinity of ligand 1 and 2 with calf thymus DNA. This will help us to understand the relationship between the increase of the number of *N*-methylpyrrole and the increase of the binding site size and the binding affinity.

2. Materials and methods

2.1. Materials

Calf thymus DNA was a commercial product purchased from the Chinese market. Ligand 1 and ligand 2

were synthesized with an efficient combination of the chloroform reaction and the DCC/HOBT coupling reaction without amino protecting and deprotecting. Their structures were identified by MS, FTIR, ^{1}H and ^{13}C NMR. All other chemicals were of analytical reagent grade.

2.2. Preparation of stock solution

Calf thymus DNA was sonicated to double helices with about 500–600 base pairs, extracted with phenol and ether sequentially after filtration, and precipitated by ethanol. The precipitate was dissolved in a buffer of pH = 7.4 with 100 mM KH_2PO_4–NaOH and 1 mM EDTA. The solution of DNA was extensively dialyzed against the same buffer. The concentration of DNA base pairs was measured spectroscopically at 260 nm with an extinction coefficient of 6600 $M^{-1}\,cm^{-1}$. Fresh solution was prepared by diluting the stock solution before experiments. Ligands 1 and 2 were dissolved in buffer before use. The buffer system mentioned above was used in all experiments except being stated.

2.3. Circular dichroism (CD) spectra

CD spectra of DNA with or without ligands were recorded by a Jasco J-500 automatic-recording spectropolarimeter (Japan) in a quartz cell with a path length of 1.0 cm. Two milliliters of DNA solution with a concentration of 4.0×10^{-5} M was put into the sample cell and was titrated by ligand 1 or 2 with a 5 μl syringe. The total volume of incremental ligands was 20 μl both for ligands 1 and 2; therefore, the DNA concentration was considered to be unchanged. The range of molar ratio of ligand to DNA was from 0 to 3. Scanning wavelength was from 380 to 220 nm. The molar ellipticities, $[\theta]$, was calculated according to Lambert–Beer's law using a DNA concentration of 4.0×10^{-5} M.

2.4. ^{1}H NMR spectra

^{1}H NMR spectra of 500 μl of ligand 1 or 2 with or without equal molar ratio of DNA were recorded on a Bruker ARX-400 NMR spectrometer operating at 400 MHz with 5 mm sample tube at room temperature. Sodium 3,3,3-trimethylsilylpropionate (TSP) was used as chemical shift reference since it presents a single proton peak only and does not interfere with the research system [15]. The sample was prepared by mixing 10% D_2O and 90% buffer solution which contained ligand and DNA. The concentration of ligand was finally 1.2 mM. H_2O resonance was suppressed with the presaturation pulse program of zgpr.

2.5. DSC analysis

DSC can be used to detect and follow thermally induced order–disorder transitions in protein, DNA and other molecules [16]. Calorimetric heat flow (mW) vs. temperature (T) profiles were directly measured by a differential scanning calorimeter of the type Micro DSC-III manufactured by Setaram Company, France. The calorimeter was connected with the CS32 controller and interfaced to a compatible Compaq computer with the software package for data acquisition and analysis. Temperature correction and baseline correction had been done before experiments. All DSC experiments were carried out under a dry atmosphere of nitrogen gas to prevent condensation of moisture. A scan rate of 1 K/min was used from 283 to 383 K throughout. 0.6 ml solutions of 1.2 mM DNA in the absence and presence of ligand 1 or 2 were kept in the sample cell and the same volume of buffer was kept in the reference cell. The area under the peak is proportional to the transition heat, which is equal to the transition enthalpy change (ΔH_{tr}) when normalized for the number of moles.

2.6. Isothermal titration calorimetry (ITC)

The measurements of the molar binding enthalpy change (ΔH_b) of ligands 1 and 2 with DNA at 293 K were carried out with an isothermal titration calorimeter from CSC Company, USA. The instrument was electrically calibrated by means of a standard electric pulse. Solution of ligand 1 or 2 was used to titrate DNA to form the ligand–DNA complex. A 250 μl syringe was used for the titrant. Typically per injection, 10 μl of 2.5 mM ligand solution was added in a single titration to the sample cell with 500 μl of 1.4 mM DNA. Mixing was carried out by stirring the sample cell at 200 rev/min. It was assumed that in every titration, ligand was bound to DNA completely since DNA was superfluous relative to the ligand itself.

The reference cell was filled with the same volume of buffer. To correct the dilution heat of ligand, the control experiments were also performed at the same temperature using similar conditions with buffer only. All solutions were degassed to reduce the noise. The heat of each reaction was determined by the integration of the peak observed after the contribution from the dilution heat of each injection was subtracted.

3. Results

3.1. CD spectra

CD spectrum of calf thymus DNA in phosphate buffer (pH 7.4) showed a typical positive peak at 272 nm and a negative peak at 243 nm (Fig. 2) which correspond to the conformation of DNA duplex [17].

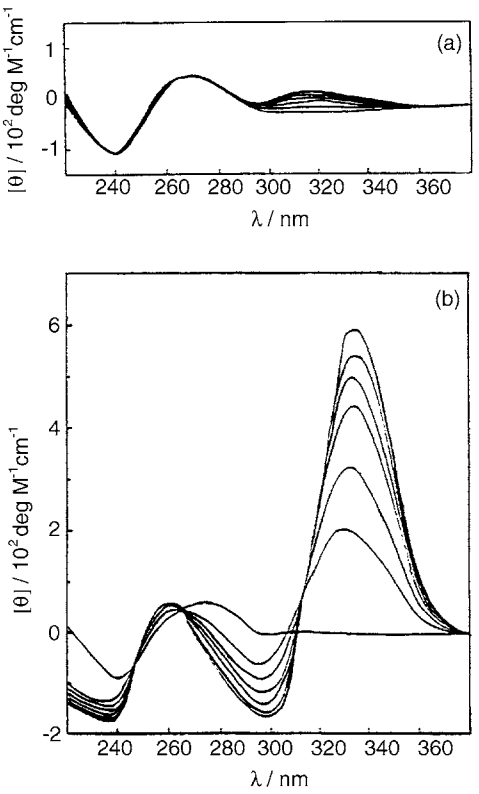

Fig. 2. CD titrations at room temperature of calf thymus DNA with ligand 1 (a) and 2 (b). Solution conditions are as described in the text. Molar ellipticities, [θ], are in units of deg M^{-1} cm^{-1}.

Neither the free DNA nor the free ligands exhibited CD signals in the 300–380 nm wavelength region. With incremental titration of ligands, in the CD spectra of mixed solutions a specific absorption peak appeared in the 300–380 nm wavelength region, which implied a binding motif of ligands with DNA. The maximum absorption signal in this region appeared at 322 nm for ligand 1 (Fig. 2a) and at 333 nm for ligand 2 (Fig. 2b). Furthermore, the maximum absorption signals were up to an ultimate value with the addition of surplus ligands. The final molar ellipticities for ligands 1 and 2 were 0.5×10^2 and 6.0×10^2 deg M^{-1} cm^{-1}, respectively.

In the DNA-absorbing wavelength region (220–300 nm), each family of CD spectra shared two isoelliptic points, which were consistent with the specific binding motif. These isoelliptic points were observed at 241 and 269 nm for ligand 1 and at 247 and 265 nm for ligand 2. Further inspection of Fig. 2 revealed that the curvature of the titration curves for ligand 2 was substantially sharper than the curvature of the corresponding curve for ligand 1.

3.2. ^1H NMR spectra

Fig. 3 shows the ^1H NMR spectra of the free ligand 1 and that of the mixture of ligand 1 with calf thymus DNA. Resonance assignments were done for the free ligand 1. One could see that before DNA was added, the ligand 1 was exposed freely in the solution. The H of amides showed a fast exchange process and their signals were not evident. The resonances at $\delta = 3.64$ and $\delta = 3.23$ ppm were from the two types of protons of EDTA and they did not alter before and after mixing of ligand 1 with DNA. The resonances of seven protons at the aromatic rings could be observed at the region of $\delta = 6.0-7.2$ ppm (Fig. 3a). However, once an equivalent molar of DNA was added to the solution containing ligand 1, the signals of H attached to the aromatic rings decreased dramatically (Fig. 3b), and a significant down-field shift was found for the aromatic protons of ligand 1. The maximum chemical shift ($\Delta\delta$) among them was up to about 0.3 ppm. Being different from the aromatic protons, the signals of the protons at the *N*-methyl of pyrroles ($\delta = 3.6-3.9$ ppm) and aliphatic protons ($\delta = 1.8-3.3$ ppm) displayed no obvious chemical shift but only decreased with the addition of DNA.

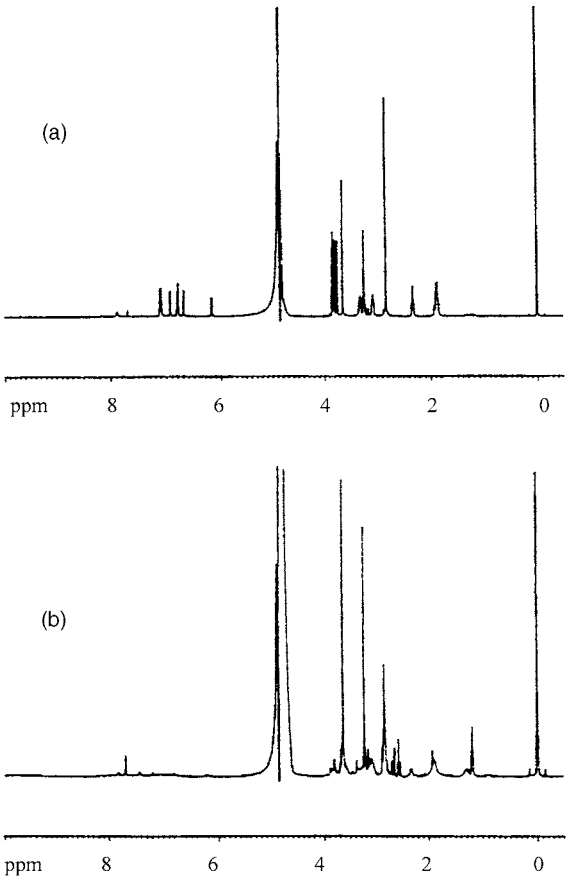

Fig. 3. ^{1}H NMR spectra of ligand 1 in the absence (a) and presence (b) of calf thymus DNA. Different peak height scale was adopted in order to observe the presence of the proton signals at aromatic rings.

For ligand 2, when DNA was added, the signals of aromatic ring protons of ligand 2 disappeared completely and its signals at the *N*-methyl of pyrroles and aliphatic protons were much weaker than that of ligand 1.

3.3. DSC data

DSC occupies an important role on the conformational stability of DNA thermal transition processes and the correlation between thermodynamic and structural features [18]. The transition enthalpy change of DNA (ΔH_{tr}) was obtained by integration of the thermal peak and the temperature of the onset point, and not of the top of the peak, was taken as DNA melting temperature (T_m) in order to avoid inaccuracy resulting from thermal lag. Under the buffer condition, calf thymus DNA showed a negative and wide thermal peak (Fig. 4a). It was an endothermic transition process of DNA itself and its T_m value was 352.6 K. With the addition of ligands 1 and 2, T_m of DNA increased gradually. When the concentration of ligand 1 was 1.2 mM, the T_m value of DNA increased to 355 K (Fig. 4b); while for the same concentration of ligand 2, the T_m value of DNA increased to 360.2 K (Fig. 4c). The ΔT_m for ligands 1 and 2 was 2.5 and 7.6 K, respectively. It indicates that the binding of ligand with DNA increases the DNA thermal stability. Table 1 also lists the results of DNA transition enthalpy change. Addition of ligands led to decreasing DNA transition enthalpy changes. Further inspection of Fig. 4 revealed that the binding of ligand 1 and 2 with DNA changed not only the DNA melting temperature, but also the transition process since the thermal peak became narrow. Especially for ligand 2 (Fig. 4c), its addition to DNA solution induced the transition of DNA from a slow process to a fast process; and its ΔT_m value was larger than that of ligand 1, which indicated that the binding affinity of ligand 2 with DNA was stronger than that of ligand 1.

3.4. The binding molar enthalpy change of ligand with DNA

The values of molar enthalpy change of ligands 1 and 2 binding to DNA were measured by an isothermal titration calorimeter. After subtracting the dilution heat of the ligands in buffer from the titration heat of the ligands in DNA solution, averaging the results of several titrations, and normalizing them for the number of moles, we gained the molar binding enthalpy changes, which were -3.4 ± 0.4 kJ/mol for ligand 1 and -15.5 ± 0.8 kJ/mol for ligand 2, respectively, listed in Table 1.

Table 1
The melting temperature (T_m), the molar transition enthalpy change (ΔH_{tr}) and the molar binding enthalpy change ($\Delta H_{b,\ 20\ ^\circ C}$) of ligands 1 and 2 with DNA[a]

Sample	T_m (K)	ΔH_{tr} (kJ mol)	$H_{b,293\ K}$ (kJ mol)
DNA	352.6 ± 0.2	25.0 ± 1.0	–
DNA + ligand 1	355.0 ± 0.3	23.2 ± 1.2	-3.4 ± 0.4
DNA + ligand 2	360.2 ± 0.3	22.6 ± 0.9	-15.5 ± 0.8

[a] The uncertainties are also given in the table.

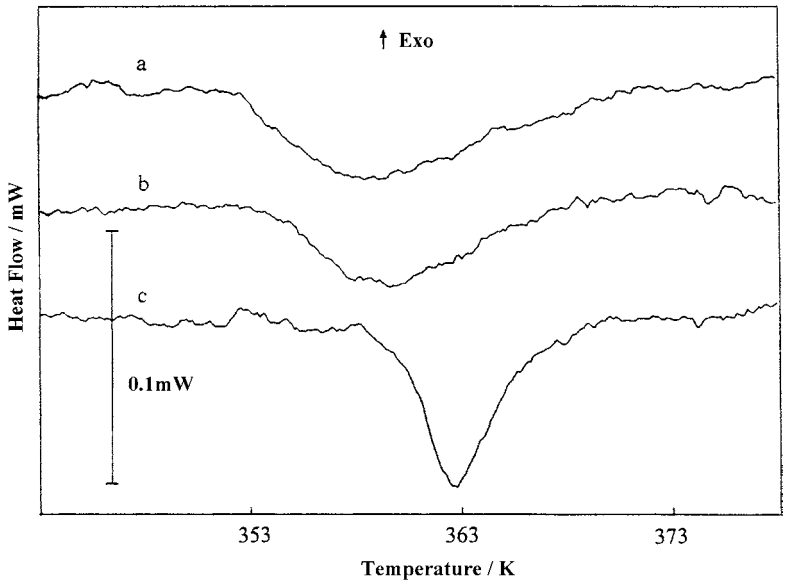

Fig. 4. The DSC curves of DNA (a), DNA with ligand 1 (b), and DNA with ligand 2 (c).

4. Discussion

The features of CD specific absorption region of ligand with DNA shows that synthetic short chain oligoamides can interact with calf thymus DNA. Inspecting the titration curvature, one could see that the final molar ellipticities for ligands 1 and 2 at the 300–380 nm region were 0.5×10^2 and 6.0×10^2 deg M^{-1} cm^{-1}, respectively. The difference in curvature is consistent with the fact that ligand 2 binds to DNA with a greater affinity than ligand 1; a correlation is substantiated by the ligand–DNA NMR spectroscopy.

In NMR spectrum, the protons of aromatic rings of ligand 1 show a down-field chemical shift after DNA was added. It discloses an interaction of minor groove binding mode between ligand 1 and DNA possibly, but not an intercalation, which often shows an upfield shift for aromatic ring protons [19]. The protons of aromatic rings of ligand 1 were buried partly in the groove and the molecular movement was cumbered. On the other hand, because ligand 1 acts like a knife placed in the minor groove, the orientation of the aromatic proton and the *N*-methyl was opposed since *N*-methyl was out-forward in the minor groove of DNA. So the signals of the *N*-methyl protons were affected less than that of aromatic protons. In the same way, the aliphatic protons had much higher degrees of freedom, their signals were observed too in the mixed solution of ligand 1 with calf thymus DNA.

Since calf thymus DNA is a macromolecule, its molecular movement is too slow to show its proton resonance. When the aromatic protons of ligand 2 were buried in the minor groove of DNA deeply, their signals could not be detected. This phenomenon implies a higher binding affinity of ligand 2 with DNA than that of ligand 1. As the chemical shift of protons at aromatic rings of ligands often prove the binding mode of ligands to DNA, the results of NMR down-field shift indicate the minor groove binding mode of ligands 1 and 2 with mainly DNA. The buried degree of aromatic protons discloses the difference of their binding affinity with DNA.

The characters of NMR disclose that the predominant binding sites of DNA by ligands may be in DNA minor groove. Both ligands 1 and 2 are chain-type molecules, one ligand needs to bind several bases of DNA, therefore ligands 1 and 2 may bind to AT-rich region as well as CG region; and other nonspecific interactions are also present. The structural change of short chain oligoamide, different from distamycin (such as the inducement of γ-aminobutyric acid), may change their binding characters to DNA.

DSC experiments discloses that ligands 1 and 2 increase DNA thermal stability and the binding affinity of ligand 2 with DNA is stronger than that of ligand 1. It was reported that distamycin ligands bind to DNA through a hydrogen bond of *N*-methylpyrrole amides with bases of DNA [20]. The molar binding enthalpy changes of ligands 1 and 2 reveal that the strength of the interaction between the synthetic compound and DNA is related to the number of formation of hydrogen bonds. In comparison with ligand 1, increment of a *N*-methylpyrrole amide for ligand 2 makes its binding affinity increase significantly. The protons at amide which take part in the formation of hydrogen bond with the acceptor on the base pair of DNA will contribute to the binding affinity and in addition the aromatic protons which are buried deeply in the minor groove to interact with the wall of the minor groove through extensive van der Waals forces also can enhance the binding affinity.

In conclusion, the experimental results have proved that the designed ligands 1 and 2 are a kind of low molecular weight DNA-binding molecules and can efficiently recognize DNA. Increasing the number of *N*-methylpyrrole will increase both the binding site size and the binding affinity.

Acknowledgements

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