Vibrational dynamics of wet-spun films of the NaDNA-netropsin complex: A Raman and infrared study

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Raman spectroscopy and infrared absorption have been used to study the vibrational dynamics of wet-spun films of the NaDNA-netropsin (NaDNA-net) complex as well as netropsin and NaDNA. The NaDNA-net complex is found to have a *substantially* different vibrational spectrum from either netropsin or NaDNA. The films of NaDNA-net appear to be more crystalline than the films of NaDNA alone. The formation of the NaDNA-net complex strongly favors the *B* conformation of the double helix. These vibrational data provide useful information for the understanding of the nature of the interaction of DNA with the antiviral, antitumor antibiotic netropsin.

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INTRODUCTION

A central goal of biophysics is to provide a microscopic understanding of the structural and dynamical properties and function of biologically important molecules and molecular assemblages. Perhaps the most important molecule to life itself is DNA, the carrier of the genetic code. Significant progress has been made in the understanding of the structural and dynamical properties of DNA [1–8]. The understanding of the microscopic nature of the interactions of DNA with other molecules is less advanced, since such problems are more complex [9].

Netropsin is an antiviral, antitumor antibiotic. Though this drug is too toxic for clinical use, it has received intensive study since it is a paradigm of base-specific yet nonintercalative (i.e., not between the bases) DNA-binding drug molecules [10–17]. Atomic-level understanding gained by vibrational studies of DNA-netropsin complexes should facilitate the design of less toxic antitumor drugs [18]. In this paper we report the results of our Raman and infrared (ir) studies of the complex of NaDNA with netropsin.

Netropsin binds tightly to *B*-DNA in AT-rich regions (AT denotes base pairs of adenine and thymine), interfering with both transcription and replication [19]. It shows little or no affinity for single-stranded DNA or RNA, or for double-stranded RNA or DNA-RNA hybrids [19], suggesting that it does not bind to the *A* double helix. Studies of oriented films of complexes of NaDNA with netropsin (NaDNA-net) as a function of relative humidity (RH) demonstrate a strong suppression of the formation of *A*-DNA at relative humidities down to about 50% [20].

Single-crystal x-ray-structure analysis by Kopka *et al.* [15] of netropsin complexed with the double-helical *B*-DNA dodecamer C-G-C-G-A-A-T-T-^{Br}C-G-C-G have revealed a wealth of information about the changes in the structure of both netropsin and DNA induced by the formation of the complex. Their work showed that a single netropsin molecule sits in the minor groove of the A-A-T-T center of the double helix, equidistant from the two walls of the groove, replacing the spine of hydration. The AT base specificity of netropsin arises from the fact that binding within the minor groove causes stearic hindrance of netropsin with the $N2$ amine group of guanine. Each of the two outermost amide NH groups of the netropsin makes one hydrogen bond to the thymine $O2$, while the amidinium makes a hydrogen bond to the thymine $N3$. In addition, there are significant interactions between other hydrogen atoms of the netropsin molecule and the oxygen and nitrogen atoms of the adenine and thymine. Though the distances involved with these atoms are too large for a true hydrogen bond, these interactions presumably have a significant hydrogen-bond character. Kopka *et al.* also conclude that each of the two pyrrole rings of the netropsin molecule is within 3° of being parallel to its own region of the minor groove. As a consequence, the two rings make an angle of 33° to one another (rather than 20° as in the free molecule), indicating that the formation of the complex significantly changes the geometry of the netropsin molecule. Both of the positively charged ends of the netropsin lie in the bottom of the groove, rather than being associated with particular phosphates.

Kopka *et al* [15] also found that virtually *no* changes were induced in the geometry of the dodecamer. The

double helix is neither wound nor unwound and the length of the double helix is not changed. There are still 10 base pairs per turn with a spacing of 3.34 Å per step along the helix. Changes in the "propeller" twist of the base pairs and the main-chain torsion angle are smaller than the variation observed within one helix itself. Kopka *et al.* also found that the minor groove in the free DNA structure is only 3.2–4.0 Å wide in this region, implying that there is barely enough space for the insertion of the flat organic ring of van der Waals thickness 3.5 Å. This would suggest that, though the structure of the double helix is unchanged, interactions between the two molecules are substantial.

EXPERIMENT

Oriented films of highly polymerized calf-thymus NaDNA were prepared via the wet-spinning method [14,21,22]. After spinning, the film on the cylinder was bathed for 3 weeks in 60 mL of 75% ethanol–25% water (by volume) with 0.03*M* NaCl and 1.429-m*M* netropsin at neutral *p*H. The uptake of netropsin was determined by measuring the optical density at 300.0 nm of netropsin in the ethanolic bathing solution before and after the bathing, and using a precalibrated curve of absorbance as a function of netropsin concentration in ethanolic baths. The drug concentration decreased in the ethanolic bathing solution during the bathing as the drug bound to the NaDNA. The drug input ratio, *r*, of this NaDNA-net sample was found to be 0.22 drug molecules per base pair. A drug-free oriented NaDNA film was also prepared from the same DNA lot using an identical protocol, but without netropsin, and was used as the reference in the Raman and infrared experiments.

For the Raman experiments, small samples were mounted in sealed cuvettes in which the RH was 75% [23]. The RH was maintained by placing a small amount of saturated $NaClO_3$ solution in the bottom of the cuvette. The samples were allowed to equilibrate for at least three days. A 90° scattering geometry was used, with the film bisecting the scattering angle, and phonons propagating perpendicular to the helical axes were probed. Less than 20 mW of 488-nm radiation from an Ar-ion laser were used to obtain the Raman spectra. Experiments on the state of hydration of NaDNA films have shown that the water content is not altered for laser powers at and below 20 mW [24]. The scattered light was focused into a Jobin-Yvon double monochromator and was detected by a photomultiplier and standard photon-counting electronics.

For the far-infrared (FIR) experiments, the NaDNA-net and the calf-thymus (CT) DNA films were placed across the 10-mm aperture on the bottom of the cold finger of an Oxford Instrument, Inc. cryostat. In the case of netropsin, the powdered sample was dissolved in twice-distilled water and drops of that solution were allowed to dry on a polyethylene cell, which was then placed over the aperture of the cryostat. The spectra shown are Fourier transforms of the CT-DNA, NaDNA-net, and netropsin-polyethylene divided by the Fourier transforms of the aperture and the polyethylene cell, respectively. All sample and reference measurements were made at 20 K and taken at nearly the same time (within a couple of hours). The thickness of the films was 58 μm. The detector was a liquid-He-cooled silicon bolometer from Infrared Laboratories.

The mid-infrared (MIR) experiments were made in a RH-controlled chamber with MIR transparent windows. The samples were mounted on the windows and equilibrated at the appropriate RH.

RESULTS AND DISCUSSION

Figure 1 shows the Raman spectra of netropsin, NaDNA, and NaDNA-net complex at 75% RH from 600 to 1700 cm^{-1}. The NaDNA spectrum is identical to previously published spectra [2] and shows the expected "*A*-form marker band" at 805 cm^{-1}. The netropsin spectrum has many relatively sharp peaks. The Raman spectrum of the NaDNA-net complex is *dramatically* different from the Raman spectra of either NaDNA or netropsin. Clearly, the vibrational dynamics of the NaDNA-net complex are *substantially* different from those of either NaDNA or netropsin. Given the dramatically altered structure of netropsin, one might expect changes of such a large scale for that molecule. Among the more detailed changes are the increased twist of the netropsin molecule (33° between the two pyrrole rings rather than 20° for the free molecule) and confinement within the narrow groove. As noted above, the overall geometry of the DNA double helix is hardly changed by the inclusion of the netropsin molecule in the minor groove. There are, however, significant contact points that will, in principle, alter vibrational displacement patterns, amplitudes, frequencies, and lifetimes. In Fig. 2 the spectrum of netropsin is numerically subtracted from the spectrum of the NaDNA-net complex [25]. If the Raman spectrum of the NaDNA-net complex was simple linear combination of the spectra of NaDNA and netropsin (i.e., if the vibrational dynamics of the two molecules were not perturbed by the formation of the complex), then this subtraction should yield the Raman spectrum of NaDNA. Comparison of the subtracted spectrum to the

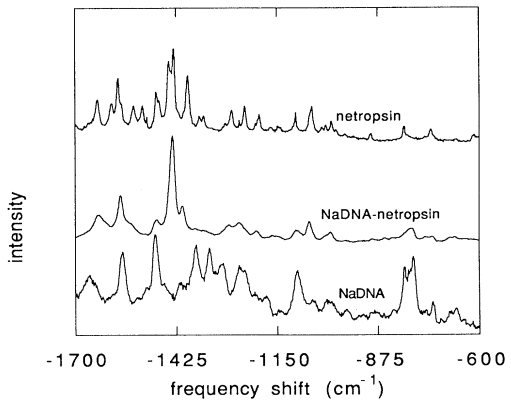

FIG. 1. The Raman spectra from wet-spun films of NaDNA-netropsin and NaDNA at 75% RH and from powdered netropsin.

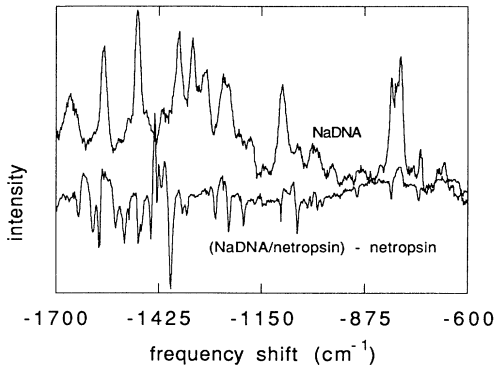

FIG. 2. Comparison of the Raman spectra of NaDNA with the spectra obtained by subtracting the netropsin spectrum from the NaDNA-netropsin spectrum.

spectrum of NaDNA alone in Fig. 2 shows that these two spectra are very different. The data shown in Figs. 1 and 2 show that the vibrational dynamics of the NaDNA double helix are modified significantly by the formation of the complex. These Raman spectra are so different that it is not possible to suggest a possible correspondence between the modes observed from netropsin, NaDNA, and the NaDNA-net complex.

Figures 3 and 4 show the low-frequency Raman spectra of the NaDNA-net complex. Weidlich *et al.* [26–28] have studied all of the Raman vibrational modes below 200 cm^{-1} in NaDNA. At 75% RH, they report five Raman modes, at 25, 34, 63, 90, and 112 cm^{-1} (the three higher-frequency modes are very broad and the uncertainty in their peak positions is about ± 3 cm^{-1}). It should be noted that the elastically scattered light observed from the NaDNA-net films (shown in Figs. 3 and 4) is more intense than reported for low-frequency Raman scattering from films and fibers of NaDNA. Consequently, our ability to resolve the lowest-frequency Raman modes of the NaDNA-net complex is diminished. In Figs. 3 and 4 only three modes are evident below 200 cm^{-1}: 24, 65, and 175 cm^{-1}. The 34-cm^{-1} mode usually appears as a shoulder on the 25-cm^{-1} mode in NaDNA

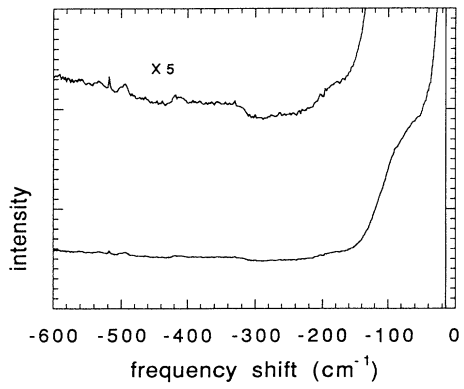

FIG. 3. The low-frequency Raman spectrum from a wet-spun film of NaDNA-netropsin at 75% RH.

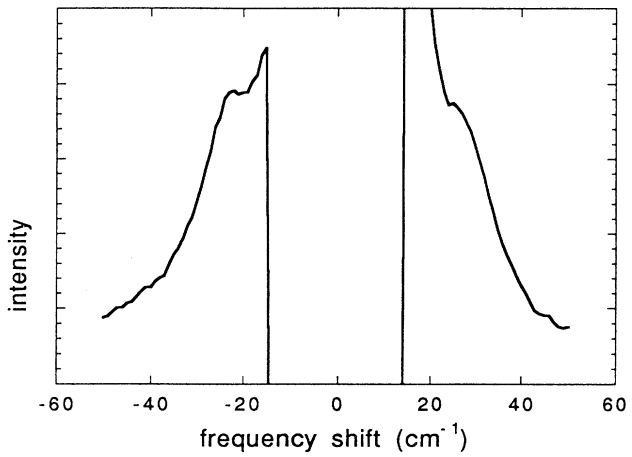

FIG. 4. Very-low-frequency Raman spectrum from a wet-spun film of NaDNA-netropsin at 75% RH.

[2]. It is possible that the 34-cm^{-1} mode is not observed in the NaDNA-net complex only because of the limits of resolution.

Of particular interest is the presence of a Raman mode at about 24 cm^{-1}, as shown in Fig. 4. In the literature, this mode has been called the "25-cm^{-1} mode" [29], as well as the "*S* mode" [5]. The nature of this mode (intramolecular, intermolecular, or plasmon) has not yet been determined [5,8,26,30,31]. Experiments by Weidlich [28] showed that the number of hydrogen bonds between the bases does not affect the frequency of this mode, arguing that motion of the two helices "beating" against one another is not the eigenvector. The frequency of this mode was found to depend on the counterion and this mode was only observed in crystalline samples. These observations support the conjecture that this mode is a lattice (or intermolecular) mode. The very simple lattice-dynamical model of Lavalle *et al.* [8] provided further support for the intermolecular nature of this mode. Urabe *et al.* [5] have reported results that suggest that this mode originates from the libration of stacked based in a column. Given the substantial modification of the vibrational dynamics of the DNA molecule shown in Fig. 1 and 2, it is unclear why no change should be induced in the librational motion of the column of bases by the formation of the complex. On the other hand, if the "25-cm^{-1} mode" was entirely intermolecular in nature, one would expect the effect of mass loading by the netropsin molecule to be observable, provided the netropsin did not perturb the effective force constants. In contrast, the experimental data for the complex show virtually no change in the frequency of this mode.

Theoretical efforts by Prohofsky and co-workers [32,33] have indicated that the vibrational modes in the 60–150-cm^{-1} region all involve motions with significant hydrogen-bond stretching. Three modes have been observed—at 63, 90, and 112 cm^{-1}—for NaDNA via Raman spectroscopy. Based on experimental evidence, Nielsen *et al.* [34,35] have proposed that the 90-cm^{-1} mode of NaDNA involves a shearing motion of the two

bases relative to one another, stretching the interconnecting hydrogen bonds. For the NaDNA-net complex, two modes are observed between 60 and 200 cm^{-1}: at 65 and 175 cm^{-1}. It is interesting to note that the 63-cm^{-1} mode of NaDNA should be virtually unchanged by the formation of the complex (just like the 25-cm^{-1} mode), while the other two modes (at 90 and 112 cm^{-1}) have been modified substantially. It is possible that the mode at 175 cm^{-1} in the NaDNA-net complex corresponds to either the 90- or 112-cm^{-1} modes of NaDNA shifted to higher frequency.

Figure 5 shows the ir spectra for NaDNA and the NaDNA-net complex from 400 to 1800 cm^{-1}. In marked contrast to the Raman spectra (where no correspondence could be identified), these ir spectra are very similar, with the distinct exception of the glycosidic region from about 1350 to 1450 cm^{-1}. Taillander and co-workers have studied the absorption of DNA-net complexes in the MIR in aqueous solution [36,37]. In the region of the vibrations sensitive to the conformation of the sugar ring (near 850 cm^{-1}) our spectra resemble their despite the large difference in the intermolecular interactions. The absorption at 835 cm^{-1} in our films indicates that the DNA is in the *B* conformation (as does their 842-cm^{-1} band). These results are consistent with earlier experimental evidence [20] that netropsin suppresses the formation of the *A* conformation. Figure 6 shows the MIR spectrum for the NaDNA-net complex at 0% RH. The 835-cm^{-1} band is present in this spectrum as well, indicating that the DNA is in the *B* conformation. This is the first observation of the *B* conformation without the presence of *any* water. This observation corroborates and validates the results of Powell *et al.* [6] on the FIR spectrum of poly(dA)-poly(dT) (dA denotes a nucleotide with deoxyribose and adenine and dT denotes a nucleotide with deoxyribose and thymine). Working in vacuum, they observed sharp low-frequency features in DNA that could only be explained by high crystallinity, which requires an intact double helix. The connection between poly(dA)-poly(dT) and NaDNA-net is that the spine of hydration in poly(dA)-poly(dT) plays the role of netropsin in NaDNA-net. A relative-humidity study of poly(dA)-poly(dT) clearly indicates the existence of ordered base

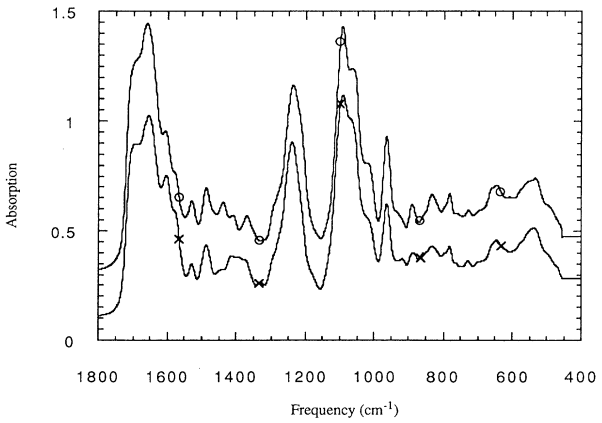

FIG. 5. MIR absorption spectrum of wet-spun films of NaDNA-netropsin (○) and NaDNA (×) at low RH.

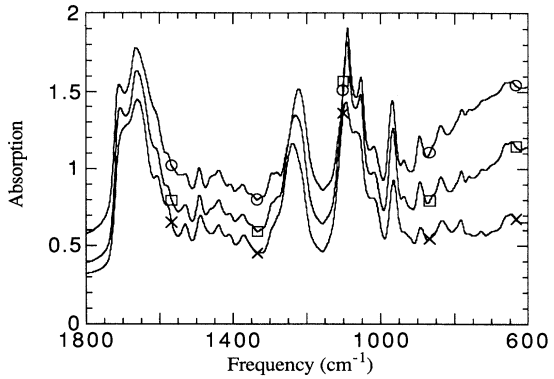

FIG. 6. MIR absorption spectra of wet-spun films of NaDNA-netropsin for 0% (×), 75% (○), and 94% RH (□). Note that the 75% and 94% RH data have been shifted by 0.4 and 0.8 units, respectively, for clarity.

stacking (as indicated by the presence of the 1714-cm^{-1} feature at 0% RH even if the sugar-ring vibrations are unusual [38].

The slight change in the shape of the nominal 1696-cm^{-1} feature upon binding of netropsin agrees with the previous results [37]. The 922-cm^{-1} band in the DNA film is greatly broadened in the spectrum from the NaDNA-net film. This is not observed in the solution studies of Liquier *et al.* [37].

The frequency region of the ir spectra corresponding to netropsin-base interactions shows marked differences between NaDNA alone and the NaDNA-net complex. From 1361 to 1440 cm^{-1} the NaDNA-net spectrum is distinctly more structured: bands exist at 1361, 1414, and 1439 cm^{-1}. The NaDNA-net band at 1414 cm^{-1} is intermediate in frequency to a 1420-cm^{-1} band observed in the copolymer dG-dC (dG denotes a nucleotide with deoxyribose and guanine and dC denotes a nucleotide with deoxyribose and cytosine) in *B* form and the 1410-cm^{-1} band of the same copolymer in the *Z* form [39]. Similarly, the NaDNA-net band at 1361 cm^{-1} is intermediate between the 1364-cm^{-1} band of the *B* form copolymer dG-dC and the 1354-cm^{-1} band of the *Z* form of the same copolymer [39]. The 1439-cm^{-1} band of the NaDNA-net complex does not appear to have a corresponding band in the copolymer dG-dC. The *Z* form of poly(dG-dme5C) exhibits a mode at 1447 cm^{-1}, but it has been assigned to a methyl deformation [39]. Poly(dA-dC)·poly(dG-dT) with $NiCl_2$ exhibits an ir band at 1434 cm^{-1} that has been interpreted as the only evidence of the *Z* conformation on the A-T base pair [39]. In the same frequency range the NaDNA film shows a very broad absorption that is dramatically weaker than the band of NaDNA-net.

Figure 7 shows the FIR spectra for netropsin, NaDNA, and NaDNA-net complex. There is much more structure in the FIR spectrum of netropsin than in NaDNA-net complex, and the FIR spectrum of the NaDNA-net complex has more structure than the spectrum of the DNA alone. Sharp FIR spectra indicate a high degree of crystallinity in the sample. The fact that the FIR spectrum of NaDNA-net complex has narrower features than the FIR spectrum from NaDNA suggests

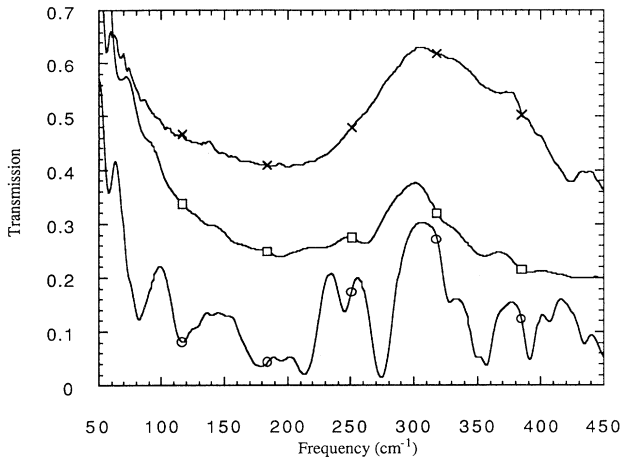

FIG. 7. FIR transmission spectra of wet-spun films of netropsin (○), NaDNA-netropsin (□), and NaDNA (×) at low RH. Note that 0.2 units have been added to the NaDNA-netropsin data for clarity.

that the NaDNA-net sample has greater crystallinity. As in the case of the Raman data, the FIR spectrum of the NaDNA-net complex is not a simple summation of the NaDNA alone with the netropsin. There are three frequencies at which absorptions exist in both netropsin and the NaDNA-net complex: 193, 356, and 390 cm^{-1}. Absorptions with small shifts (<5 cm^{-1}) between netropsin and the NaDNA-net complex (respectively) are observed for two modes: 138 and 142 cm^{-1}, and 323 and 327 cm^{-1}. The netropsin modes at 116, 178, 246, 406, and 433 cm^{-1} are not observed in the NaDNA-net complex. The mode at 272 cm^{-1} in netropsin is probably evident in the NaDNA-net complex at 261 cm^{-1}. Whether the shift to lower frequency is due to mass loading or weakening of the force constant cannot be determined without detailed knowledge of the eigenvector.

Weidlich *et al.* [40] and Bennett *et al.* [41] reported changes in the FIR spectra of poly(rI)-poly(rC) (rI denotes a nucleotide with ribose and inosine and rC denotes a nucleotide with ribose and cytosine) and salmon testes DNA for several counterions: Li, Na, K, Rb, and Cs. Extracting the frequencies of modes for the different counterions from the published data and comparing them to the NaDNA-net data reported here (Fig. 7) provides an alternative explanation for the FIR NaDNA-net spectrum. The Na-poly(rI)-poly(rC) spectrum has few features and is very similar to the NaDNA spectrum shown in Fig. 7. The K-, Rb-, and Cs-poly(rI)-poly(rC) show modes at 230 and 260 cm^{-1}, similar to the observations from the NaDNA-net complex. This suggests that netropsin's effect on the FIR spectrum is similar to the effects of these (K, Rb, and Cs) counterions.

CONCLUSIONS

The high-frequency Raman data from netropsin, NaDNA, and the NaDNA-net complex are very different, indicating a substantial modification of the vibrational dynamics by the formation of the complex. The low-frequency Raman, MIR, and FIR data for the three systems all show smaller, but still significant, differences. The FIR data suggest that the NaDNA-net films have regions of higher crystallinity. Some of the FIR modes observed from the NaDNA-net films are similar to those described in earlier reports involving nucleic acids with K, Rb, and Cs counterions. This is unexpected given the enormous differences in the chemical structure and the binding sites of the counterions. The profound effect of netropsin on stabilizing the *B* conformation has been corroborated: the *B* conformation is observed at 0% RH. These data provide important information for understanding the microscopic nature of the interaction between DNA and therapeutic drugs. It is hoped that these data will be used in theoretical calculations of the vibrational dynamics of this important system.

ACKNOWLEDGMENTS

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