NMR Studies of a Plant Flavonoid – DNA Oligonucleotide Complex

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A structural model of the solution complex between a flavonoid and a DNA dodecamer containing the *E. coli wild-type lac* promoter sequence (TATGTT) was obtained using simulated annealing for refinement. The distance constraints were derived from NOESY NMR spectra. The minor groove binding of this flavonoid displays possible hydrogen bonds to the DNA, and these can take part in complex formation. This work is the first description of how a molecule of this class of natural compounds may interact with DNA.

The flavonoids[1] are ubiquitous in vascular plants. Their functions are often associated with protection from UV light, insects, fungi, bacteria and viruses.[2] Pharmacological effects of flavonoids include their ability to inhibit the release of histamines,[3] the adhesion of blood platelets,[4] acting as an antitumor drug with activity against murine and human solid tumors *in vitro* and *in vivo*,[5] blocking the inflammatory effects of hepatotoxins,[6] and acting as a heart stimulant.[7] The variety of effects, and the markedly different activities of various flavonoids, suggest that a range of targets are involved. For instance, some flavonoids have been found selectively to inhibit viral RNA replication; however, the precise mechanism remains largely unknown.[8]

On the basis of the various biological effects of flavonoids the compound kaempferol 7-neohesperidoside, K 7-neo (Fig. 1 top), was chosen for an interaction study with a bacterial promoter DNA, the *E. coli wild-type lac* sequence TATGTT. This promoter sequence has been investigated[9] as part of the dodecamer d(GCGTATGTTGCG) · d(CGCAACATACGC). In the previous study the DNA ^{1}H NMR spectrum was assigned, and distance geometry calculations showed that the structure is within the B-DNA family. Spectral overlap of resonances in several sugar residues precluded the determination of an unique *wt-lac* promoter DNA structure. Hence, in this study idealized DNA coordinates[10] were used in the distance geometry calculations to obtain a model of the kaempferol 7-neohesperidoside/DNA complex (K 7-neo/DNA) in solution.

The two-dimensional nuclear Overhauser enhancement NMR (NOESY) experiment is capable of revealing spatial relationships among protons in a molecule or in a complex of molecules.[11] This experiment utilizes the dipolar interaction between protons closer in space than about 5 Å (an r^{-6} relationship, where r is the distance between protons). The dipolar interaction also depends on how rapidly the molecule tumbles in solution, and thus on molecular mass. A low molecular mass molecule tumbles rapidly in (a non-viscous) solution, and will be monitored by NMR experiments in the extreme narrowing (motional) regime. A molecule in the extreme narrowing regime will give rise to negative NOESY cross-peaks. Conversely, a high molecular mass molecule will be monitored by NMR experiments in the slow motion regime, where NOESY cross-peaks are positive.[11,13] The mixing time in the NOESY experiment is the time period when the dipolar interaction among protons in the molecule takes place. At short NOESY mixing times proton pairs can be approximated to be in the initial buildup rate of cross-relaxation, and then the cross-peak volumes can be converted (by the r^{-6} relationship) to obtain a proton–proton distance.[12] The cross-peak volume of a proton pair with a known, fixed (covalent) distance separation may be used as a yardstick.

Experimental

The DNA sample was synthesized as described previously.[9] About 19 mg of the DNA (duplex) were dissolved in 0.4 ml 20 mM phosphate buffer (pH 7) and 50 mM NaCl. K 7-neo (purchased from Carl Roth GmbH, Karlsruhe, Germany) was used without further purification. Deuterated water was found to be a poor solvent for pure K 7-neo (less than 0.04 mg dissolved in 0.4 ml D_2O). The K 7-neo/DNA complex was obtained by adding K 7-neo to the DNA sample in a 1 : 1 molar

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 1  2  3  4  5  6  7 8  9 10 11 12
5'- G-C-G-T-A-T-G-T-T-G-C-G
    C-G-C-A-T-A-C-A-A-C-G-C -5'
24 23 22 21 20 19 18 17 16  15 14 13
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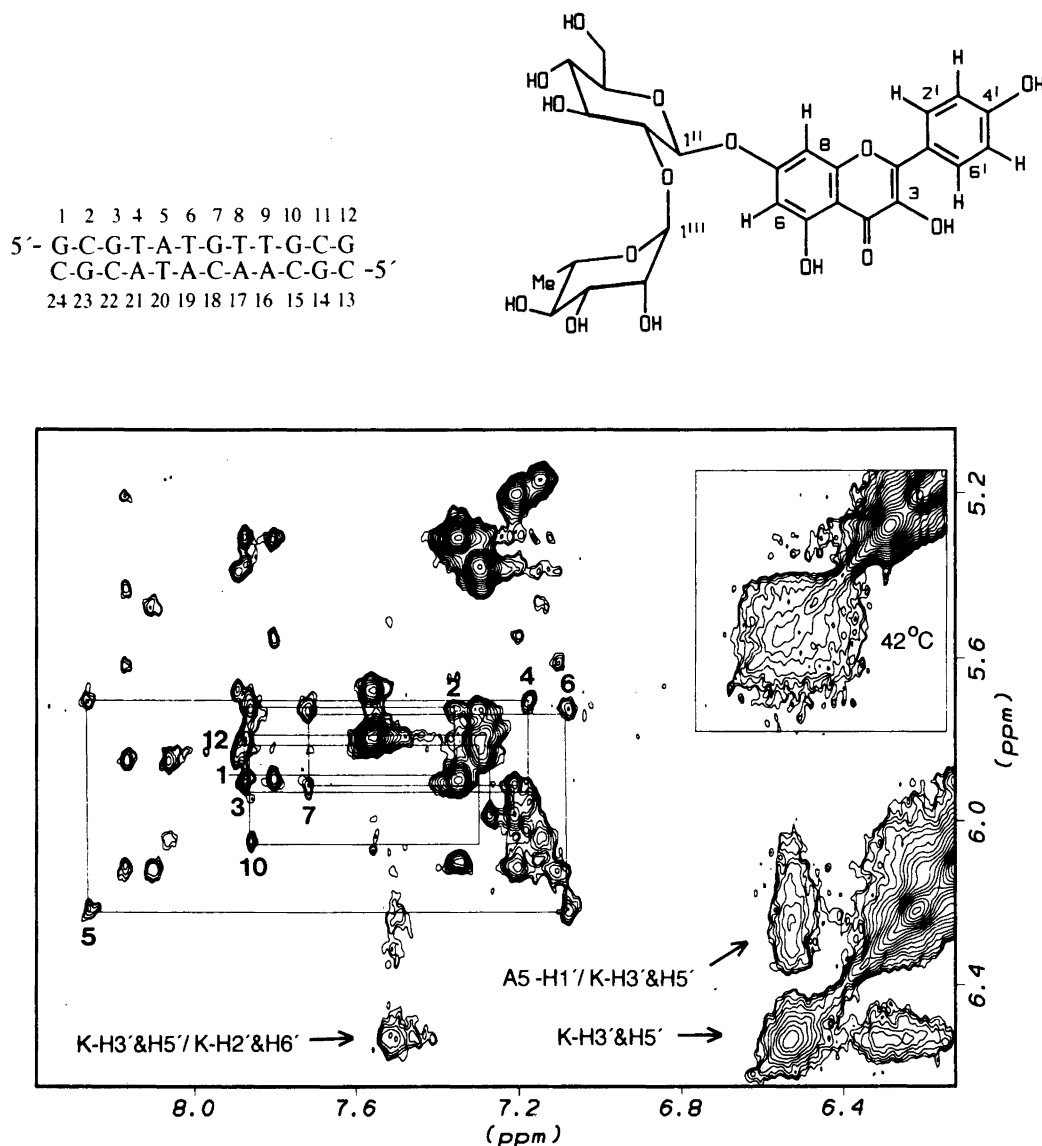
Fig. 1. Portion of the NOESY spectrum of the dodecamer DNA/kaempferol 7-neohesperidoside complex at 23°C (mixing time 0.18 s) containing the DNA base-proton to DNA sugar-H1' 'sequential walk' region. The molecular skeleton of kaempferol 7-neohesperidoside (K 7-neo) and the base sequence of the DNA are given on top of the figure. The 'sequential walk' for the d(GCGTATGTTGCG)-strand (residues 1–12) is indicated by vertical and horizontal lines connecting cross-peaks. Intra-residue base-proton to sugar-H1' cross-peaks are labelled by numbers (with the exception of residues 8, 9 and 11), and the unlabelled cross-peaks in the 'sequential walk' are inter-residue, base-proton (residue n) to sugar-H1' (residue n − 1) cross-peaks. The corresponding assignments for residues 13–24 were carried out analogously; however, they are not indicated in the figure for reasons of clarity. These assignments were consistent with those obtained for the DNA alone. K 7-neo displays one intra-aglycone cross-peak (H3' to H2' overlapped with H5' to H6') labelled K-H3' & H5'/K-H2' & H6', and one intermolecular cross-peak to adenine(5)-H1' labelled A5-H1'/K-H3' & H5'. The K-aglycone H3' (and H5') diagonal peak is labelled K-H3' & H5'. The box inserted in the upper right corner displays the diagonal region of the K-H3' & H5' and DNA A5-H1' resonances in the corresponding NOESY spectrum at 42°C. This demonstrates the exchange broadening of the K-aglycone resonances.

ratio. The 1D NMR experiments on the exchangeable protons were carried out on the K 7-neo/DNA sample dissolved in 90% H_2O and 10% D_2O. The ^1H NMR experiments were carried out at 400.13 MHz on a Bruker AM-400 instrument. The residual ^1H signal from the solvent was used as secondary chemical shift reference. NOESY spectra were obtained at 23°C (0.10, 0.18 and 0.25 s) and 42°C (0.18 s), respectively (mixing times are

given in parentheses), with the method of time-proportional phase increments. The NOESY spectra were acquired with a spectral width of 3495 Hz, 2048 complex points, 620–844 free induction decays (FIDs) and 96 transients per FID (the experiments at 9°C and the 0.10 s mixing time experiment at 23°C) or 64 transients per FID. The data were processed on a Silicon Graphics SGI-4D/25 workstation with the program FELIX (Hare

Research, Inc., Woodinville, WA). The distance geometry program[9,14–16] DSPACE (Hare Research, Inc.) was used in determining the structure of the K 7-neo/DNA complex. The starting structure for the calculations was K 7-neo separated by 10 Å from the DNA in the idealized coordinates.[10] The structure of K 7-neo is based on X-ray crystallographic data.[17–20] The DNA coordinates were kept fixed during the calculations, and the K 7-neo coordinates were moved (optimized) by simulated annealing and conjugate gradient refinement.[9]

Results and discussion

The ¹H NMR-spectrum of K 7-neo was assigned both in methanol and in water (not shown), and the rapid tumbling[13] of this low molecular mass compound (595 amu) was confirmed by the existence of weak negative two-dimensional nuclear Overhauser (NOESY) NMR cross-peaks in methanol. The molecular mass of the DNA dodecamer, on the other hand, is 7000 amu, and the tumbling in solution of this macromolecule is slower i.e. the intramolecular DNA cross-peaks are strong and positive. Upon addition of the flavonol to the DNA sample (in a 1 : 1 molar ratio), both K 7-neo intramolecular cross-peaks and intermolecular K 7-neo to DNA corss-peaks

were observed (Fig. 1). The positive sign of these cross-peaks confirmed that a slow tumbling K 7-neo/DNA complex was formed.

Assignments of the cross-peaks were carried out by a 'sequential walk' in the base-proton to deoxyribose (DNA) 1'H region (Fig. 1) and the base-proton to deoxyribose 2'H and 2"H regions (not shown) in the NOESY contour map. Apart from some minor chemical shift differences, these assignments are in full agreement with those of this DNA in the pure state at 42°C. The fact that the inter-residue cross-peaks in the 'sequential walk' (Fig. 1) are in agreement with the corresponding cross-peaks of this DNA alone, indicates that no base-pair separation occurs in the K 7-neo-bound DNA. Thus K 7-neo binds in a non-intercalative way to this dodecamer.[21] This is also supported by the NMR spectrum of the exchangeable DNA protons. Figure 1 shows the NOESY spectral region in which intramolecular K 7-neo aglycone (K-aglycone) cross-peaks and K-aglycone to DNA cross-peaks are found at a sample temperature of 23°C. At a higher temperature (42°C) the K-H3' and H5' diagonal peak of the resolved K-aglycone is broadened due to exchange, and all cross-peaks from this diagonal peak are broadened beyond detection (see inserted box in Fig. 1). The lifetime of K 7-neo bound
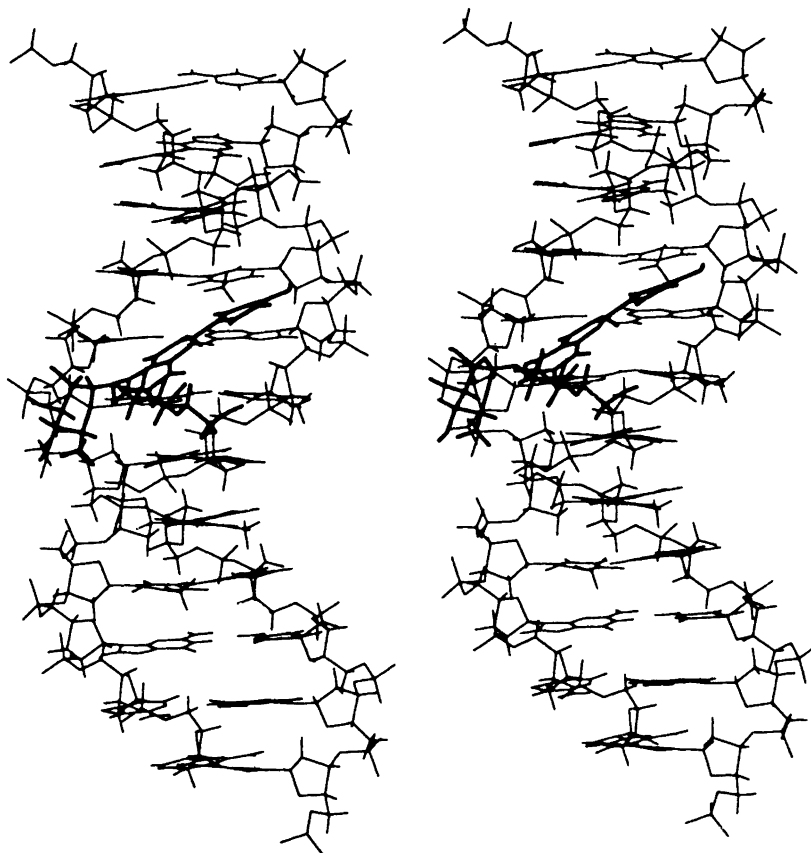

Fig. 2. Stereo-view of the molecular model of kaempferol 7-neohesperidoside (bold lines), K 7-neo, located in the minor groove of the dodecamer DNA. The structure satisfies observed NOESY cross-peaks and is without intermolecular van der Waals overlaps.

to the DNA dodecamer is in the range of tens of milliseconds, estimated from the lineshapes.[22]

The A5-H1'/K-H3' & H5' cross-peak (Fig. 1) locates the K-aglycone in the minor groove in vicinity of adenine (5). More precisely, this cross-peak shows that the distance between K-H5' (or K-H3') and A5-H1' is shorter than 5 Å. Owing to chemical shift overlap (see peaks labelled K-H3' and H5' in Fig. 1) and the effects of chemical exchange, obtaining an accurate K-H5' to A5-H1' (or K-H3' to A5-H1') distance from the cross-peak volume by the r^{-6} relationship is not possible. However, the A5-H1'/K-H3'&H5' cross-peak was calculated to correspond to a single proton pair separated by a distance of 2.5–3.5 Å. The width of the minor groove in idealized B-DNA can not accomodate the K-aglycone when both the K-H5' and K-H3' are equally close to the adenine (5)-H1', i.e. the short K-aglycone axis spans more than the width of the minor groove. In order to find the probable orientation(s) of the K-aglycone in the minor groove, molecular modelling was carried out by two different rounds of distance geometry calculations.[14–16,23,24] First the K-H3' to A5-H1' distance of 2.5–3.5 Å and second the K-H5' to A5-H1' distance of 2.5–3.5 Å was used as the experimental constraint in the calculations. The molecular models from these distance geometry calculations showed that the favourable orientation of the K-aglycone in the *wt-lac* minor groove is such that K-H5' is close to A5-H1', and K-H3' is at the outside of the minor groove (Figs. 2 and 3). Furthermore, the way in which the deoxyribose-H1' is positioned in the minor groove of (idealized) B-DNA necessitates K-aglycone to

be oriented along the DNA strand of residues 1–12 in the 3'-direction of adenine(5).

The detailed structural features in the model of the K 7-neo/DNA complex shown in Fig. 3 explain the observed adenine(5)/K-aglycon cross-peaks, A5-H1'/K-H5' and A5-H2/K-H6', in that these intermolecular distances are 3.2 and 2.8 Å, respectively. (The A5-H2/K-H6' cross-peak is close to the NOESY diagonal in a region not displayed in Fig. 1.) The model also shows the feasibility of H-bond formation between K 7-neo and DNA O2 in the complex; the K-HO3' to O2 distance in thymine(6) is 2.8 Å (see arrow in Fig. 3). Hydrogen bonds between other K-OH groups and DNA bases and DNA phosphate groups may also take part in the complex formation; these are not explicit in the model (Figs. 2 and 3). Furthermore, the K 7-neo intra-sugar cross-peaks are exchange-broadened at 23°C, and at 42°C these positive cross-peaks are line-narrowed (not shown). This demonstrates higher mobility of the K 7-neo sugars compared to the K-aglycone when K 7-neo is associated to the DNA dodecamer, thus supporting the structural features of the model (Fig. 2). A similar mode of DNA binding has earlier been reported for some antibiotics,[25–27] and the finding in this study should promote the search for potent antibiotics/antitumor agents among plant metabolites such as the flavonoids. Future studies will undoubtly include possible structural modifications of idealized B-DNA in complexes such as the present one.
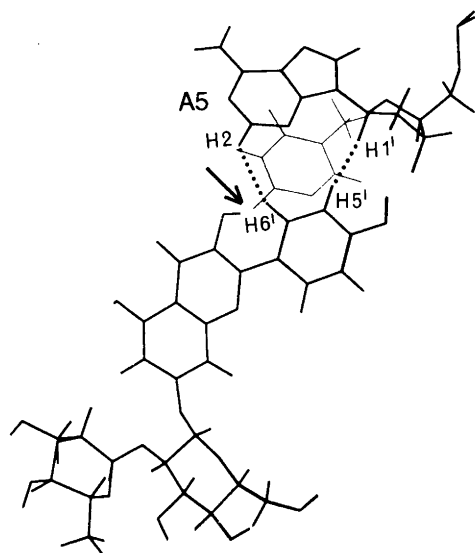

Fig. 3. Structural details of the kaempferol 7-neohesperidoside located in the minor groove in vicinity of adenine(5) of the DNA. The two dotted lines indicate intermolecular proton-pair distances that correspond to observed NOESY cross-peaks; the left between A5-H2 and K-H6', and the right between A5-H1' and K-H5'. Arrow points to the K-O3'H ··· O2-T6 hydrogen bond that is feasible in the model.

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