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Carbohydrate Research 338 (2003) 1523–1525
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Note

Crystal structure of chartreusin derivative A132

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Received 24 February 2003; accepted 15 April 2003

Abstract

The crystal structure of chartreusin derivative A132 (benzilidene chartreusin) has been determined by single-crystal X-ray diffraction. The space group is $C2$ with unit cell dimensions, $a = 18.482(4)$, $b = 8.749(3)$, $c = 43.906(2)$ Å, $\beta = 94.87(2)^\circ$, and the structure was refined to R-factors of 0.2365 (6585 all unique reflections) and 0.087 (2914 reflections with $F_o > 4\sigma(F_o)$) by a full-matrix least-squares method. There are two molecules in an asymmetric unit. Both molecules have similar structures, which are favorable to bind with DNA in the minor groove. A modeling study of the A132–DNA complex based on the X-ray structures suggests that the sugar moiety of A132 may play an important role in recognizing the sequence of DNA base pairs.

Keywords: Crystal structures; DNA; Intercalation; Chartreusin

Chartreusin is an antitumor antibiotic isolated from *Streptomyces chratreusis* that has,[1] a chromophore ring and a sugar moiety with D-fucose and digitalose, as shown in Fig. 1. It has been revealed that chartreusin inhibits transcription and replication by binding to DNA[2–5] with the specificity to the base pair sequence of 5′-(CGC)-3′.[6] Since chartreusin has not been developed clinically because of rapid excretion into the bile, many derivatives of chartreusin have been synthesized for clinical trials. A132 is one of such derivatives developed by Ishihara Sangyo Kaisya Ltd. Company.[7] A132 has an additional benzilidene group to chartreusin, as shown in Fig. 1, and it exhibits its antitumor activity by binding to DNA, as does chartreusin.[7] Structural information is very useful to understand the DNA-binding mode of A132 and chartreusin. Here we report the crystal structure of A132 and the modeling of the A132–DNA complex.

The structure of A132 can be divided into a planar chromophore ring and a sugar moiety, having two pyranose rings and one phenyl ring. For clear discussion, four rings are defined as ring A (chromophore), B (D-fucose), C (digitalose) and D (phenyl) as shown in Figs. 1 and 2.[8] Two molecules (Mol-1 and Mol-2) exist in an asymmetric unit, making stacking interactions between planar chromophore rings and a hydrogen bond between OH-4′ and OH-7, as shown in Fig. 2. To compare overall structures of Mol-1 and Mol-2, the dihedral angles between pairs of rings were measured by calculating the best plane of each ring. Four selected angles are listed in Table 1. Interestingly, the angles involving A, B and C of Mol-1 are almost the same as those in Mol-2, giving a similar overall structure for this domain to both molecules. Since no strong hydrogen bond is present among these rings, methyl and hydroxyl groups in a sugar moiety may fix the conformation of the two glucosidic bonds in A–B and B–C. Remarkable differences between Mol-1 and Mol-2 are found in the orientation of the phenyl rings: the interplanar angle between D and C in Mol-1 is 21°, while that in Mol-2 is 65°. In the crystal, each phenyl ring stacks on the pyranose ring of the other molecule differently. Perhaps this packing effect is the reason why the phenyl rings have different orientations.

Daunomycin and adriamycin are also DNA-binding compounds having a chromophore ring and a sugar moiety. The X-ray structure of the daunomycin–d(CGTACG) complex[9] shows that the chromophore

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0008-6215/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0008-6215(03)00200-3

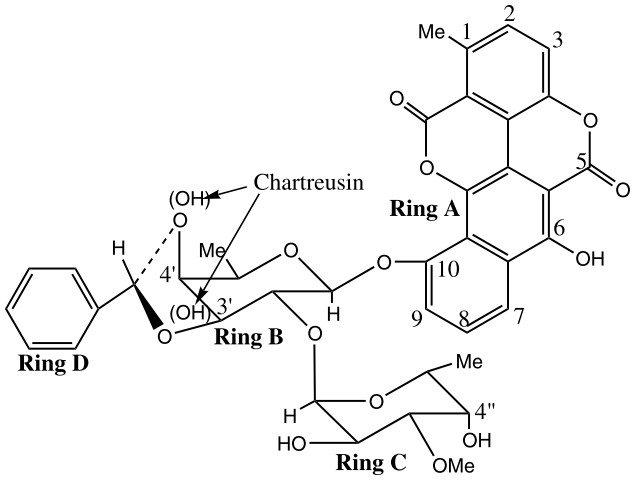

Fig. 1. Chemical structures of chartreusin and A132 are shown. Four rings are defined as ring A (chromophore), ring B (D-fucose), ring C (digitalose) and ring D (phenyl). Selected atom numbering is also indicated.

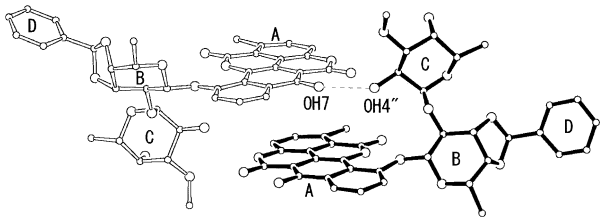

Fig. 2. Two molecules in an asymmetric unit are illustrated by the program ORTEP.[8] Mol-1 and Mol-2 are shown by solid and open bonds, respectively. For clarity, hydrogen atoms and solvent molecules are not illustrated. Carbon and oxygen atoms are shown by small and large circles, respectively. A hydrogen bond between Mol-1 and Mol-2 is indicated by a dotted line.

Table 1
Selected interplanar angles (°)

Planes	Mol-1	Mol-2
A–B	57	47
A–C	106	93
B–C	52	47
B–D	21	65

rings intercalated between base pairs of 5′-CG-3′ and that a sugar moiety interacts with the minor groove of DNA. Considering its structural similarity with daunomycin, A132 is also expected to intercalate between the base pairs from the minor groove of DNA. Based on the X-ray structures of A132 and the daunomycin–d(CGTACG)$_2$ complex,[9] modeling of the A132–d(CGTACG)$_2$ complex was attempted by structural energy minimization using the program CNS.[10] Since Mol-1 and Mol-2 have similar structures except for the orientation of the phenyl ring, using Mol-1 and/or Mol-2 as a starting model gave an almost equivalent model for the A132–DNA complex. This model is shown in Fig. 3. The chromophore ring intercalates between the base pairs of 5′-(CG)-3′, and the sugar moiety nicely fits to the minor groove of DNA. A hydroxyl group (OH-4′) of the digitalose (ring C) possibly hydrogen bonds with the amino group (N-2) of guanine and the carbonyl group (O-2) of cytosine in the base pair on one side of intercalation. Additionally, a hydroxyl group (OH-7) of the chromophore hydrogen bonds with the O-4 atom of deoxyribose to support the binding of A132 to DNA. Since there is no specific hydrogen bond between the chromophore and the base pairs, the sugar moiety is thought to be responsible for the base pair sequence specificity of A132. Further, as in the cases of daunomycin and adriamycin,[11] no hydrogen bonds are found between A132 and the neighboring base pairs in our model. Further investigations are required to reveal the DNA-binding specificity of chartreusin to 5′-(CGC)-3′.

1. Experimental

The synthesis of A132 has already been reported.[7] Crystals were prepared by dissolving 20 mg of A132 in acetone (4 mL). By slow evaporation of this solution at 20 °C, suitable crystals for X-ray data collection were obtained. Diffraction data up to $2\theta = 140°$ were collected by a Rigaku AFC7R diffractometer on a Rigaku rotating anode generator with a graphite monochromated Cu K_α radiation using the $2\theta/\omega$ scan mode.

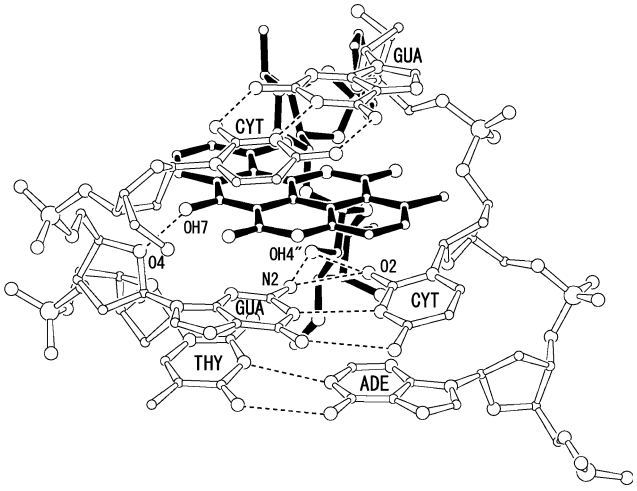

Fig. 3. The modeling structure of A132–d(CGTACG)$_2$ viewing from the major groove of DNA is illustrated by the program ORTEP[8] with the selected atom labels. For clarity, only the intercalation site is illustrated. A132 and DNA are shown by solid and open bonds, respectively. Possible hydrogen bonds are indicated by dotted lines.

Crystal data are: $C_{39}H_{36}O_{14}$–CH_3OH–H_2O, space group $C2$, $a = 18.482(4)$, $b = 8.749(3)$, $c = 43.906(2)$ Å, $\beta = 94.87(2)^\circ$, $V = 7074(3)$ Å^3, $Z = 8$. Data processing and the initial phase angle determination were performed by the teXsan system.[12] Some electron density for solvent molecules were found in a difference Fourier map, and these were assigned to methanol and water molecules. Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were introduced by geometrical calculations and not refined. The crystal structure was refined to $R = 0.2365$ (6585 reflections, all unique reflections) and $R_1 = 0.087$ (2914 reflections with $F_o > 4\sigma(F_o)$) by a full-matrix least-squares method using the program SHELX-97,[13] based on 974 parameters.

2. Supplementary information

Full crystallographic details, excluding structure features, have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 206702. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk. Web: http://www.ccdc.cam.ac.uk/conts/retrieving/html.

Acknowledgements

We thank the X-ray crystallography laboratory at Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan, for data collection.

References

1. Leach, B. E.; Calhoun, K. M.; Johnson, L. E.; Teeters, C. M.; Jackson, W. G. *J. Am. Chem. Soc.* **1953**, *75*, 4011–4012.
2. Li, L. H.; Clark, T. D.; Murch, L. L.; Wooden, J. M.; Pschgoda, L. M.; Krueger, W. C. *Cancer Res.* **1978**, *38*, 3012–3018.
3. Yagi, M.; Nishimura, T.; Suzuki, H.; Tanaka, N. *Biochem. Biophys. Res. Commun.* **1981**, *98*, 642–647.
4. Uramoto, M. T.; Kusano, T; Nishio, T.; Isono, K.; Shishida, K.; Ando, T. *FEBS Lett.* **1983**, *153*, 325–328.
5. Krueger, W. C.; Pschigoda, L. M. *J. Antibiotics* **1986**, *39*, 1298–1303.
6. Uramoto, M.; Kusano, T.; Isono, K.; Shishido, K.; Ando, T. *FEBS Lett.* **1983**, *153* (2), 325–328.
7. Kon, K.; Sugi, H.; Tamao, K.; Ueda, Y.; Yamada, N. *J. Antibiotics* **1990**, *43*, 372–382.
8. Johnson, C.K. ORTEP2, REPORT ORNL-5138, Oak Ridge National Laboratory, Tennessee, 1976.
9. Wang, A. H.-J.; Ughetto, G.; Quigley, G. J.; Rich, A. *Biochemistry* **1987**, *26*, 1152–1163.
10. Brünger, A. T.; Adams, P. D.; Clore, G. M.; DeLano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J. S.; Kuszewski, J.; Nilges, M.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren, G. L. *Acta Crystallogr. Sect. D* **1998**, *54*, 905–921.
11. Frederick, C. A.; Williams, L. D.; Ughetto, G.; van der Marel, G. A.; van Boom, J. H.; Rich, A.; Wang, A. H.-J. *Biochemistry* **1990**, *29*, 2538–2549.
12. teXsan, Crystal Structure Analysis Package, Molecular Structure Corporation, 1992.
13. Sheldrick, G.M. SHELX-97, Program for Crystal Structure Refinement, University of Göttingen, Germany, 1997.