Structural Basis for Reaction Mechanism and Drug Delivery System of Chromoprotein Antitumor Antibiotic C-1027

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Antitumor antibiotic C-1027 that is regarded as a natural model of drug delivery system, consists of a carrier apoprotein (Apo) and an enediyne chromophore (Chr). We have compared three solution structures of the DNA-Chr complex, Apo-Chr complex, and free Chr determined by high-resolution NMR experiments. The guest molecule, C-1027 chromophore, showed two distinct binding modes fitted to binding sites of the hosts (target DNA and carrier Apo). The novel Chr interacts with DNA through its benzoxazolinate and aminosugar moieties, and also with Apo through the benzoxazolinate and macrocyclic moieties. The superposition of Chrs in these three states clearly revealed conformational deviation of the 16-membered macrocyclic moiety containing intra-chlorophenol ring. *Ab initio* calculations supported good correlation between the reactivity and the conformational alteration of Chr induced in hosts. The present results provide molecular basis and implication for the host-recognition mode, the reaction mechanism, and drug delivery system of chromoprotein C-1027.

Key words: Antitumor antibiotic/ Enediyne/ Drug delivery system/ NMR structure/ DNA cleavage

To understand the molecular basis for unique biological activity of C-1027, three-dimensional structures of DNA-Chr complex, Apo-Chr complex, and free Chr are essential. We clarified solution structures of free Chr and the complex formed between the drug and DNAoligomer by NMR techniques. The NOESY measurements of Chr-DNA oligomer complex yielded a total of 144 DNA intramolecular, 33 drug-intramolecular, and 36 DNA-drug intermolecular NOEs that we were able to clearly assign. Free Chr in D_2O solution provided 14 intramolecular ROES in the ROESY. The solution structures of DNA-Chr complex and free Chr obtained with distance-restrained molecular dynamics computations gave relevant modeds that are fully consistent with the observed NMR-derived distance date. In the unbound state, restrained molecular dynamics simulation illustrated that free Chr can't fix its benzoxazolinate (BO) and aminosugar (AS) moieties in one specific monfomation without characteristic interaction. Indeed, ROEs of the BO and AS parts were

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Scope of research

The major goal of our laboratory is to elucidate the molecular basis of the activity of various bioactive substances by biochemical, physicochemical, and synthetic approaches. These include studies on the mechanism of sequence-specific DNA cleavage by antitumor or carcinogenic molecules, studies on the DNA recognition of zinc-finger proteins, and model studies on the action of ion channels. In addition, artificial designed peptides have also been developed as useful tools in molecular biology and potentially in human medicine.

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rarely detected. Since most of observed intra-ROEs of Chr arose from the 16-membered macrocyclic part (MC), the conformation of MC was restrained. Average pairwise RMS deviations among final structures were 0.75 Å for the DNA-Chr complex and 0.77 Å for free Chr. The generated structures of DNA-Chr complex and free Chr were averaged and energy-minimized.

In the Chr-DNA heptamer complex, the inter-NOEs of BO and AS moieties with DNA were in the majority (75%) of all observed intermolecular peaks. The proton chemical shit perturbations of these functionla moieties were also large upon binding to DNA. Thus, both the BO and AS parts play important roles for the recognition and binding of Chr to DNA. The DNA-Chr complex model evidently revealed that C1027-Chr interacts with each tetranucleotide of the d(C3C4A5T6)/d(A0T10G11G12) duplex through unique intercalation of the BOmoiety at the d(C3C4)/d(G11G12) step and also minor groove binding of the AS part at d(A5T6)/d(Q9T10G11). This complex is stabilized by the stacking interaction through intercalation and by the backbone helical and minor groove contacts through van der Waals interactions. The intercalation of the BO moiety was demonstrated by the NOEs connecting 1''-NH with H6, H1', H2', 2'' of C3 and H6, NH_2 of C4; OMe with H8, H1, H1' of G11; H6'' with H3' of G12. One of the two six-membered rings of C3 and C4, while the one containing methoxy group stacks on the purine rings of G11 and G12. The protruding methoxy group of BO moiety makeds van der Waals contacts with H1' of G11 sugar. Furhermore, the inter-NOEs of the AS moiety with the DNA oligomer were obzerved connecting of β-Hs (H2', H3', H4', and 5'-Meβ) with H1' and H4' of T6 and H2 of A5; α-Hs (4'-NMe and 5'-Meα) with H2 of A9, H1' and H4' of T10 and H4', H5', and H5'' of G11. There are backbone heical sugur-phosphate backbones of A5T6 and A9T10G11strands on other side. The orientation of the AS moiety permits its α^1Hs and β^1HS to face toward the A9T10-strand and A5T6-strand, respectively. A set of contacts between Chr and DNA minor groove floor was detected. These contacts include van der Waals interaction of AS H3' with DNA A5(H2) and A9(H2) and a hydrogen bond supports our previous data that the guanine 2-amino group of the central base in trinucleotide 5'-AGG plays a key role in the recognition of the DNA oligomerby by Chr. In addition, the observation of several NOEs showed the backbone helical binding between the aromatized AEB ring (H5,H6, and H8) and the DNA backbone [C4(H1'), A5 (H1' and H4'), and T6(H5')]. An intermolecular NOE cross peak between Chr-H6 and A5-H1' or -H4' revealed the prozimity of these protons. The Chr-H3 is situated close to H1' of G12, as indicated by the constructed model. Therefore, it is reasonably proposed that the DNA lesions caused by the C-1027 Chr are due to the abstraction of hydrogen atoms from A5-C1' or -C4' by the Chr-C6 radical and from G12--C1' by the Chr-C3 radical. In fact, our previous gel electrophoretic analysis evidently showed that the DNA domage occurs at CCA/TGG with a two nucleotide 3'-stagger of the reactive biradical atoms(C3 and C6) of the enediyne Chr to the vicinity of deoxyribose hydrogen atoms (H1' or H4') of the DNA backbone (A5 and G12). The AS assists in winding of the Chr around the minor groove of the DNA oligomer.

We evaluated the influence of spatial configuration of the neighboring aromatic ring on the reactivity of the biradical by an *ab initio* method (23,24). Recently, Chen and co-workers pointed out that the reactivity of p-benzyne type bieadicals depends upon the energy splitting between the singlet (S) ground state and triplet (T) excited state; the lager the S-T splitting becomes, the less reactive the singlet biradical dose. On the basis of this information, we istimated and conpared teh S-T splittings of both biradical-aromatic ring systems that are confomationally similar to C1027-Chrs in DNA and Apo. The S-T splitting of the biradical-aromaric ring system in DNA was slightly smaller than that in Apo (Table III). Accordingly, the p-benzyne type biradical in DNA is more reactive than that in Apo, because of the conformational alteration of the MC part containing chlirophenoling. It is possible for the drug to control the reactivity of hydrogen-abstraction reactions by subtle structural perturbation of the intramolecular cholorphenol ring adjacent to the reacting AEB system . On the other band, neocarzinostatin (NCS), esperamicin, calcheamicin, and kedarcidin require external cofactor (thiol) as trigger of the Bergmann reaction.