

Antitumor Eneidyne Chromoprotein C-1027: Mechanistic Investigation of the Chromophore-Mediated Self-Decomposition Pathway

Masayuki Inoue,^{*,†,‡} Toyonobu Usuki,[†] Nayoung Lee,[†] Masahiro Hiramata,^{*,†} Toshiyuki Tanaka,[§] Fumihito Hosoi,[⊥] Shinji Ohie,[⊥] and Toshio Otani[⊥]

Contribution from the Department of Chemistry, Graduate School of Science, Tohoku University, Sendai 980-8578, Japan, Research and Analytical Center for Giant Molecules, Graduate School of Science, Tohoku University, Sendai 980-8578, Japan, Institute of Applied Biochemistry, University of Tsukuba, Tsukuba 305-8572, Japan, and Hanno Research Center, Taiho Pharmaceutical Co., Ltd., Saitama 357-8527, Japan

Received January 31, 2006; E-mail: inoue@ykbsc.chem.tohoku.ac.jp; hiramata@ykbsc.chem.tohoku.ac.jp

Abstract: C-1027 is an extremely potent antitumor agent that causes double-stranded DNA cleavages. It is a unique small molecule–protein complex composed of a highly reactive enediynes chromophore, which upon binding reacts with its target molecule DNA through radical-mediated hydrogen abstraction and an apoprotein that encapsulates the chromophore serving as its carrier to reach DNA. Although C-1027 has favorable properties as an effective drug delivery system, it slowly self-decomposes due to the reactivity of the chromophore toward the apoprotein. Understanding how the C-1027 destroys itself may enable design of its analogues that overcome this limitation. In this paper, mechanistic insights into the self-reactivity of C-1027 that facilitates its own decomposition are described. We provide evidence that the formation of the Gly96 radical, which promotes the oxidative protein scission and the subsequent chromophore release, is the major pathway for the self-decomposition of C-1027. On the basis of the newly isolated products of the self-decomposition, we propose that the apoprotein effectively protects two different structural elements of the chromophore that are essential for its biological activity: the nine-membered enediynes moiety (necessary for DNA cleavage) and the benzoxazine moiety (necessary for DNA intercalation). Using an engineered apoprotein analogue kinetically more stable toward the chromophore radical, we show that enhanced overall properties can be achieved for the natural C-1027 with respect to stability and antitumor activities. The results present the first example of a rationally designed C-1027 analogue reported to display superior in vitro antitumor activity to the natural C-1027. Our findings may have implications for design of proteins that can stably encapsulate highly reactive small molecules.

Introduction

C-1027 is an extremely potent antitumor antibiotic isolated from *Streptomyces globisporus* C-1027. It has been shown to display highly effective cytotoxicity against cultured cancer cells as well as marked growth inhibitory effect against transplantable mouse and human tumor cells inoculated into nude mice.¹ The cytotoxicity of C-1027 is believed to originate from its highly selective DNA-binding property and the DNA-damaging reactivity. Like other macromolecular enediynes antibiotics, such as neocarzinostatin,^{2,3} kedarcidin,⁴ and maduropeptin,⁵ C-1027 is composed of a highly reactive enediynes chromophore in complex with an apoprotein (**1**, Figure 1).^{6–9} The apoprotein

(10489 Da) is a single polypeptide chain of 110 amino acid residues cross-linked by two disulfide bonds:¹⁰ **1** is bound noncovalently in a cleft of the apoprotein. The solution NMR structure of the complex formed between **5** and the apoprotein (Figure 2)¹¹ showed that most of the chromophore–apoprotein contacts are made between the hydrophobic groups of chro-

[†] Department of Chemistry, Graduate School of Science, Tohoku University.

[‡] Research and Analytical Center for Giant Molecules, Graduate School of Science, Tohoku University.

[§] Institute of Applied Biochemistry, University of Tsukuba.

[⊥] Hanno Research Center, Taiho Pharmaceutical Co., Ltd.

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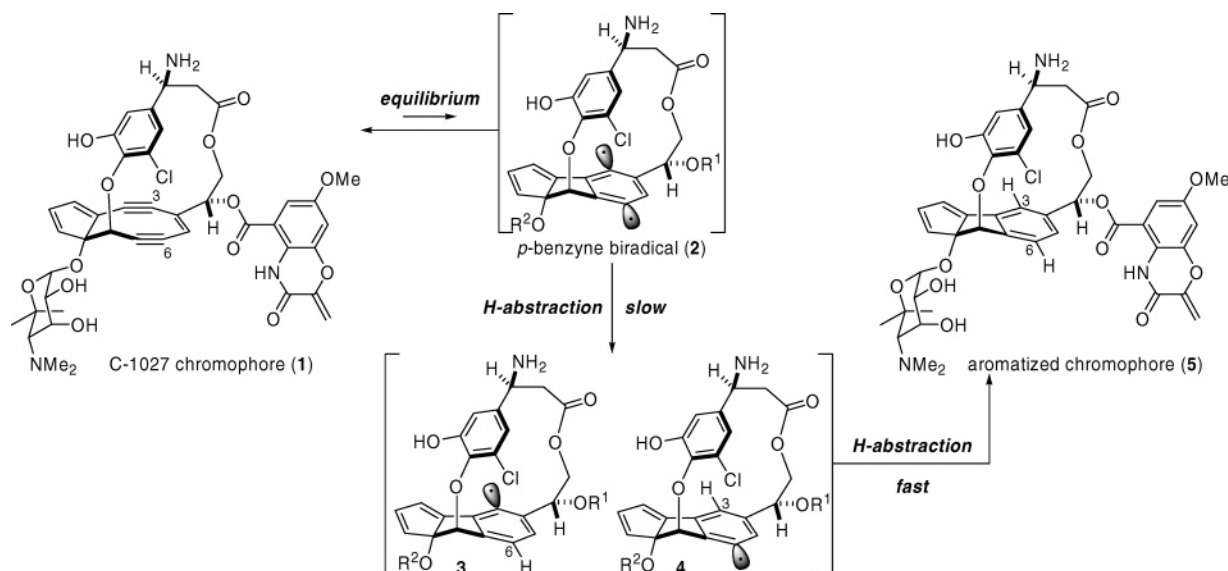


Figure 1. Structures of the C-1027 chromophore (1), *p*-benzyne biradical (2), phenyl radicals (3, 4), and the aromatized chromophore (5).

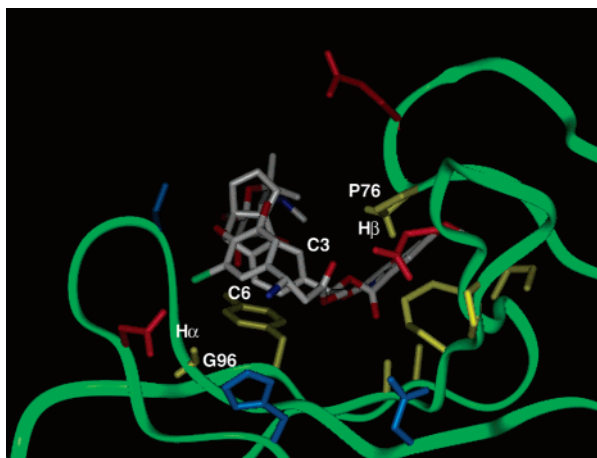


Figure 2. NMR structure of the aromatized chromophore (5) binding site in the C-1027 apoprotein (ref 11, PDB accession number 1HZL). The side chains that protrude into the binding site are shown, and acidic, hydrophilic, and hydrophobic residues are colored red, blue, and yellow, respectively. Radical centers (C3 and C6) and possible hydrogen sources (H α of Gly96 and H β of Pro76) are labeled.

mophore **1** (the macrocyclic core and the benzoxazine) and the hydrophobic side-chains of the apoprotein.¹²

Chromophore **1** is a member of the enediynes structural family, which is characterized by two acetylenic groups conjugated to a double bond within a nine- or ten-membered ring.^{13–15} In contrast to all other enediynes natural products known, which

require external activators such as nucleophiles to initiate the electronic rearrangement, the nine-membered enediynes moiety of **1** spontaneously aromatizes at room temperature via a Masamune–Bergman rearrangement¹⁶ without an activator (**1**→**2**→**3/4**→**5**, $t_{1/2} = 0.8$ h in ethanol). Consequently, when not bound to the apoprotein, **1** displays the highest reactivity among the enediynes family. The Masamune–Bergman cyclization step is known to be rate-limiting for synthetic noncyclic enediynes.^{16b,c} On the other hand, **1** displays unique reactivity as suggested by our previous studies of **1** and related model compounds that it is in equilibrium with **2** and that the hydrogen abstraction by the cyclized *p*-benzyne intermediate (**2**→**3/4**) is the rate-limiting step.^{17–19}

Structural and biochemical studies have revealed that two structural elements of chromophore **1**, the benzoxazine moiety and the nine-membered enediynes moiety, play crucial roles in effecting the biological activity of C-1027. Sugiura et al.

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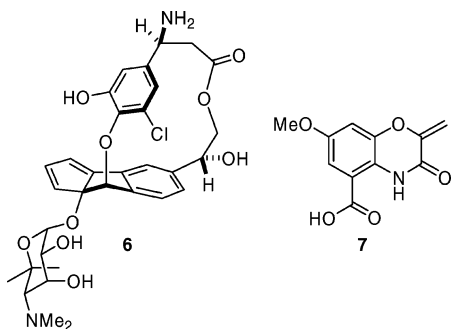


Figure 3. Structures of the hydrolyzed aromatized chromophore (**6**) and the benzoxazine moiety (**7**).

determined the high-resolution NMR structure of the complex between the aromatized chromophore **5** and double-stranded DNA²⁰ and proposed that the enediyne portion of **1** is positioned in the minor groove of the DNA and the benzoxazine moiety is oriented parallel to the DNA bases. The importance of the benzoxazine moiety **7** (Figure 3) was shown by Dedon that its removal from **1** (see **6**)^{6a} results in loss of DNA binding affinity by ~400-fold.²¹ Chromophore **1** interacts with DNA in a highly sequence-selective manner. The *p*-benzyne biradical **2**, generated through the rearrangement, abstracts hydrogens of the DNA backbone sugar accessible in the minor groove. This ultimately leads to oxidative cleavage of the double-stranded DNA.²² Goldberg reported that the double-strand lesions are specific to certain five-nucleotide sequences, such as CTTT/AAAAG, ATAAT/ATTAT, CTTA/TAAAG, CTCT/AAGAG, and especially GTTAT/ATAAC (cutting sites are underlined).²³

C-1027 is remarkable because the apoprotein stabilizes the radical-generating chromophore by tight binding, and yet the chromophore can be released to react upon reaching its target DNA in the cell nucleus. While the reactivity of the chromophore by itself in organic solvent or toward DNA is relatively well studied, the ways in which apoprotein modulates the highly reactive chromophore in biological settings is not well understood.

Considering that **1** constantly generates **2** even when bound to the apoprotein, hydrogen abstraction from the apoprotein by **1** might be expected to lead to its facile decomposition before reaching its biological target. The NMR structure of the complex formed between **5** and the apoprotein suggests that the radical σ -orbitals at C3 and C6 of **2** would be positioned perpendicular to the proximal hydrogens (H β -Pro76 and H α 1-Gly96) such that the hydrogen abstraction is unfavorable (Figure 2).²⁴ By positioning the *p*-benzyne radical with low accessibility to hydrogen sources, the apoprotein kinetically stabilizes the enediyne moiety, thereby preventing the chromophore–protein complex from self-decomposition. Once encapsulated stably in the apoprotein, the highly reactive **1** can be transported by the apoprotein through the cells to its target, double-stranded

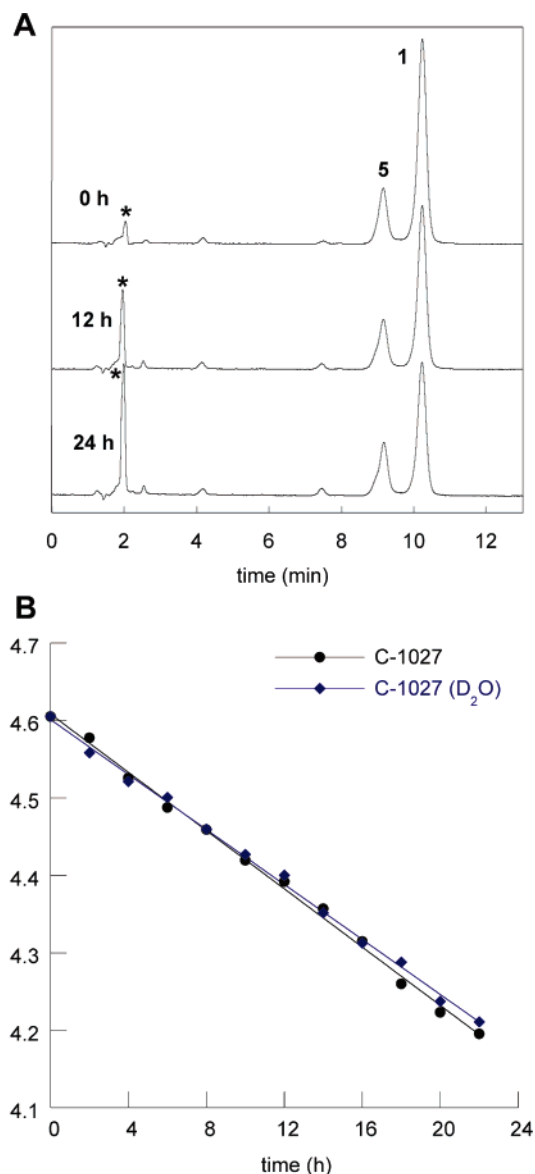


Figure 4. Time course of C-1027 chromophore (**1**) content when C-1027 was incubated at 37 °C in phosphate buffer (pH 6.8). (A) HPLC profiles of the contents of C-1027 [Cosmosil 5C18-MS (2.5 mm × 150 mm); eluent, 50% MeCN/20 mM phosphate buffer (pH 6.8); flow rate, 0.2 mL/min; detection, 350 nm; temperature, 40 °C]. (B) Plots of time against $\ln([1]/[1]_0 \times 100)$ content of C-1027 in H₂O buffer (black circles), and in D₂O buffer (blue squares).

DNA.²⁵ Thus, the apoprotein appears to function both as a stabilizer and as an effective carrier.²⁶ These features make it a favorable drug delivery system (DDS).

Despite these potentially ideal properties as a DDS for a reactive antitumor agent, C-1027 is known to undergo aging.²⁷ The apoprotein is presumably not able to completely inhibit the radical-mediated reactions of **2**, and C-1027 slowly decom-

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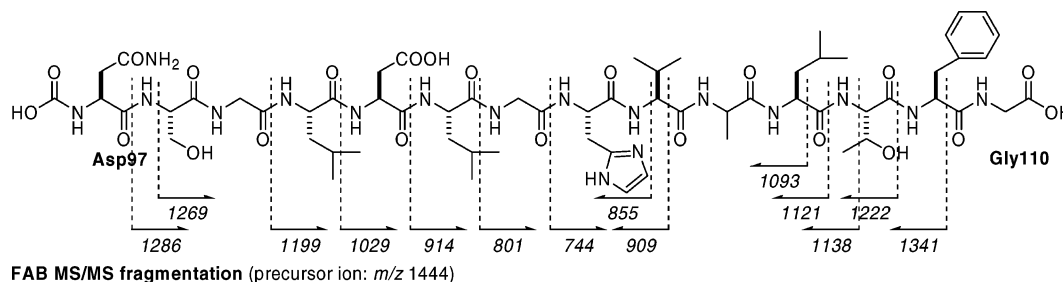


Figure 5. Mass spectroscopy data of the peptide fragment **8**.

poses, losing its potent bioactivity.^{24,28,29} In this paper, we elucidated a detailed self-decomposition mechanism of C-1027 and discuss a dually protective function of the apoprotein. The mechanistic insights thus obtained furthermore allowed us to design and prepare a kinetically stabilized analogue of C-1027 by engineering a mutant apoprotein more resistant toward radical-mediated reactions.³⁰ Direct comparison of the stability and the *in vitro* antitumor activity of the natural and the analogue showed that suppressing the self-decomposition pathway provides a basis for enhanced properties for C-1027.

Results and Discussion

Self-Decomposition Pathway of C-1027. To study the ability of the C-1027 apoprotein to protect chromophore **1**, we have previously evaluated the stability of C-1027 using reverse-phase HPLC. C-1027 was incubated in neutral buffer at 37 °C, and the same quantity of solution was injected into HPLC system every 2 h [ODS column; eluent, 50% acetonitrile in phosphate buffer], where **1** and **5** were extracted from the protein complex. As shown in Figure 4A, the amount of chromophore **1** decreased in a time-dependent manner.³¹

The chromophore content over time was found to obey pseudo-first-order kinetics (Figure 4B). To exclude the possibility that water was acting as hydrogen-donor, the C-1027 apoprotein was incubated in D₂O solution. The slope of the graph is almost indistinguishable from that in H₂O buffer. The absence of kinetic isotope effects (KIE) demonstrates that solvent water is not involved in chromophore decomposition. These studies together support the hypothesis that the direct reaction of **1** with the apoprotein in C-1027 results in the overall decomposition of C-1027. The rate constant of the reaction ($k_H = 5.22 \times 10^{-6} \text{ s}^{-1}$) was determined by fitting the data to $\ln([1]/[1]_0 \times 100) = -k_H t$. The half-life of **1** (37 h) is significantly longer in the apoprotein than in ethanol (0.8 h), indicating that the apoprotein is inefficient as a hydrogen donor. These results illustrate the ability of the apoprotein to effectively stabilize the radical-generating enediynes portion of **1**, having low reactivity as a hydrogen donor to biradical **2**.

Although these preliminary studies provided the kinetic basis for the role of the apoprotein in preservation of the reactive

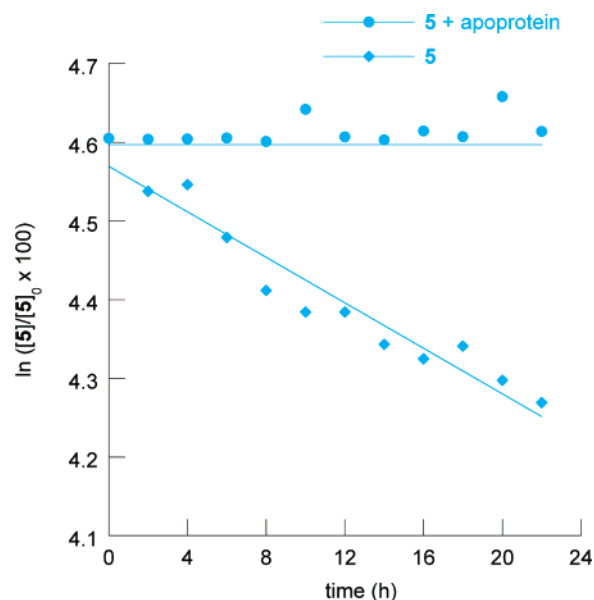


Figure 6. Plots of time against the aromatized chromophore (**5**) concentration in the presence (blue squares) and in the absence (blue triangles) of the apoprotein in H₂O buffer at 37 °C.

chromophore, observation that an unaccounted species arises as a consequence of C-1027 decomposition prompted us to further investigation. During the HPLC analysis of the C-1027 decomposition, it came to our attention that, while the amount of the aromatized chromophore **5** was constant over time, the peak height at 2 min in the HPLC profile increased in a time-dependent fashion (Figure 4A, peak marked with *). Therefore, we next examined more closely the end products of the C-1027 decomposition pathway. To isolate and characterize the unknown species that give rise to the HPLC peak (*), 500 mg of the natural C-1027 was incubated in water at 37 °C for 72 h. MALDI-TOFMS analyses of the lyophilized residue showed a new strong peak at 9086 Da as well as the apoprotein at 10489 Da.²⁷ Radical-mediated cleavage of the apoprotein appears to occur upon incubation.

The residue of C-1027 after 72 h incubation was purified using the ODS column (eluent, 20%→50% MeCN/1% AcONH₄ (pH 6.8)). From the various minor unidentified components, we successfully isolated four major species: the aromatized chromophore **5**, the hydrolyzed products **6** (ca. 4% yield) and **7** (ca. 5%), and peptide fragment **8** (m/z 1444.7067, ca. 5%). As shown in Figure 5, **8** was revealed to be the Asp97-Gly110 peptide fragment with a carbamic acid N-terminus,³² which is considered to originate from radical-mediated oxidative cleavage at Gly96.³³ Furthermore, it was also found that the unaccounted for HPLC peak (Figure 4A, peak with *) mainly consisted of

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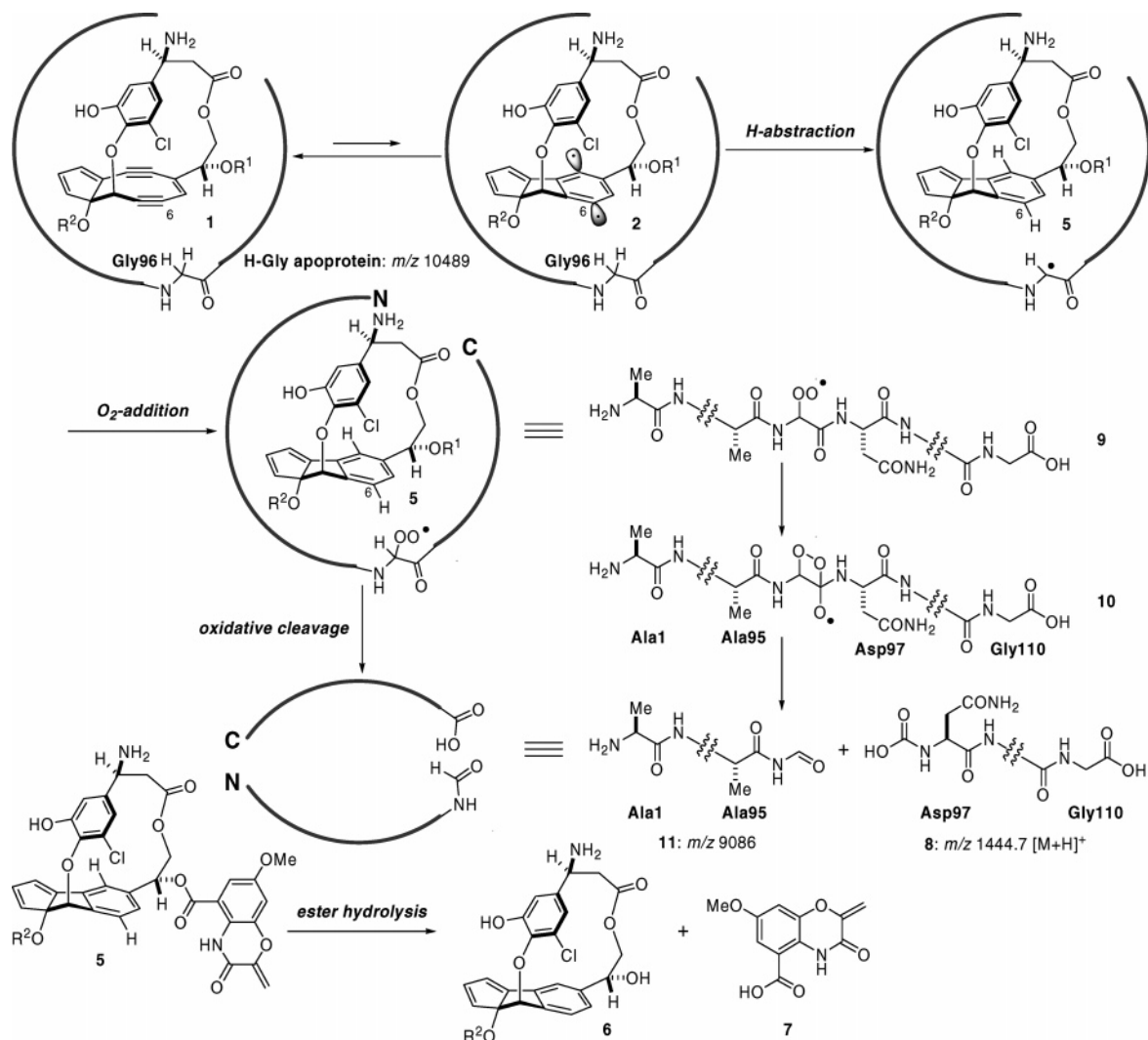


Figure 7. Proposed mechanism of self-decomposition of C-1027. The apoprotein is represented as a circle. Labels N and C shows the N- and C-terminus, respectively.

6, 7, and peptide 8. Taken together, the generation of compounds 6–8 correlates with the consumption of 1.

Spontaneous hydrolysis of the aromatized chromophore 5 to give 6 and 7 had not been reported previously. Since 6 and 7 were isolated from the C-1027 residues after the solution incubation experiments, we wondered if the apoprotein was involved in their formation. Therefore, the stability of 5 in the absence of or in the presence of the apoprotein was investigated in separate experiments. Incubation of 5 by itself in the buffer (pH 6.8) at 37 °C for 72 h afforded 6 and 7 in 54% and 25% yield (based on recovered 5), respectively. The rate of hydrolysis of 5 in the buffer was determined by HPLC analysis to be $4.60 \times 10^{-6} \text{ s}^{-1}$, which coincides well with the rate of the decay of 1 (Figure 6). On the contrary, incubation of an equimolar

mixture of 5 and the apoprotein under the same conditions failed to generate the hydrolyzed compounds, indicating that 5 binds the apoprotein tightly and as a result, is protected from hydrolysis. It suggests that 6 and 7 are not generated directly from the apoprotein-bound form of 1 or 5, but are produced only after 5 is released from the apoprotein upon its scission. These results are not only consistent with the three-dimensional structure (Figure 2), in which the benzoxazine is deeply buried within the binding cleft of the apoprotein, but also provide the first evidence that the apoprotein kinetically prevents the hydrolytic loss of the benzoxazine moiety from chromophore 1.

Consideration of all the above data led us to propose a mechanism of the self-decomposition of C-1027 as shown in Figure 7.³⁴ First, *p*-benzyne 2, which is in equilibrium with chromophore 1, abstracts the hydrogen of Gly96 to generate the Gly radical.³⁵ Next, peroxy radical 9 is formed through

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(34) We note that there could be other minor degradation pathways in addition to the major one in Figure 7. For instance, the generated Gly radical may add back to the chromophore before reacting with oxygen, leading to chromophore–protein adducts, or the phenyl radical such as 3 could react with molecular oxygen instead of hydrogen. However, attempts to detect the protein adduct or the oxidized chromophore were unsuccessful.

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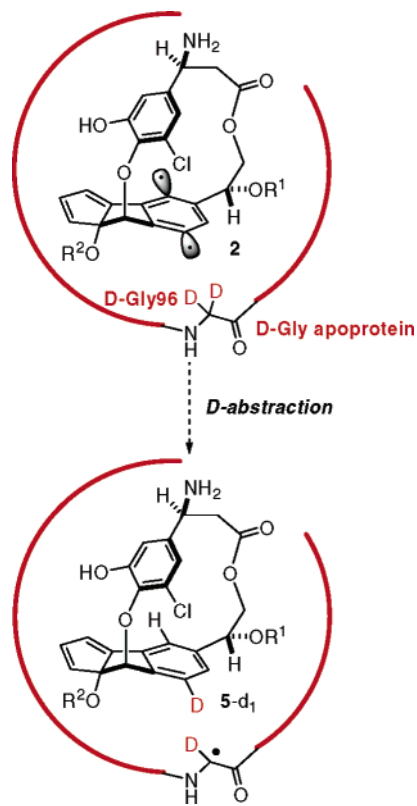


Figure 8. Design of D-Gly C-1027.

oxygen addition,³³ and this is followed by the peptide cleavage via dioxetane intermediate **10**; these steps explain the presence of **8** and **11**, both of which were detected by mass spectroscopy. The apoprotein cleavage we propose is consistent with the spatial proximity of the α -protons of Gly96 and the C6-hydrogen of **5**, as determined by high-resolution NMR (Figure 2). This protein scission liberates **5**, whose ester linkage is hydrolyzed at a similar rate to its generation; thus, the amount of **5** remains constant.

Intriguingly, what emerges from the present mechanistic studies of the C-1027 decomposition is an additional role of the apoprotein in protecting chromophore **1**: it not only kinetically stabilizes the nine-membered enediene that reacts with the DNA backbone sugar but also prevents hydrolysis of the benzoxazine that intercalates into the double-stranded DNA. Therefore, the apoprotein plays 2-fold protective functions as a carrier of **1**. Both of these functions are apparently required for delivery of the unstable **1** to its target DNA sequences.

Kinetically Stabilized Analogue of C-1027. On the basis of our earlier hypothesis that the formation of the Gly96 radical plays a major role in the self-decomposition of C-1027, it was envisioned that a simple substitution of Gly96 α -protons with deuterium would stabilize C-1027 by virtue of KIE in the hydrogen abstraction step (Figure 8).³⁶ The D-Gly analogue of C-1027 (D-Gly C-1027) was expected to meet the requirements that the modified apoprotein should increase the stability of **1** while retaining the binding affinity between the apoprotein and the chromophore. We have thus reported the preparation and a preliminary evaluation of the D-Gly C-1027.³⁰ The D-Gly apoprotein was obtained by isotopic labeling of the bacterially expressed C-1027 apoprotein using glycine-*d*₅ (98% D) added medium.³⁷ Multiple deuteration of the recombinant D-Gly apoprotein was confirmed by its MALDI-TOFMS (*m/z* 10968).³⁸

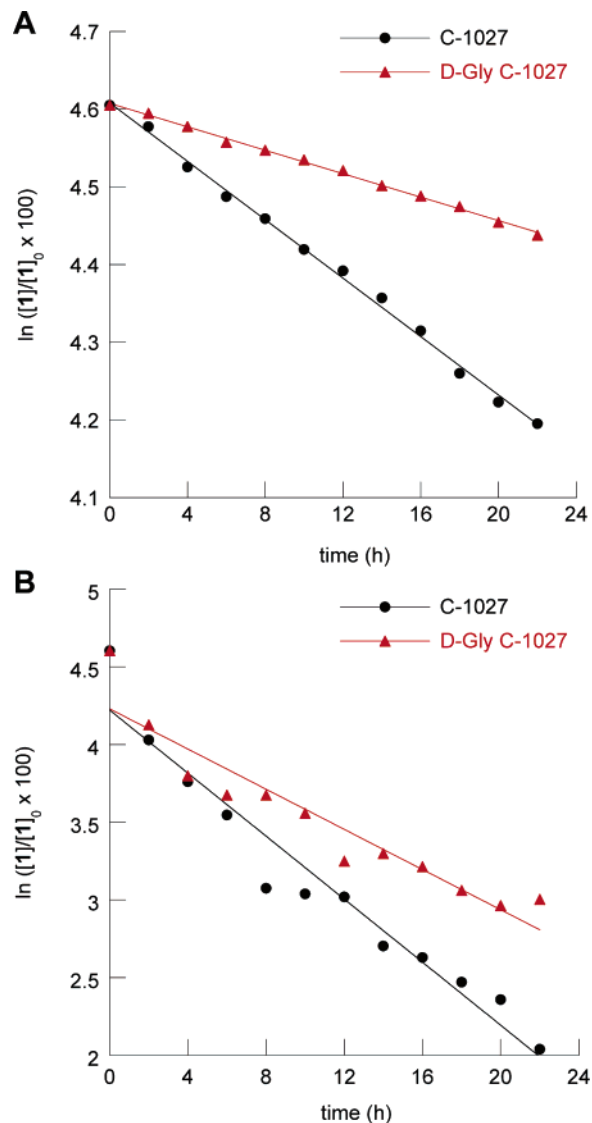


Figure 9. Plots of time against chromophore (**1**) content of (A) natural C-1027 (black circles) and D-Gly C-1027 (red triangle) in H₂O buffer at 37 °C, and (B) natural C-1027 (black circles) and D-Gly C-1027 (red triangles) in solid form at 37 °C.

Importantly, all deuterated amino acids, except Gly96 were distant from the reaction site¹¹ and considered to be of no consequence in further stability experiments. Isotopic incorporation of 78% was calculated from the relative integral volume of the cross-peaks of the glycine α -protons on 2D NMR spectra.

As shown in Figure 9A, we found that the recombinant D-Gly apoprotein possesses an improved chromophore-stabilizing activity. The KIE (k_H/k_D) is determined to be 4.1; the chromophore survives 4 times longer in the D-Gly apoprotein ($t_{1/2}$

(36) Isotope effects on the DNA cleavage by the enediynes have been reported. Neocarzinostatin: (a) Kappen, L. S.; Goldberg, I. H.; Wu, S. H.; Stubbe, J.; Worth, L., Jr.; Kozarich, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 2797. (b) Frank, B. L.; Worth, L., Jr.; Christner, D. F.; Kozarich, J. W.; Stubbe, J.; Kappen, L. S.; Goldberg, I. H. *J. Am. Chem. Soc.* **1991**, *113*, 2271. Calicheamicin: (c) Hangeland, J. J.; De Voss, J. J.; Heath, J. A.; Townsend, C. A.; Ding, W.; Ashcroft, J. S.; Ellestad, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 9200.

(37) Sakata, N.; Ikeno, S.; Hori, M.; Hamada, M.; Otani, T. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 1592.

(38) D-Gly apoprotein contains four extra vector-derived amino acids (Gly-Ser-His-Met) at the N-terminus. For the control experiment, we prepared the nonenriched recombinant apoprotein (*m/z* 10911), and confirmed that the four additional amino acids in the recombinant protein do not affect the chromophore-stabilizing ability. See Supporting Information.

= 151 h) than in the natural apoproteins ($t_{1/2}$ = 37 h). This KIE is even larger than those seen in organic solvents (2.8–3.8),^{17a} confirming the high site-specificity of the radical abstraction. The kinetic significance of the Gly96 proton abstraction in these experiments again supported the conclusion that consumption of **1** occurs mainly through cycloaromatization and not via the hydrolysis of the benzoxazine moiety.

The natural and the D-Gly C-1027 isoforms were also incubated in solid form at 37 °C (Figure 9B). Although the experimental errors are rather high, there is a clear difference in the reaction rates, and the KIE (k_H/k_D) was calculated to be approximately 1.8. Intriguingly, both C-1027 isoforms have shorter half-lives when solid than in buffer solution (natural C-1027: 7 h; D-Gly C-1027: 12 h). The smaller KIE value in the solid state than in solution suggests that hydrogen abstraction of **1** at Gly96 is less selective in the former case. This can be attributed to the protein conformation of the apoprotein being different in the solid and in solution, and the effective conformation to stabilize **1** can only be realized by the hydration of the apoprotein, although this hypothesis cannot be verified in the absence of structural data on the solid C-1027. Because C-1027 displays its biological activities in water in physiological conditions, the higher chromophore-stabilizing ability in solution is intrinsically important for its antitumor activity.

Isolation of the deuterated aromatized chromophore (**5-d**₁) as a product of the self-decomposition of the D-Gly C-1027 incubated in solution was not successful previously,³⁰ and thus we were unable to directly correlate the KIE to the formation of **5-d**₁ as a product of deuterium abstraction from D-Gly96. This is because the D-Gly C-1027 is too stable in solution to supply a reasonable amount of **5-d**₁ and any **5-d**₁ formed under these conditions is quickly hydrolyzed (vide supra). Therefore, we turned to analyze the self-decomposition products formed upon incubation of the D-Gly C-1027 in a solid format, which should generate sufficient amounts of the products. Thus, the D-Gly C-1027 in a solid format was incubated at 37 °C for 5 days. Almost all of **1** was consumed as a result of self-decomposition, allowing the LC-ESI-TOFMS analyses of the resultant compounds. Isotope incorporation of the aromatized chromophore was found to be 35% D from the isotope patterns (Figure 10). This is lower than the maximal value (78%), concordant with the low selectivity of **1** for D-Gly96 in the solid state. Nevertheless, the results confirmed that the abstraction of deuterium from D-Gly96 by the chromophore occurred as the major pathway. Our proposed self-decomposition pathway was reinforced by the MALDI-TOFMS peaks of the peptide fragments that corresponded to the oxidative cleavage of the D-Gly C-1027 at the D-Gly96 residue (m/z 9518).

Biological Evaluation of the D-Gly C-1027. To evaluate if the enhanced stability of the D-Gly C-1027 is reflected on the biological activity, we performed in vitro toxicity assays using various cancer cell lines. Both the natural and the D-Gly C-1027 isoforms were shown to possess extreme in vitro toxicity toward various cancer cell lines. The results are recorded in Table 1, alongside an IC₅₀ comparison of the clinically prescribed anticancer agents taxol and adriamycin. The multiple deuteration of the apoprotein apparently did not affect the cytotoxic activity exerted by chromophore **1**. It indicates that, while the D-Gly apoprotein encapsulates the reactive chromophore more stably than the natural H-Gly apoprotein, it releases the chromophore

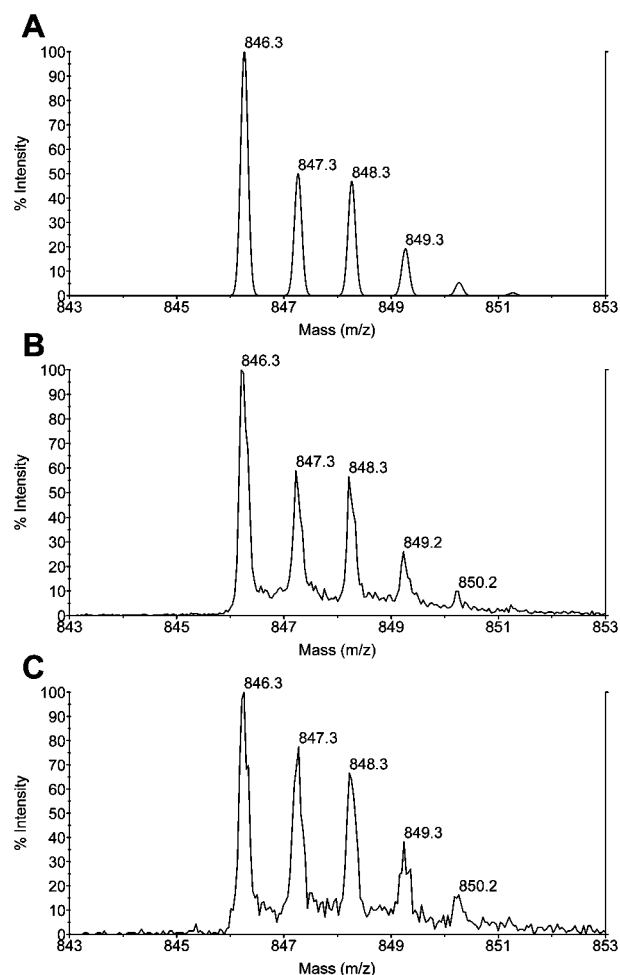


Figure 10. ESI-TOFMS spectra of the aromatized chromophore (**5**). (A) Simulated spectrum of **5** (m/z 846.3 [M+H]⁺). (B) MS spectrum of **5** after solid natural C-1027 was incubated at 37 °C for 3 days. (C) MS spectra of **5** and **5-d**₁ (m/z 847.3 [M + H]⁺) after solid D-Gly C-1027 was incubated at 37 °C for 5 days.

Table 1. In Vitro Cytotoxicity (IC₅₀, pM) of Various Agents against Cancer Cell Lines

cell line	IC ₅₀ (pM)			
	C-1027	D-Gly C-1027	taxol	adriamycin
JHH-7	6.1	5.6	7.2×10^3	11×10^3
AZ521	11	15	6.1×10^3	—
PANC1	19	27	—	—
HT-1376	62	52	—	—
KB	15	19	1.7×10^3	9.0×10^3

just as efficiently as the natural apoprotein. Both of the C-1027 isoforms exhibited markedly greater potency (1000-fold) than taxol and adriamycin.

We next tested if the stabilities of the C-1027 isoforms are correlated with changes in their cytotoxicity over time. First, the isoforms were incubated at 37 °C for 24 or 48 h in pH 6.8 buffer. Then, the in vitro cytotoxicity of the isoforms against human hepatocellular carcinoma JHH-7 was examined. As shown in Table 2, in a sharp contrast to a significant loss of activity observed for the natural C-1027 after 48 h of incubation (6.1 to 176 pM), the D-Gly C-1027 maintained its activity (IC₅₀ = 30 pM) albeit with a 3-fold decrease. The D-Gly C-1027 clearly possesses a prolonged lifetime as an anticancer agent compared to the natural C-1027. In conclusion, we have thus

Table 2. Cytotoxicity Data (IC_{50} , pM) for C-1027 and D-Gly C-1027 against Human Hepatic Carcinoma JHH-7 after Incubation at 37 °C in Phosphate Buffer (pH 6.8)

time	IC_{50} (pM)	
	C-1027	D-Gly-C-1027
0	6.1	5.6
24	34.4	11.5
48	176.0	30.4

demonstrated that simple design of the C-1027 apoprotein analogue that stabilizes the reactive chromophore achieves enhanced stability for the overall small molecule–protein complex and hence improved anticancer activity. Our findings may have implications for design of proteins that stably encapsulate highly reactive small molecules.

Conclusion

We have shown that the formation of the Gly96 radical and the subsequent oxidative peptide cleavage are the major pathway for the self-decomposition of C-1027. Direct evidence that the chromophore triggers radical-mediated oxidative cleavage of the apoprotein is provided. On the basis of newly isolated products of the C-1027 self-decomposition, a second role for the apoprotein as a chromophore carrier is proposed. The apoprotein effectively stabilizes two functional units of the chromophore

that are essential for its DNA cleaving activity: the eneidyne moiety for the hydrogen abstractions and the benzoxazine moiety for the DNA intercalation. Using the D-Gly C-1027, we show that the kinetic stabilization of the reactive chromophore provides an enhanced overall stability for the small molecule–protein complex, thereby achieving more effective antitumor activities compared to that of the natural C-1027. C-1027 represents a unique drug delivery system such that the protein stably encapsulates a highly reactive small molecule yet is capable of unleashing it when the target DNA molecule is in close proximity. The mechanistic insights into the self-decomposition pathway for C-1027 described here should aid our understanding on how the protein interacts with the reactive small molecule to modulate its reactivity.

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Supporting Information Available: Materials and methods, and analytical details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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