A PROPOSED REACTION MECHANISM FOR THE ENZYMATIC REDUCTIVE CLEAVAGE OF GLYCOSIDIC BOND IN ANTHRACYCLINE ANTIBIOTICS

Sir:

The reductive glycosidic cleavage of anthracyclines in mammals is unique since it involves reduction at the glycosidic bond. This reaction is important for the metabolism of anthracycline antibiotics[1~4]. In the case of aclacinomycin A, we demonstrated the production of 7-deoxyaklavinone and reductively condensed 7-deoxyaklavinone dimer, 7,7′-bis(7-deoxyaklavinone), by microsomal NADPH-cytochrome P-450 reductase (EC 1.6.2.4) (Fig. 1)[1,2]. In this paper, through the studies on anthracycline-stimulated NADPH oxidase reaction and on the reductive glycosidic cleavage reaction by several redox enzymes, we propose a reaction mechanism for the enzymatic reductive glycosidic cleavage reaction of anthracyclines.

Clark-type oxygen sensor was the product of Beckmann, 76365. Catalase (EC 1.11.1.6) and xanthine oxidase (EC 1.2.3.2) purified chromatographically were obtained from Worthington Biochemical Corporation and Sigma, Co., respectively. Other enzymes were prepared from adult male rat liver. Microsomal NADPH-cytochrome P-450 reductase was purified as previously reported[1,2]. Mitochondrial NADH dehydrogenase (EC 1.6.99.3) was partially purified by the method of SANADI *et al.*[5], to the step of sonicated heavy and light mitochondria, and DT-diaphorase was prepared by the method of ERNSTER[6] to ammonium sulfate fractionation step. Protein concentration was determined by the method of LOWRY *et al.*[7], using bovine serum albumin as standard.

In Fig. 2, anthracycline antibiotic-dependent NADPH oxidase reaction by NADPH-cytochrome P-450 reductase is shown, and the product was confirmed as H_2O_2 by the reversal of oxygen consumption which occurred upon the addition of catalase. These results and the action of NADPH-cytochrome P-450 reductase on quinone compounds[8~12] indicate that the interaction of anthracycline antibiotics with NADPH-cytochrome P-450 reductase under aerobic conditions follows the reduction(semiquinone)-oxidation(quinone) cycle of quinone moiety of the antibiotics.

The reductive glycosidic cleavage reaction of anthracyclines by xanthine oxidase, mitochondrial NADH dehydrogenase, and DT-diaphorase is shown in Table 1. As in the case of NADPH-cytochrome P-450 reductase[1,2], these enzymes required anaerobic conditions for the reaction and produced 7-deoxyaklavinone and 7,7′-bis (7-deoxyakavinone) from aclacinomycin A and only 7-deoxyadriamycinone from adriamycin. Although the natural substrate is different among these enzymes, a common property of these redox enzymes is the reactivity with quinone compounds[1,2,8~15]. Then, the above results indicate that the first step of the reductive glycosidic

Fig. 1. Reductive glycosidic cleavage reaction of aclacinomycin A.

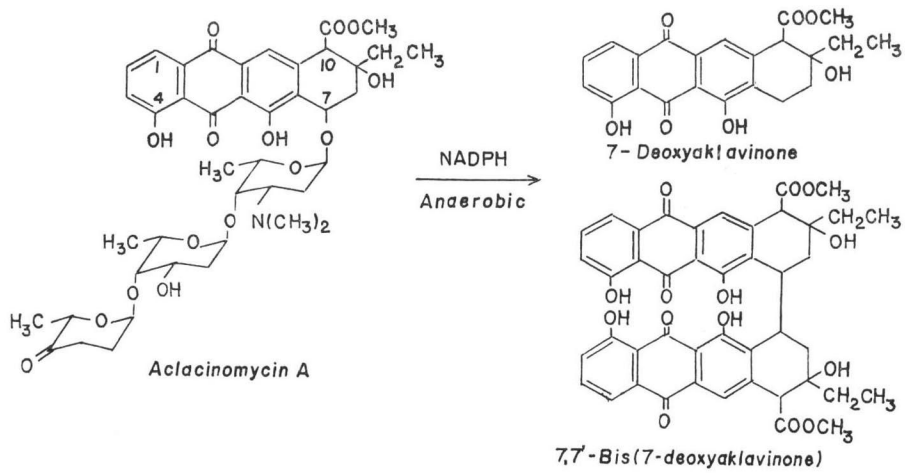


Table 1. Reductive glycosidic cleavage reaction by xanthine oxidase and by some enzymes.

Enzyme	Substrate	Electron donor	Product	(nmoles/tube)
Xanthine oxidase	Aclacinomycin A	Xanthine	7-deoxyaklavinone	6.6
			7,7′-bis(7-deoxyaklavinone)	27.8
〃 〃	Aclacinomycin A	〃 (air)*	None	0
〃 〃	Adriamycin	〃	7-deoxyadriamycinone	28.4
〃 〃	Daunomycin	〃	7-deoxydaunomycinone	45.6
Mitochondrial NADH dehydrogenase	Adriamycin	NADH	7-deoxyadriamycinone	28.6
〃	〃	〃 (PCMB)**	〃	21.3
DT-diaphorase	Adriamycin	NADH	7-deoxyadriamycinone	9.4
〃	〃	〃 (dicoumarol)***	〃	0.8

The reaction was carried out as reported previously[1,2]. In the case of xanthine oxidase, the reaction mixture consisted of 50 mM potassium phosphate buffer, pH 7.5, 0.2 mM xanthine, 0.2 mM substrate, and 60 μg of enzyme. In the case of mitochondrial NADH dehydrogenase, 0.2 mM NADH and 0.93 mg of protein were used and the reaction was carried out at 37°C. In the case of DT-diaphorase, 0.2 mM NADH and 0.89 mg of enzyme were used and the reaction was carried out at 37°C.

* The reaction was carried out under air.

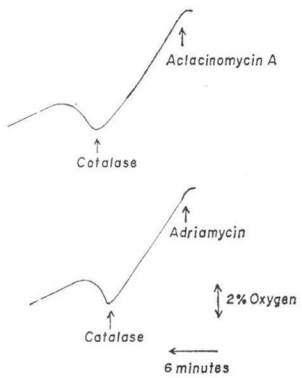
** *p*-Chloromercuribenzoic acid (PCMB), 0.1 mM, was added to the reaction mixture to eliminate the effect of contaminated sulfhydryl enzyme.

*** To check the enzyme activity, 1.0 mM dicoumarol was added to the reaction mixture.

Fig. 2. Anthracycline antibiotic-dependent NADPH oxidase reaction by NADPH-cytochrome P-450 reductase.

The reaction mixture, in a total volume of 3.5 ml, consisted of 0.14 M potassium phosphate buffer, pH 7.5, 0.3 mM NADPH, and 21 μg of purified enzyme, and oxygen consumption was recorded.

Anthracycline and catalase were added at indicated position at a concentration of 30 μM and 40 μg protein, respectively.



cleavage reaction of anthracyclines is the reduction of the quinone moiety of anthracyclines to semiquinone form as under aerobic conditions. Considering the presence of reducible quinone oxygen in anthracyclines, the reductive glycosidic cleavage reaction of anthracyclines is the consequence of the electron flow of semiquinone form in the aglycone molecule, from semiquinone to C-7 position (Fig. 3). And the rest of the free radical at quinone moiety is transferred to C-7 position to produce 7-deoxyaglycone dimer, 7,7′-bis(7-deoxyaklavinone), or accept the second electron to produce 7-deoxyaklavinone. In the case of daunomycin and adriamycin, the phenolic hydroxyl group at C-11 position may interfere with the radical transfer from semiquinone moiety to C-7 position by the equilibrium as shown in Fig. 4, so there is no production of 7-deoxyaglycone dimer.

The proposed reaction mechanism of reductive glycosidic cleavage reaction of anthracyclines explains the following properties of anthracycline antibiotics; 1) stimulation of oxidase reaction under aerobic conditions as observed in Fig. 2, 2) requirement of anaerobic conditions for reductive glycosidic cleavage reaction and catalysis of the same reaction by several redox emzymes which react on quinone compounds, 3) formation of 7-deoxyaglycone dimer in the reductive

Fig. 3. Proposed reaction mechanism of reductive glycosidic cleavage reaction of aclacinomycin A.

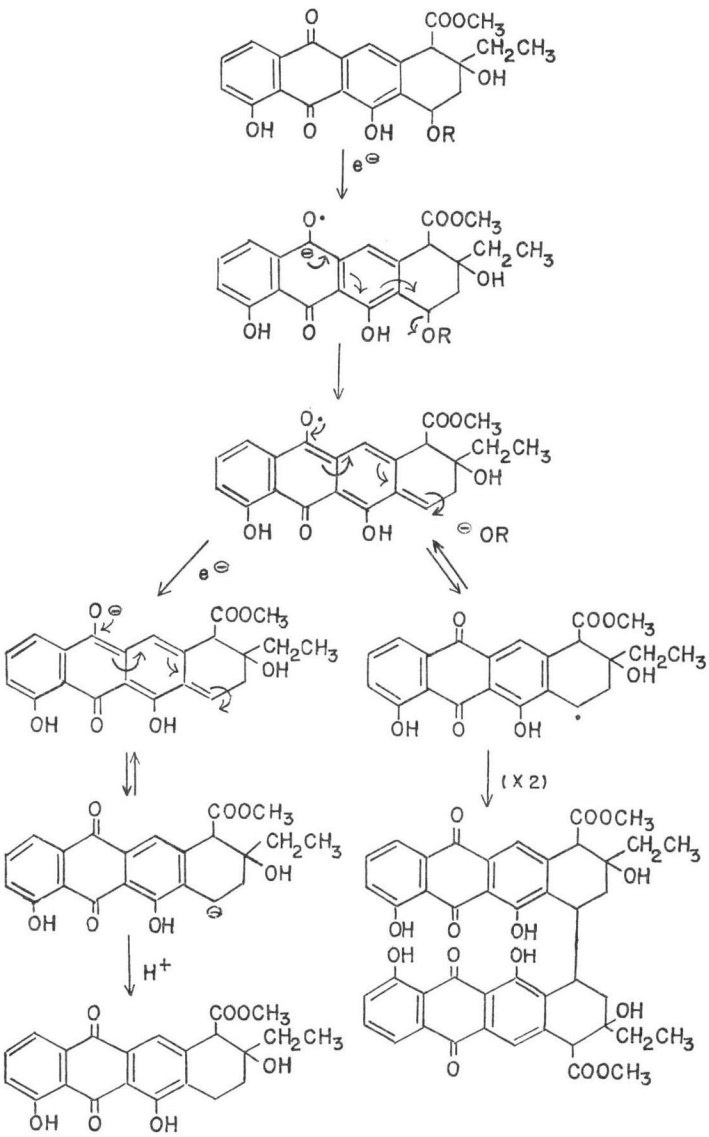
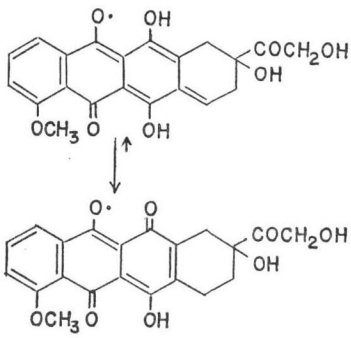


Fig. 4. Interference of radical transfer in 7-deoxyadriamycinone.



glycosidic cleavage reaction of aclacinomycin A and no production of it from the compounds which possess hydroxyl group at C-11 position, and 4) mutagenicity[16~18], carcinogenicity[19], stimulation of lipid peroxidation[12], and strand scission of DNA[20]. These are related to the radical reactions of anthracycline antibiotics.

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References

1) OKI, T.; T. KOMIYAMA, H. TONE, T. INUI, T. TAKEUCHI & H. UMEZAWA: Reductive cleavage of anthracycline glycosides by microsomal NADPH-cytochrome *c* reductase. J. Antibiotics 30: 613~615, 1977
2) KOMIYAMA, T.; T. OKI, T. INUI, T. TAKEUCHI & H. UMEZAWA: Reduction of anthracycline glycoside by NADPH-cytochrome P-450 reductase. Gann, in press, 1979
3) BACHUR, N. R. & M. V. GEE: Microsomal reductive glycosidase. J. Pharmacol. Exp. Ther. 197: 681~686, 1976

4) ASBELL, M. A.; E. SCHWARTZBACH, F. J. BULLOCK & D. W. YESAIR: Daunomycin and adriamycin metabolism *via* reductive glycosidic cleavage. J. Pharmacol. Exp. Ther. 182: 63~69, 1972
5) SANADI, D. R.; R. L. PHARO & L. A. SORDAHL: NADH-CoQ reductase-assay and purification. *In* Methods in Enzymology (R. W. ESTABROOK & M. E. PULLMAN *eds*), Vol. 10, pp. 297~302, Academic Press. New York & London, 1967
6) ERNSTER, L.: DT-diaphorase. *In* Methods in Enzymology (R. W. ESTABROOK & M. E. PULLMAN *eds*), Vol. 10, pp. 309~317, Academic Press. New York & London, 1967
7) LOWRY, O. H.; N. J. ROSENBROUGH, A. L. FARR & R. J. RANDALL: Protein measurement with the FOLIN phenol reagent. J. Biol. Chem. 193: 265~275, 1951
8) NISHIBAYASHI-YAMASHITA, H. & R. SATO: Vitamin K_3-dependent NADPH oxidase of liver microsomes. Purification, properties and identity with microsomal NADPH-cytochrome *c* reductase. J. Biochem. 67: 199~210, 1970
9) IYANAGI, T. & I. YAMAZAKI: One-electron-transfer reactions in biochemical systems. III. One-electron reduction of quinones by microsomal flavine enzymes. Biochim. Biophys. Acta 172: 370~381, 1969
10) HANDA, K. & S. SATO: Stimulation of microsomal NADPH oxidation by quinone group containing antitumor chemicals. Gann 67: 523~528, 1976
11) GOODMAN, J. & P. HOCHSTEIN: Generation of free radicals and lipid peroxidation by redox cycling of adriamycin and daunomycin. Biochem. Biophys. Res. Commun. 77: 797~803, 1977
12) BACHUR, N. R.; S. L. GORDON, M. V. GEE & H. KON: NADPH-cytochrome P-450 reductase activation of quinone anticancer agents to free radicals. Proc. Natl. Acad. Sci. U.S.A. 76: 954~957, 1979
13) MURAOKA, S.; H. ENOMOTO, M. SUGIYAMA & H. YAMASAKI: The mechanism of the reduction of cytochrome *c* by xanthine oxidase with electron acceptors. Biochim. Biophys. Acta 143: 408~415, 1967
14) NAKAMURA, S. & I. YAMAZAKI: One-electron-transfer reactions in biochemical systems. IV. A mixed mechanism in the reaction of milk xanthine oxidase with electron acceptors. Biochim. Biophys. Acta 189: 29~37, 1969
15) IYANAGI, T. & I. YAMAZAKI: One-electron-transfer reactions in biochemical systems. V. Difference in the mechanism of quinone reduction by the NADH dehydrogenase and the NAD(P)H dehydrogenase (DT diaphorase). Biochim. Biophys. Acta 216: 282~294, 1970
16) SEINO, Y.; M. NAGAO, T. YAHAGI, A. HOSHI, T. KAWACHI & T. SUGIMURA: Mutagenisity of several class of antitumor agents to *Salmonella typhimurium* TA98, TA100, and TA92. Cancer Res. 38: 2148~2156, 1978
17) UMEZAWA, K.; M. SAWAMURA, T. MATSUSIMA & T. SUGIMURA: Mutagenisity of aclacinomycin A and daunomycin derivatives. Cancer Res. 38: 1782~1784, 1978
18) BENEDICT, W. F.; M. S. BAKER, L. HAROUN, E. CHOI & B. N. ANOS: Mutagenisity of cancer chemotherapeutic agents in the *Salmonella*/microsome test. Cancer Res. 37: 2209~2213, 1977
19) MARQUARDT, H.; F. S. PHILIPS & S. S. STERNBERG: Tumorigenicity *in vivo* and induction of malignant transformation and mutagenesis in cell cultures by adriamycin and daunomycin. Cancer Res. 36: 2065~2069, 1976
20) LOWN, J. W.; S-K. SIM, K. C. MAJUMDAR & R-Y. CHANG: Strand scission of DNA by bound adriamycin and daunomycin in the presence of reducing agents. Biochem. Biophys. Res. Commun. 76: 705~710, 1977