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Generation of semiquinone radical from carbazilquinone by NADPH-cytochrome P-450 reductase

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Introduction

NADPH-cytochrome P-450 reductase (EC 1.6.2.4) is an important enzyme for xenobiotic metabolism by its catalysis of the electron transfer from NADPH to cytochrome P-450. In the previous studies on the metabolism of several anticancer quinone drugs, we have found that NADPH-cytochrome P-450 reductase contributes to the quinone drug metabolism and carbazilquinone (Fig. 1) was also an effective substrate for the enzyme as well as aclacinomycin A, adriamycin, mitomycin C, and streptonigrin [1–4]. These studies revealed that the semiquinone form produced by one electron reduction of the drug with NADPH-cytochrome P-450 reductase is an important intermediate of the drug action and/or drug metabolism and recent studies supported this hypothesis [5, 6].

Carbazilquinone consists of quinone part, two aziridine groups, and two alkyl side chains, and is effective against leukemia, lymphoma, and some solid tumors. The compound is classified as an alkylating agent because of its action mechanism, and *in vitro* study has indicated that the reduction of the drug with sodium borohydride showed the enhanced effect on DNA binding activity [6–11].

In this paper, we would like to report the direct e.s.r. evidence for the generation of the semiquinone radical from carbazilquinone by purified NADPH-cytochrome P-450 reductase.

Materials and Methods

NADPH-cytochrome P-450 reductase was obtained from rabbit liver microsomes. Solubilization with Emulgen 913 and purification of the enzyme was carried out by the method reported by Taniguchi *et al.* [12]. Carbazilquinone was the kind gift of Sankyo Co. (Japan). X-band e.s.r. spectrum was measured with Jeol JES-FE3X using a quartz flat cell at 25°. The sample was prepared using the Thunberg tube, because the reduced drug is easily reoxidated by air [4]. NADPH solution was placed in the side vessel and all other reagents were placed in the main tube. The partial pressure of oxygen was first reduced by evacuation before the introduction of nitrogen to the tube. After the addition of NADPH solution to the main tube, the sample solution was immediately placed into the cell under nitrogen, and the e.s.r. spectrum was measured.

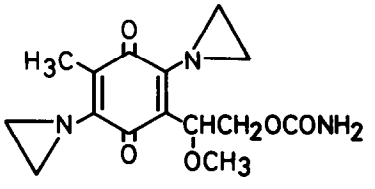

Fig. 1. Structure of carbazilquinone.

Results and Discussion

The high modulation spectrum of semiquinone radical from carbazilquinone which was generated by purified NADPH-cytochrome P-450 reductase is shown in Fig. 2(a). The signal at $g = 2.005$ appeared to consist of five intensive peaks. Figure 2(b) shows the low modulation spectrum, on the other hand, the 16 hyperfine structures in the spectrum probably originated in two nitrogens of the aziridinyl groups at positions 2 and 5 and a set of three equivalent protons of methyl group at position 6. The e.s.r. signal appeared

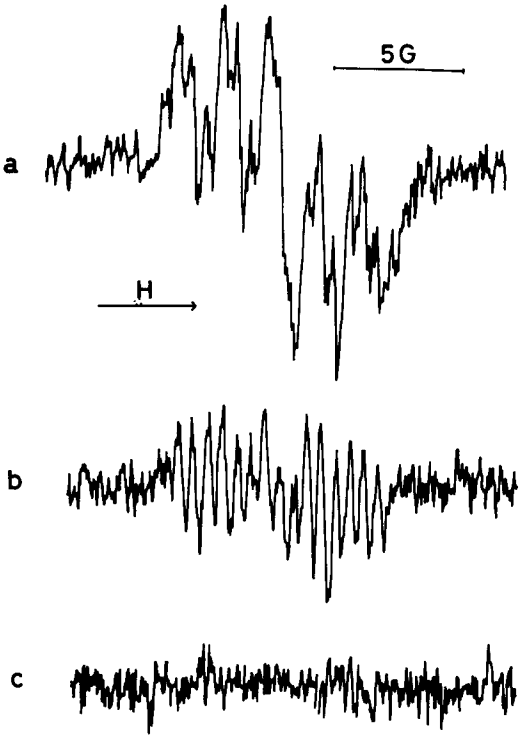

Fig. 2. The e.s.r. spectrum of carbazilquinone in the presence of NADPH-cytochrome P-450 reductase. The incubation mixture consisted of 0.1 M Tris-HCl, pH 7.5, 1.8 mM carbazilquinone, 2.4 mM NADPH, and 80.5 μg/ml NADPH-cytochrome P-450 reductase, and the preparation procedure is described in the Materials and Methods section. (a) Modulation amplitude 1.0 G, microwave power 10 mW, time constant 0.1 sec. (b) The same conditions as (a) except for the modulation amplitude which was 0.25 G. (c) The same conditions as (a) except for the spectrum which was measured after 15 min.

immediately after the reaction, slowly disappeared, and after 15 min, was completely gone [Fig. 2(c)]. When the sample lacked the enzyme or the drug, or air was bubbled through it the e.s.r. signal did not appear. The disappearance of the e.s.r. signal with time or by bubbling air through the sample is explained by further reduction or reoxidation of the semiquinone form of the drug, respectively.

Sato *et al.* observed the similar low modulation spectrum which was generated by NADPH-microsome system [13], and they tried computer simulation taking into consideration the above mentioned two nitrogens and a set of three equivalent protons of methyl group. Therefore, the enzyme that catalyzed the generation of semiquinone radical of carbazilquinone in their system must be NADPH-cytochrome P-450 reductase.

The study of DNA binding experiment *in vitro* indicated that the reduction of carbazilquinone increased the binding activity of the drug [11], and the theoretical study also supported the reductive activation of the drug [14]. However, the enzyme that catalyzed the reductive activation of the drug was obscure. As indicated in a previous paper [4], carbazilquinone was an effective substrate for NADPH-cytochrome P-450 reductase, and the present e.s.r. result clearly showed that NADPH-cytochrome P-450 reductase generated the semiquinone radical from carbazilquinone. In addition to our previous works [1–4], recent studies indicate the contribution of NADPH-cytochrome P-450 reductase on drug action [5, 6]. The present result also strongly suggests the importance of NADPH-cytochrome P-450 reductase on the *in vivo* activation and action mechanism of carbazilquinone.

Faculty of Pharmaceutical Science TAKANOBU KIKUCHI
Kyoto University YUKIO SUGIURA
Sakyo-ku
Kyoto 606
Japan

Niigata College of Pharmacy TADAZUMI KOMIYAMA*
5829 *Kamishin'ei-cho*
Niigata 950-21
Japan

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* Author to whom correspondence should be addressed.