BIOORGANIC CHEMISTRY 9, 434–439 (1980)

Investigation of the Interaction of Superoxide Ion with Adriamycin and the Possible Origin of Cardiotoxicity of the Anthracycline Anticancer Antibiotics

I. B. AFANAS'EV, N. I. POLOZOVA, AND G. I. SAMOKHVALOV

All-Union Research Vitamin Institute, Nauchny proezd, 14A, 117246, Moscow, USSR

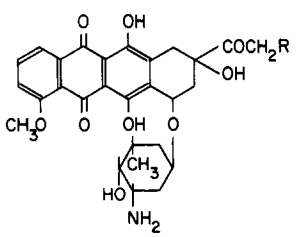
Received April 16, 1979

Interaction of superoxide ion with adriamycin in an aprotic medium has been studied. It was shown that superoxide ion reacts irreversibly with adriamycin, giving a diamagnetic product (the dimer or oligomer of semiquinone) which can be reoxidized to adriamycin. This product was also obtained when adriamycin reacted with benzosemiquinone, ubisemiquinone, and the semiquinones of tocopherylquinone and vitamin K_1. It is suggested that the cardiotoxicity of adriamycin and other anthracycline anticancer antibiotics is caused by the high electron-attracting properties of these antibiotics, while the ability of natural quinones to reduce cardiotoxicity and to induce recovery of respiration in mitochondria is due to their interaction with the semiquinone states of the antibiotics.

INTRODUCTION

In 1975 Handa and Sato (*1*) found that the anthracycline anticancer antibiotics adriamycin and daunorubicin (**Ia, Ib**) initiated sulfite oxidation during incubation with rat liver microsomes. The authors suggested that these drugs form the semiquinone anion radicals in microsomes. These results were later supported by studying the antibiotic effects on rat liver microsomes (*2, 3*), submitochondrial particles (*4*), and cytochrome *P*-450 reductase (*5*).

It is known that the compounds **Ia, Ib** show high cardiotoxicity. Bachur *et al.*



Ia, R = OH, adriamycin

Ib, R = H, daunorubicin

(*3*) suggested that the anthracycline antibiotics enter the single-electron transfer chain at a point between NADPH and cytochrome *P*-450, forming semiquinones which reduce molecular oxygen to the superoxide ion. An analogous mechanism was proposed by Thayler (*4*). Goodman and Hochstein (*5*) suggested that

0045-2068/80/040434-06$02.00/0

cardiotoxicity of the anthracycline antibiotics is caused by superoxide production, which leads to initiation of lipid peroxidation.

It is therefore important for the elucidation of the origin of cardiotoxicity of these drugs to investigate the mechanism of interaction of adriamycin with the superoxide ion. In addition, it is of interest to study the possibility of the interaction of adriamycin with natural quinones which are believed to be able to reduce the cardiotoxicity of the antibiotics (*6*).

MATERIALS AND METHODS

Adriamycin HCl (Farmitalia) was used without purification. Ubiquinone Q_9 and vitamin K_1 had a stated purities of 98 and 94%, respectively. Tocopherylquinone was prepared by oxidation of α-tocopherol with ferric chloride (*7*). Its spectral and thin-layer chromatography properties were in good agreement with literature data (*7*).

The O_2^- solutions in dimethylformamide (DMF) were prepared by electrochemical reduction of molecular oxygen (*8*). The supporting electrolyte was tetrabutylammonium perchlorate. The cell had a mercury cathode and a platinum anode. The anode and cathode compartments were separated by a cock. DMF was dried over anhydrous potassium carbonate and was twice vacuum distilled. For experiments a middle fraction (~50% of the total amount of DMF) was used. Solutions of the superoxide ion, 0.001–0.05 *M*, were freshly prepared prior to each experiment. (Time of electrolysis was equal to 15–70 min.) Under these conditions a half-life of the superoxide ion was equal to ~20 hr (*9*).

After argon flushing, the solutions of reactants were transported in a quartz cell of a spectrophotometer under argon. Spectra were recorded on a Specord uv-VIS spectrophotometer and a Jes-ME-3X (Jeol) esr spectrometer.

RESULTS

The absorption spectra of adriamycin HCl and a product of the reaction of adriamycin with O_2^- at room temperature are shown in Fig. 1. The spectrum of adriamycin HCl in DMF is very similar to that of the adriamycin monocation in water (λ_{max} = 483, 503(lg ϵ 4.08), 537, and 589 nm in DMF; 475, 495(lg ϵ 4.00), 540 nm in water (*10*)). When solutions of adriamycin and O_2^- were mixed at the molar ratio $\leq$2, a spectrum with maxima at 559, 618, and 645 nm was immediately generated. The spectrum did not change when pure oxygen bubbled through the reaction mixture.

In order to study the possibility of reoxidation of the reaction product, an excess of benzoquinone was added to the reaction mixture. As is seen from Fig. 2, it caused regeneration of adriamycin.[1] When the O_2^- solution was repeatedly

[1] The amount of adriamycin recovered depended on the amount of benzoquinone added. In the experiment cited in Fig. 2, the recovery of adriamycin was about 70%.

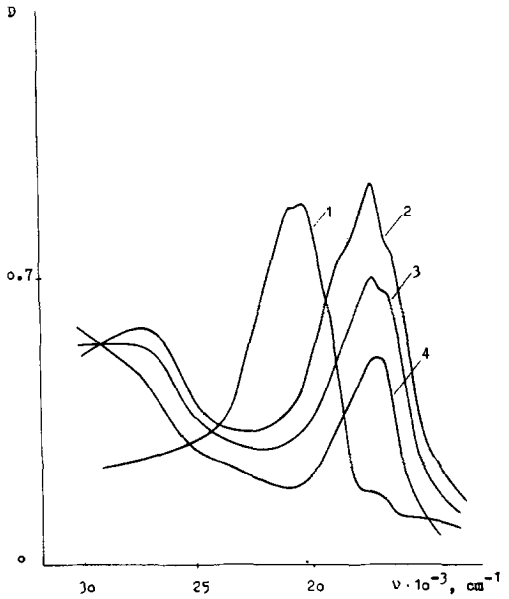


FIG. 1. Curve (1) Absorption spectrum of adriamycin HCl; [adriamycin] = 0.8×10^{-4} *M*. (2) Absorption spectrum of *X*; [adriamycin] = 0.7×10^{-4} *M*, $[O_2^+] = 1.8 \times 10^{-4}$ *M*. (3) The same at [adriamycin] = 0.5×10^{-4} *M*, $[O_2^+] = 1.8 \times 10^{-4}$ *M*. The same at [adriamycin] = 0.35×10^{-4} *M*, $[O_2^+] = 3.5 \times 10^{-4}$ *M*. 3.5×10^{-4} *M*.

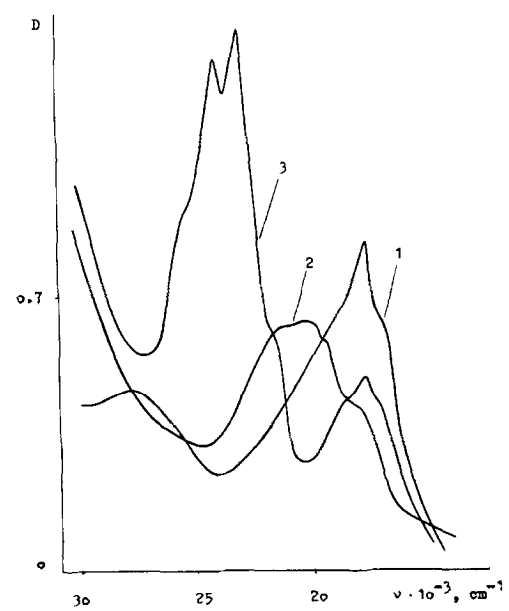


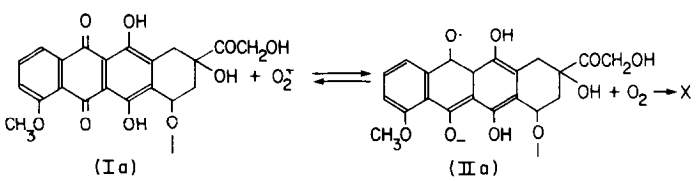
FIG. 2. Curve (1) Absorption spectrum of *X*; [adriamycin] = 0.8×10^{-4} *M*, $[O_2^+] = 1.7 \times 10^{-4}$ *M* (the first solution). (2) Absorption spectrum obtained by mixing 2.7 ml of the first solution with 0.7 ml of 0.01 *M* benzoquinone solution (the second solution). (3) Absorption spectrum obtained by mixing 1.5 ml of the second solution with 1.0 ml of 4.4×10^{-4} *M* solution of O_2^-.

added, the spectrum of reaction product reappeared quantitatively together with that of benzoquinone, the latter being formed as the result of reduction of benzoquinone with the superoxide ion. However, it should be mentioned here that in the experiments carried out with a large excess of the superoxide ion, the addition of benzoquinone to reaction mixture caused the formation of new product(s) (this was indicated by increase of the optical density at 500–600 nm) instead of regenerating adriamycin.

Further, we studied the competitive interaction of superoxide ion with the double mixture of adriamycin and quinones: benzoquinone, ubiquinone Q_9, tocopherylquinone, and vitamin K_1. Earlier we had shown (*11*) that the reaction of O_2^- with these quinones in DMF occurs to form the corresponding semiquinone anion radicals. In the presence of adriamycin, semiquinones however did not form; and in all cases only a spectrum of the product of the reaction of O_2^- with adriamycin was quantitatively produced. This product was also formed in the reaction of adriamycin with benzosemiquinone or ubisemiquinone, which were prepared by the interaction of the superoxide ion with corresponding quinones.

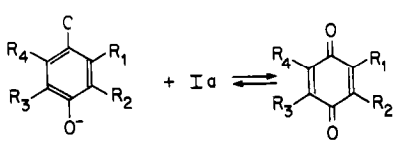
DISCUSSION

Our findings thus show that the interaction of adriamycin with the superoxide ion is practically irreversible, i.e., the equilibrium ([1]) is completely displaced to the right.



[1]

However, by reason of the absence of esr spectrum, the end product of Reaction [1] is not a semiquinone anion radical (**IIa**). Nonetheless, we believe that *X* is formed in electron-transfer process and is not the base or the anion of adriamycin. (The latter could be formed for example through deprotonation with O_2^-.) Indeed, this product is also formed in the electron-transfer reaction between semiquinones and adriamycin (Reaction [2]), and its spectrum is completely different from those of the base or the anion of adriamycin (*10*).



[2]

(For benzoquinone, $R_1 = R_2 = R_3 = R_4 = H$, ubiquinone Q_9, $R_1 = R_2 = CH_3O$, $R_3 = CH_3$, $R_4 = H(CH_2C(CH_3) = CHCH_2)_9-$).

We found that *X* can be reoxidized into adriamycin with a large excess of benzoquinone. Therefore, it seems to be the dimer or oligomer of semiquinone (**IIa**). It is possible that *X* is a mixture of two or three compounds as the ratio of maxima at 559, 618, and 645 nm depended on the experimental conditions. For example, this ratio was equal to 1 : 1.3 : 1 at $[O_2^{\cdot}]/[\text{adriamycin}] \leq 2$, whereas the maxima were poorly separated and their ratio was equal to about 1 at a large excess of $O_2^{\cdot}$. This behavior is probably caused by the existence of different configurations of the semiquinone complexes with hydrogen bonds of varying types. In addition, deprotonation of the semiquinone complexes by $O_2^{\cdot}$ was possible when the reaction was carried out with an excess of the superoxide ion.

That adriamycin is able to react with superoxide ion in aprotic medium opens the question of the possibility of reduction of molecular oxygen by adriamycin in mitochondria and microsomes. As is known, Equilibrium [3] is determined by the difference between one-electron redox potentials of adriamycin and molecular oxygen:

$$\mathbf{IIa} + O_2 \rightleftarrows \mathbf{Ia} + O_2^{\cdot}. \qquad [3]$$

One-electron redox potential of oxygen is equal to -0.11 to -0.15 V vs SHE (at $[O_2] = 1\ M$) (*12*). The first redox potential of adriamycin is equal to -0.63 to 0.66 V vs SCE at pH 7.1–7.5 (*2, 13*); but this is a two-electron potential, and it therefore cannot be used for the calculation of the equilibrium constant for Reaction [3]. Besides, one must take into account that the formation of sufficiently stable complexes of the adriamycin semiquinone displaces Equilibrium [3] to the left even though the difference between redox potentials $E_0(\mathbf{Ia/IIa}) - E_0(O_2/O_2^{\cdot}) < 0$.

Therefore another mechanism for stimulation of the superoxide production by adriamycin must exist. It is evident that $O_2^{\cdot}$ can be formed by oxidation of completely reduced adriamycin with molecular oxygen. But Thayler (*4*) showed that during incubation of adriamycin and NADH with submitochondrial particles, NADH oxidation accompanied by the formation of the superoxide ion caused an insignificant decrease of the adriamycin concentration. It is clear that the stationary concentration of the adriamycin semiquinone would be significant if adriamycin was a true one-electron carrier between NADH and O_2. We therefore believe that the participation of adriamycin in stimulation of the superoxide formation can be indirect. For example adriamycin may modify the properties of components of the respiratory chain (*4*).

Thus our experiments show that adriamycin may oxidize the semiquinones of ubiquinones, tocopherylquinone, vitamin K, and even benzoquinone. Cardiotoxicity of adriamycin can therefore be caused by its high electron-attracting properties, as practically all mitochondrial one-electron carriers must be oxidized by adriamycin. Indeed, adriamycin partially inhibits respiration in mitochondria and submitochondrial particles *in vitro* (*14, 15*). It is possible that the effect of natural quinones to decrease the cardiotoxicity of adriamycin (*6*) is accounted for by their ability to displace equilibriums ([2]) to the left and by this means to restore respiration in mitochondria.

ACKNOWLEDGMENT

We are very grateful to Dr. V. D. Kuznetzov for a sample of adriamycin.

REFERENCES

1. K. HANDA AND S. SATO, *Gann* **66,** 43 (1975).
2. S. SATO, M. IWAIZUMI, K. HANDA, AND Y. TAMURA, *Gann* **68,** 603 (1977).
3. N. R. BACHUR, S. L. GORDON, AND M. V. GEE, *Mol. Pharmacol.* **13,** 901 (1977).
4. W. S. THAYER, *Chem. Biol. Interact.* **19,** 265 (1977).
5. J. GOODMAN AND P. HOCHSTEIN, *Biochem. Biophys. Res. Commun.* **77,** 797 (1977).
6. C. E. MEYER, W. MCGUIRE, AND R. YOUNG, *Cancer Treat. Rep.* **60,** 961 (1976); M. GHIONE AND C. BERTAZZOLI, "Biomedical and Clinical Aspects of Coenzyme Q" (K. Folkers and Y. Yamamura, Eds.), p. 183. Elsevier, Amsterdam/Oxford/New York, 1977; G. ZBINDEN, E. BACHMANN, AND H. BOLLINGER, ibid., p. 219; E. P. CORTES, M. GUPTA, C. CHOU, M. PATEL, A. MUNDIA, AND K. FOLKERS, ibid., p. 267.
7. C. CASSAGNE AND J. BARAUD, *Bull. Soc. Chim. Fr.*, 1470 (1968).
8. I. B. AFANAS'EV, S. V. PRIGODA, T. YA. MAL'TSEVA, AND G. I. SAMOKHVALOV, *Int. J. Chem. Kinet.* **6,** 643 (1974).
9. I. B. AFANAS'EV, S. V. PRIGODA, AND G. I. SAMOKHVALOV, *Zh. Obshch. Khim.* **47,** 2507 (1978).
10. R. J. STURGEON AND S. G. SCHULMAN, *J. Pharm. Sci.* **66,** 958 (1977).
11. I. B. AFANAS'EV AND N. I. POLOZOVA, *Khim. Pharm. Zh.*, N 4, 16 (1979).
12. J. DIVISEK AND B. KASTENING, *J. Electroanal. Chem.* **65,** 603 (1975); Y. A. ILAN, G. CZAPSKI, AND D. MEISEL, *Biochim. Biophys. Acta* **430,** 209 (1976).
13. G. M. RAO, J. W. LOWN, AND J. A. PLAMBECK, *J. Electrochem. Soc.* **125,** 534 (1978).
14. M. GOSALVEZ, M. BLANCO, J. HUNTER, M. MIKO, AND B. CHANCE, *Eur. J. Cancer* **10,** 567 (1974).
15. Y. IWAMOTO, I. L. HANSEN, T. H. PORTER, AND K. FOLKERS, *Biochem. Biophys. Res. Commun.* **58,** 633 (1974).