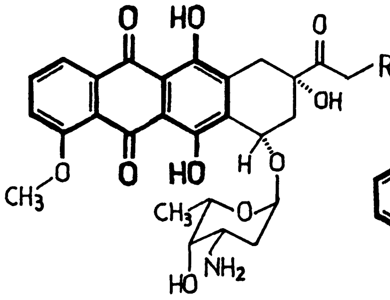
CHEMISTRY LETTERS, pp. 933-936, 1987.

An EPR Study of a Model System for the Initial Oxygen Activation Process Caused by Adriamycin-Iron System

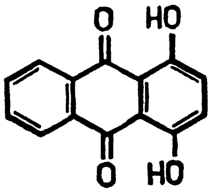
Masahiko TACHIBANA, Shozo TERO-KUBOTA,
and Masamoto IWAIZUMI*
Chemical Research Institute of Non-Aqueous Solutions,
Tohoku University, Katahira 2-1-1, Sendai 980

1,4-Dihydroxyanthraquinone, a model compound for Adriamycin, is reduced by Fe^{2+}-ADP complex to generate semiquinone, while by Fe^{2+}-phosphate complex to hydroquinone. The difference is explained in terms of the higher redox potential of the latter reducing agent. Similar reduction reactions were observed in the case of the drug.

Adriamycin (ADM, 1a), one of the anthracycline antibiotics widely used in cancer chemotherapy, is known to cause damage in organs with high O_2 pressure. Sugioka et al. have reported that in the presence of Fe^{2+} ion and adenosine 5'-diphosphate (ADP) the drug produces activated species of oxygen, which would cause lipid peroxydation. They have shown that the reaction of the oxygen activation proceeds in different manners dependent on the coordination environment around Fe^{2+} ion.1) Though the semiquinone of ADM has been proposed to be the initial transient species, direct evidence is stil lacking. Recently several authors reported that anaerobic reduction of Daunomycin (1b) causes reductive elimination of the aminosugar moiety through two-electron reduced intermediates.2) For the use of such a strong reducing agent as nicotinamide adenine diphosphate hydride (NADH), and for the unstability of the reduced form of ADM, their experimental systems seem to be too complex to know details of the reduction. So, we attempted to use 1,4-dihydroxyanthraquinone (1,4-DHAQ, 2) as a model compound for ADM, Fe^{2+} complexes as rather moderate reducing agents, and dimethylsulfoxide (DMSO) instead of H_2O as a solvent for simplification of the system. This paper shows that slight difference in Fe^{2+} coordination environment causes drastic changes in anaerobic reduction behavior.



R=OH: 1a
H ; 1b



2

Adriamycin was isolated from Adriacin , kindly supplied from Kyowa Hakko Kogyo Co. Ltd., and further purified by recrystallization from ethanol. Leucoquinizarin (LQ) was purified by sublimation. Other reagents were of the highest grade available and used without further purification. The Fe^{2+} complex solutions used as a reducing agent were prepared by dissolving $FeSO_4$ in H_2O containing six-fold excess of ADP or KH_2PO_4. Reduction was initiated by addition of the reducing agent to a DMSO solution of 1,4-DHAQ or ADM in the ratio of DMSO/H_2O=95/5 (v/v). The concentration of the reagents in the resulting solutions were 1 mM for 1,4-DHAQ or ADM and 1 to 3 mM for the Fe^{2+} complexes. The sample solutions were prepared under Ar and/or degassed condition by a vacuum line to protect from oxygen. EPR spectra were recorded on a JEOL JES-FE2XG or Varian E-112 spectrometer in X-band. UV-Visible spectra were recorded on a Shimadzu UV-240 spectrophotometer. All the measurements were made at room temperature.

Overall reactions were examined by UV-Visible spectroscopy. Time-course measurements of the reaction of 1,4-DHAQ with Fe^{2+}-ADP is shown in Fig. 1. Immediately after addition of the reducing agent, yellowish-orange color of the solution turned brownish-orange, which was caused by gaining absorption intensity at 490 nm over the original absorption having maxima at 460 and 480 nm of 1,4-DHAQ. Since the anion radical of 1,4-DHAQ has a sharp absorption maximum at 497 nm,$^{3)}$ the resultant spectral pattern can be interpreted as arising from a mixture containing a large amount of the starting material and a little one-electron reduced compound. The characteristic feature of the spectra did not change for a long period of time. It means that the system quickly reaches an equilibrium, i.e., it does within 5 min at most.

Similar experiments were made by use of the reducing agent Fe^{2+}-phosphate. In this case, the spectra showed gradual decrease of the absorption intensity at 460-480 nm range accompanying appearence of a new absorption maximum at 490 nm and increase of two absorption maxima at 397 and 412 nm (Fig. 2). The spectral

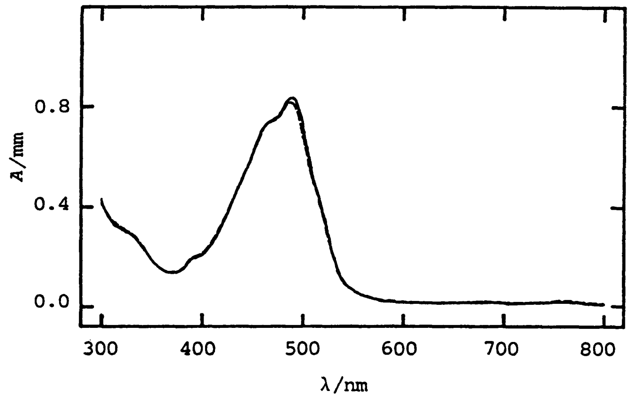


Fig. 1. UV-Visible spectra of 1,4-DHAQ reduced by Fe^{2+}-ADP complex. [1,4-DHAQ]= 1 mM; [Fe^{2+}]= 2 mM; ——— : 5 min later; – – – : 3 h later

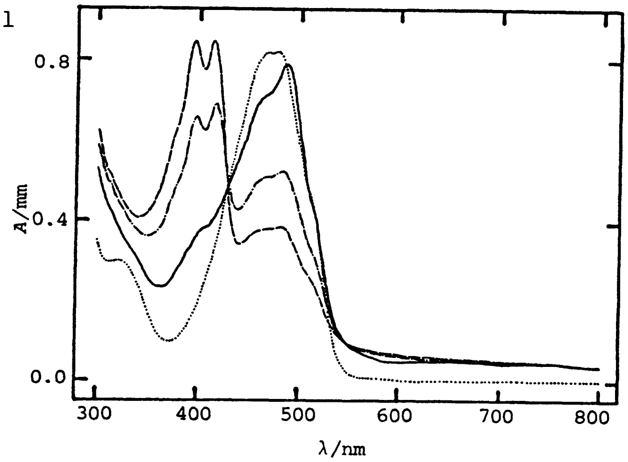


Fig. 2. UV-Visible spectra of 1,4-DHAQ reduced by Fe^{2+}-phosphate complex. [1,4-DHAQ]= 1 mM; [Fe^{2+}]= 3 mM; ·········· : before redution; ——— : 5 min later; —·— : 1 h later; – – – : 5 h later

pattern in the short wavelength region is identical to that for LQ, the two-electron reduced compound of 1,4-DHAQ. It is clear, therefore, that the Fe^{2+}-phosphate complex produces the hydroquinone in slow reaction rate as well as the semiquinone radical.

EPR measurements were made during the cource of reduction in the both Fe^{2+}-ADP and Fe^{2+}-phophate cases. The EPR spectra observed for the both cases have the same hyperfine splitting pattern, which clearly shows couplings with four sets of two equivalent protons and the splitting constants are in agreement with reported values for the 1,4-DHAQ semiquinone radical.[4)] The spectral intensities show, however, different time-dependent changes between the two cases (Fig. 3). When Fe^{2+}-ADP was used, the signal intensity exhibited no apparent change from few minites after the addition of the reducing agent. On the other hand, in the reduction with Fe^{2+}-phosphate, the signal gradually intensified with the increase of LQ concentration, which was detected by UV-Visible spectra.

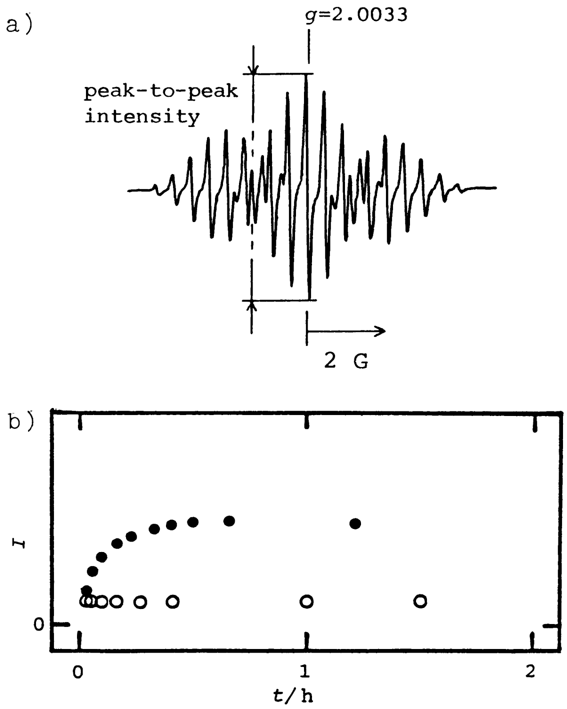


Fig. 3. a) EPR spectra of 1,4-DHAQ semiquinone anion radical, b) time-dependent change of EPR signal intensities.

○ : reduced by Fe^{2+}-ADP

● : reduced by Fe^{2+}-phosphate

These observations suggest that the semiquinone radical may be converted from LQ produced by two-electron reduction. The following experiment indicates that the comproportionation reaction of LQ and 1,4-DHAQ is the most probable reaction for generation of the semiquinone radical, i.e., a DMSO solution of LQ and 1,4-DHAQ under Ar atmosphere, if it contains a small amount of water, generates the radical at a reaction rate so slow as it takes few hours to complete the reaction. The fact that water is necessary to proceed the reaction shows that an acid-catalyzed process is included in the reaction.

The difference in redox behavior between the two Fe^{2+} complexes is interpreted in terms of the redox potentials. Oxidation potentials for the Fe^{2+}-ADP and Fe^{2+}-phosphate complexes in aqueous solution are -0.25 V and -0.84 V vs. S.C.E., respectively.[1)] The former is 0.34 V higher than the one-electron reduction potential of -0.59 V for 1,4-DHAQ in DMSO,[5)] so the redox equilibrium

$$\mathrm{DHAQ} + \mathrm{Fe^{2+}\text{-}ADP} \rightleftharpoons \mathrm{DHAQ^{\overline{\cdot}}} + \mathrm{Fe^{3+}\text{-}ADP}$$

is driven only a little amount to the right hand side. The latter is nearly equal to the two-electron reduction potential of -0.86 V for 1,4-DHAQ, being enough to produce a large amount of LQ.

The reduction of ADM by the two reducing agents was also examined. UV-

Visible spectral changes with time were so little that it was hard to distinguish the product from the substrate, unlike the case of model compound. However, the EPR spectra showed formation of the anion radical of ADM for the both reducing agent systems, i.e. Fe^{2+}-ADP and Fe^{2+}-phosphate complexes, though the EPR signals were not very strong. The time-dependent changes of EPR spectral intensities are shown in Fig. 4. When Fe^{2+}-ADP was used, the radical species appeared immediately after the addition of the reducing agent and the intensities decreased monotonically. The feature of the time-dependent changes of the Fe^{2+}-ADP system is quite different from that of the Fe^{2+}-phosphate system where the radical species gradually appears and reaches the maximum in 30 min. These time-dependent behaviors are similar to those observed in the 1,4-DHAQ system presented above, and it seems to be a reflection of the difference between the redox capability of the iron complexes. Reduction potentials for ADM in DMSO are -0.70 V for one-electron reduction and -0.98 V vs. S.C.E. for two-electron reduction, more negative than those for 1,4-DHAQ, making it difficult to generate the reduced species.[5] However the radical formation mechanism can be explained in the same way as the case of 1,4-DHAQ. Fe^{2+}-ADP produces rather unstable semiquinone only, while Fe^{2+}-phosphate produces quasi-stable hydroquinone which is the possible source for the semiquinone radical through an acid-catalyzed comproportionation.

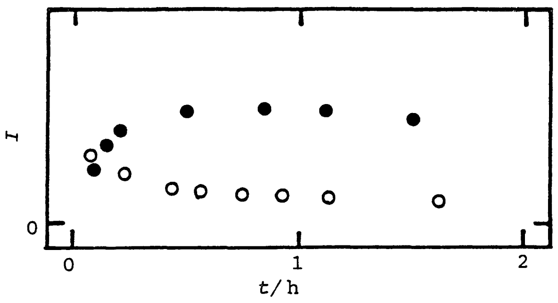


Fig. 4. Time-dependent change of EPR signal intensities of semiquinone radical produced in ADM-Fe^{2+} complex system.
○ : reduced by Fe^{2+}-ADP
● : reduced by Fe^{2+}-phosphate

It should be noted that Fe^{2+} complexes, which have been viewed as a mediator of electron from reduced anthracycline to oxygen in an oxygen activation reaction, in fact reduce the anthracycline by itself under certain conditions.

References

1) K. Sugioka, H. Nakano, M. Nakano, S. Tero-Kubota, and Y. Ikegami, Biochim. Biophys. Acta, 753, 411 (1983).

2) J. Fisher, K. Ramakrishnan, and J. E. Becvar, Biochemistry, 22, 1347 (1983); D. L. Kleyer and T. Koch, J. Am. Chem. Soc., 106, 2380 (1984); J. Fisher, B. R. J. Abdella, and K. E. McLane, Biochemistry, 24, 3562 (1985); D. J. Brand and J. Fisher, J. Am. Chem. Soc., 108, 3088 (1986).

3) J. Anne and J. Moiroux, Nouv. J. Chim., 8, 259 (1984).

4) J. Gendell, W. R. Miller, Jr., and G. K. Fraenkel, J. Am. Chem. Soc., 91, 4369 (1969); D. Lunny, J. Bailes, and J. D. Memory, J. Chem. Phys., 53, 3387 (1970).

5) A. Ashnagar, J. M. Bruce, P. L. Dutton, and R. C. Prince, Biochim. Biophys. Acta, 801, 351 (1984).

(Received March 2, 1987)