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Study of the Redox Properties of Biological Radicals Produced by Reaction with Short-lived Inorganic Species

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The electrochemical properties of radicals produced in aqueous solutions of DNA components may be related to their role in the mechanism of radiation sensitization as applied to the therapy of tumours.

The half-wave potential of polarographic curves is a characteristic of a particular substance in a given solution. For reversible electrode processes, *i.e.* when the equilibrium between the oxidized and reduced form is established within the experiment time-scale, the half-wave potential is equal to the formal standard potential. Due to the short life time of the radicals studied, the reversible stage is hardly reached in the present experiments. The half-wave potential of irreversible electrode processes is more negative for reduction and more positive for oxidation reactions than the formal standard potential.

Since the radiosensitization action of electron affinic compounds is based on the oxidation of the radicals, the half-wave potential of irreversible oxidation of the radicals gives upper threshold values for the formal standard potential of compounds which can act as sensitizers and therefore is of great interest.

We report results, in buffered solutions at pH = 7, on the polarographic behaviour of the radicals produced by the reactions of uracil with the primary products of water radiolysis, OH, H and e_{aq}^- [1].

The experimental data have been obtained by marrying a fast coulostatic technique to pulse radiolysis [2]. The coulostatic change produced by faradaic current across the polarographic cell is given by

$$\Delta E = \frac{Q}{C_{dl}} = \frac{1}{C_{dl}} \int_0^t \sum_{X = A,B,C\ldots} i_X \, dt$$

where Q is the charge deposited at the DME, t the time, i_X the contribution of species X to total current and C_{dl} the specific differential capacity.

The radiation source was a 2 MeV Febetron accelerator which provided an intense 30 ns duration X-ray pulse. The dose delivered to the system was 1 Krad.

Experimental data, in the coordinates voltage–time, were processed using a desk calculator for the simultaneous occurrence of more than one electrochemical reaction and for effects such as those caused by the reactions of intermediates in pairs.

The energy transfer from ionizing radiation to the water molecules produces high reactive intermediates and molecular products:

$$H_2O \rightsquigarrow H_3O^+, e_{aq}^-, H, OH, H_2O_2, H_2 \qquad (1)$$

The yields of the highly reactive intermediates H, OH and e_{aq}^-, expressed as number of molecules produced by each 100 eV of energy absorbed by the system, G, are $G_{(e_{aq}^-)} = 2.9$, $G_{(OH)} = 2.8$, $G_{(H)} = 0.5$. In N_2O saturated solutions, the hydrated electron is converted to OH radical:

$$N_2O + e_{aq}^- \xrightarrow{H_2O} OH + OH^- + N_2 \qquad (2)$$

so that the initial intermediate is almost entirely OH.

Using N_2O saturated solution at pH = 7, 10^{-3} *M* phosphate buffer, and adding 10^{-3} *M* uracil, U, the observed potential change is caused almost entirely by the product of reaction 3, *i.e.* the uracil–OH adduct:

$$U + OH \longrightarrow U\text{–}OH \qquad (3)$$

The curve obtained, Fig. 1, shows for U–OH one oxidation wave with half-wave potential $E_{1/2} = -0.45$ V *vs.* SCE and two reduction waves with $E_{1/2} = -0.6$ V *vs.* SCE and $E_{1/2} = -0.92$ V *vs.* SCE. The wave at *ca.* -1.1 V *vs.* SCE is due to the reduction of H_2O_2. The results indicate that in reaction 3 at least two different U–OH adducts are formed with different electrochemical behaviours and their yields are in the ratio 1:4. Previous workers [3] have

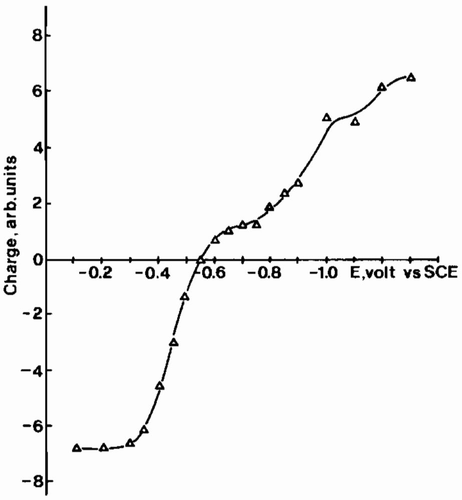

Fig. 1. Charge *vs.* potential curve for N_2O saturated solution 0.5 *M* Na_2SO_4, 10^{-3} *M* uracil in phosphate buffer 10^{-3} *M*, pH = 7. Data refer to 50 μs of elapsed time.

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indicated that, at neutral pH, two different radicals can be produced by OH attack at positions C-5 and C-6.

In argon-saturated solutions the radicals which can contribute to the faradaic process at the electrode are the U–OH and the uracil electron adduct U^-. In 10^{-3} *M* uracil solution the OH radicals are completely scavenged in both N_2O and Ar saturated solutions, therefore the contribution of U–OH to the change in interfacial potential in the latter system can be calculated from the yields of OH radical in the two solutions. Figure 2 displays the experimental curve and the calculated contribution of U–OH and U^- adducts.

The half-wave potential of the electron adduct, $E_{1/2} = -1.3$ V *vs.* SCE, indicates that this radical is a powerful reducing agent and can react with the U–OH in reaction which regenerates uracil. Another back-reaction which the results indicate as thermodynamically possible is the disproportionation of uracil–OH adducts. Furthermore compounds with a redox potential more positive than ~–0.2 V *vs.* SCE oxidize all the radicals produced in uracil solution and might be expected to be sensitizing agents for anoxic cells.

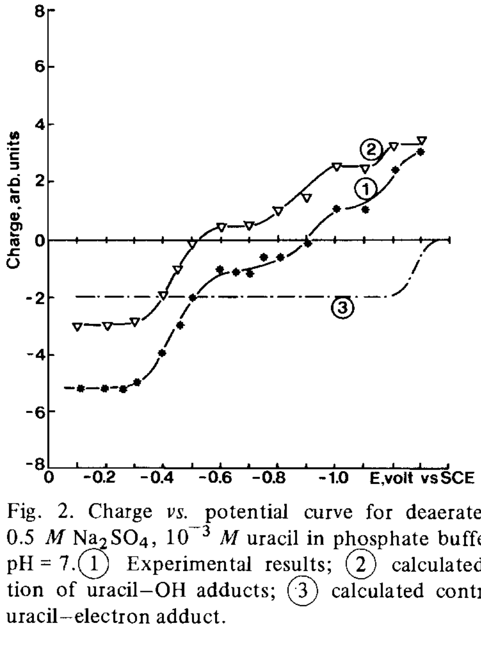

Fig. 2. Charge *vs.* potential curve for deaerated solution 0.5 *M* Na_2SO_4, 10^{-3} *M* uracil in phosphate buffer 10^{-3} M, pH = 7. ① Experimental results; ② calculated contribution of uracil–OH adducts; ③ calculated contribution of uracil–electron adduct.

The reported results prove that the coulostatic technique is a convenient method for the determination of the electrochemical behaviour of radicals of biological interest and gives reliable information on the mechanism of radiation induced biological damage. Further work is in progress.

References

1 a) E. Barrow, P. Johnson and A. Cobure, *Radiation Res., 1,* 410 (1954).
b) G. Scholes, J. F. Ward and J. J. Weiss, *J. Mol. Biol., 2,* 379 (1960).
c) J. Holian and W. M. Garrison, *Nature, 212,* 394 (1966).
d) G. Scholes, P. Shaw, R. L. Willson and M. Ebert in 'Pulse Radiolysis', M. Ebert, J. P. Keene, A. J. Swallow and J. H. Baxendale, Eds., Academic Press, New York, 1965, p. 281.
e) G. Scholes, in 'Radiation Chemistry of Aqueous Systems', G. Stein, Eds., Weizmann Science Press of Israel, Jerusalem, 1968, p. 259.
f) C. L. Greenstock, J. W. Hunt and M. Ng, *Trans. Farad. Soc., 65,* 3279 (1969).
g) C. L. Greenstock, *Trans Farad. Soc., 55,* 2541 (1970).
h) K. M. Bansal and R. M. Sclder, in 'Fast Processes in Radiation Chemistry and Biology', G. E. Adams, E. M. Fielden and B. D. Michael, Eds., Wiley, 1973, p. 259.
i) C. L. Greenstock and R. H. Wiebe, in 'Oxygen and Oxy-Radicals in Chemistry and Biology', M. A. J. Rodgers, E. L. Powers, Eds., Academic Press, New York, 1981, p. 119.
2 F. Barigelletti, F. Busi, M. Ciano, V. Concialini, O. Tubertini and G. C. Barker, *J. Electroanal. Chem., 97,* 127 (1979).
3 G. Nucifora, B. Smaller, P. Remko and E. C. Avery, *Radiation Res., 49,* 96 (1972).