Free Rad. Res. Comms., Vol. 5, No. (4–5), pp. 253–258
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SINGLE-ELECTRON-TRANSFER IN THE REDUCTION OF 1,2-DIOXETANES BY BIOLOGICALLY ACTIVE SUBSTRATES.

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(Received June 3, 1988)

Substances of low oxidation potential, which can also make available protons and hydrogen atoms, e.g. phenothiazines, NADH, and ascorbic acid efficiently reduce 1,2-dioxetanes to their vic-diols by single-electron-transfer; a significant side reaction is catalytic decomposition of dioxetanes into the corresponding ketone fragments

KEY WORDS: Ascorbic acid; N-benzyldihydronicotinamide; Chlorpromazine; 1,2-Dioxetanes; NADH; Phenothiazine

The implication of 1,2-dioxetanes in photobiological processes is well established.[1] This is expected in view of their unique ability to generate efficiently triplet excited carbonyl products on thermal decomposition (eq. 1). However, only recently[2–5] has the genotoxicity of this unusual class of cyclic peroxides been demonstrated. Surprising was the modest genotoxic activity displayed by the dioxetanes and uncertain was the photochemical origin of the DNA damage. Thus, as peroxide structures it is inherent that they may oxidize critical cell components and thereby cause "oxidative stress".[6] The living cell is equipped against such damages, the most prominent defense mechanism entails detoxification through reduction by glutathione. Indeed, we recently reported[7] that biological thiols such as glutathione and cysteine efficiently reduce 1,2-dioxetanes to their vic-diols, the thiols being oxidized to the corresponding disulfides. A single-electron-transfer (SET) process was proposed for this novel redox reaction of dioxetanes, in which the peroxide serves as electron acceptor and the sulfur compound as electron donor, leading to a radical ion pair. If the resulting radical cation of the sulfur substrate cannot readily supply a hydrogen atom or proton, as is the case for alkyl sulfides, oxygen transfer and C–H bond insertion prevail. A significant side reaction is also catalytic decomposition of the dioxetane.[8] The purpose of the present study was to confirm the involvement of single-electron-transfer (SET) in the reaction of 1,2-dioxetanes **1** with biological reductants.

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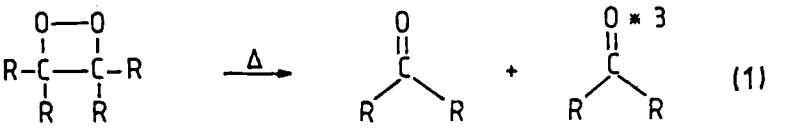


FIGURE 1

MATERIALS AND METHODS

General Aspects

Boiling and melting points are uncorrected, the latter were taken on a Reichert Thermovar Kofler apparatus. Infrared (IR) spectra were obtained on a Perkin-Elmer 1420. ^{1}H NMR spectra were run either on a Bruker AW 80 (80 MHz) with temperature control or a Bruker AC 200 (200 MHz) spectrometer, using p-dichlorobenzene or hexamethyldisiloxan as internal standard. ^{13}C NMR spectra were taken on a Bruker AC 200 (50 MHz). The chemical shifts (δ) are reported in ppm. The UV/Vis spectral studies were carried out on a U-3200 Hitachi spectrometer with Replay and Time Scan Program. Mass Spectra (MS) were measured on a Varian MAT CH7. Combustion analyses for elemental composition were obtained inhouse.

Thin-layer chromatography (TLC) was run on Polygram SIL/G/UV (40 × 80 mm) from Machery and Nagel Co. Column chromatography utilized silica gel 32–64 mesh ASTM (activity III). Commercial reagents and solvents were purchased from standard chemical suppliers and purified by double destillation, when necessary from EDTA. Known compounds were prepared according to literature procedures and purified to match reported physical and spectral data.

Stirring was performed magnetically; room temperature (RT) was ca. 20°C; drying after aqueous work-up was carried out over anhydrous $MgSO_4$ and rota-evaporation was performed at aspirator pressure (15–20 torr) at 0°C.

Phenothiazine and chlorpromazine were purchased from Fluka AG, NADH and ascorbic acid from Sigma, tocopherol from Merck, N-Benzyldihydronicotinamide was prepared and purified as described.[10]

*3,3,4,4-Tetramethyl-1,2-dioxetane (**1a**).*

A 50-ml, round-bottomed flask, provided with magnetic spinbar and Claisen and vacuum distilling adapters, was charged with 2.00 g (10.0 mmol) each of 2-bromo-2,3-dimethyl-3-hydroperoxybutane[11] and silver succinimide, suspended in 8 ml of dibutyl phthalate, while stirring at 40°C under vacuum (1 torr) for 3 h. The volatile products were collected into a dry ice-methanol cooled receiver. The condensate was crystallized from pentane at −20°C to give 0.500 g (4.35 mmol) (44%) of yellow needles, m.p. 76–78°C (lit.[12] 76°C).

*3-Hydroxymethyl-3,4,4-trimethyl-1,2-dioxetane (**1b**)*

Was prepared via base-catalyzed cyclization of the 2,3-dimethyl-1,2-epoxy-3-hydroperoxybutane[13]. Instead of using the recommended tetramethylammonium hydroxide as phase transfer base, we found that mere KOH afforded higher yields and purer products[14].

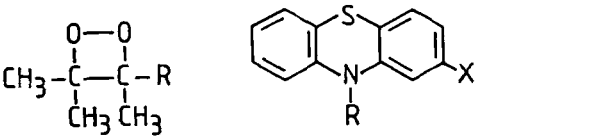
General procedure for the reduction of dioxetanes ***1a*** *and* ***1b*** *by the biological substrates*

A 5-ml, round-bottomed flask, provided with magnetic spinbar and nitrogen inlet and outlet tubes, was charged with 0.050 mmol of the particular dioxetane **1a** or **1b** in 1 ml solvent and cooled to the desired reaction temperature by means of a MeOH bath, employing a cryostat. A solution of 0.10 mmol of the biological substrate (0.050 mmol in the case of chlorpromazine and ascorbic acid) in 1 ml of the same solvent was added within 10 min. The reaction course was monitored by UV-Vis spectrometry, TLC and ^{1}H NMR until all the dioxetane was consumed. The product composition of the crude reaction mixture was determined by quantitative ^{1}H NMR using the appropriate internal standard.

RESULTS

We chose phenothiazine (**2a**) and chlorpromazine (**2b**) because their radical cations are sufficiently stable for direct UV-Vis spectrophotometric detection.[15,16] As anticipated, phenothiazine (**2a**) and chlorpromazine (**2b**) both gave transient absorptions at λ_{max} 521 nm and 525 nm, respectively, characteristic for the corresponding radical cations.[15,16] A product study of the reaction of phenothiazine (**2a**) with dioxetane **1b** ($CHCl_3$ or CH_3CN, -20°C) showed that **1b** was reduced to its triol and **2a** converted to its diphenothiazinyl cation **3**,[17] the stable product of the radical cation of **2a** to the extent of 43%. The remainder (57%) of the reaction course could be accounted for in terms of catalytic decomposition of the dioxetane **1b** into acetone and hydroxyacetone.

Besides the glutathione defense mechanism against hydroperoxides[6], the cell contains other efficient reductants to detoxify such hazardous oxidants, e.g. dihydronicotinamide adenine dinucleotide (NADH), riboflavin adenosine diphosphate ($FADH_2$) and ascorbic acid, to mention the more important ones. Indeed, in H_2O at 25°C NADH was oxidized to NAD by dioxetane **1b** and the latter reduced to its triol in 34% yield; the remainder of the reaction (66%) was accounted for in terms of the already mentioned carbonyl products (catalytic decomposition) of the dioxetane. The reaction course was conveniently monitored by following the decrease of the NADH absorption (λ_{max} = 339 nm) and the increase in the NAD absorption (λ_{max} = 260 nm). Similarly, as NADH model compound, N-benzyldihydronicotinamide[18,19] led in MeOH at -25°C to clean reduction of dioxetane **1a** to pinacol.



1a (R = CH_3) 2a (R = X = H)

1b (R = CH_2OH) 2b (R = $(CH_2)_4OH$, X = Cl)

FIGURE 2

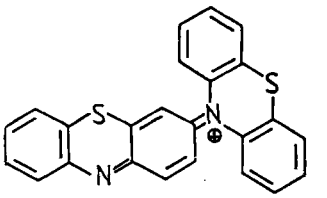


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FIGURE 3

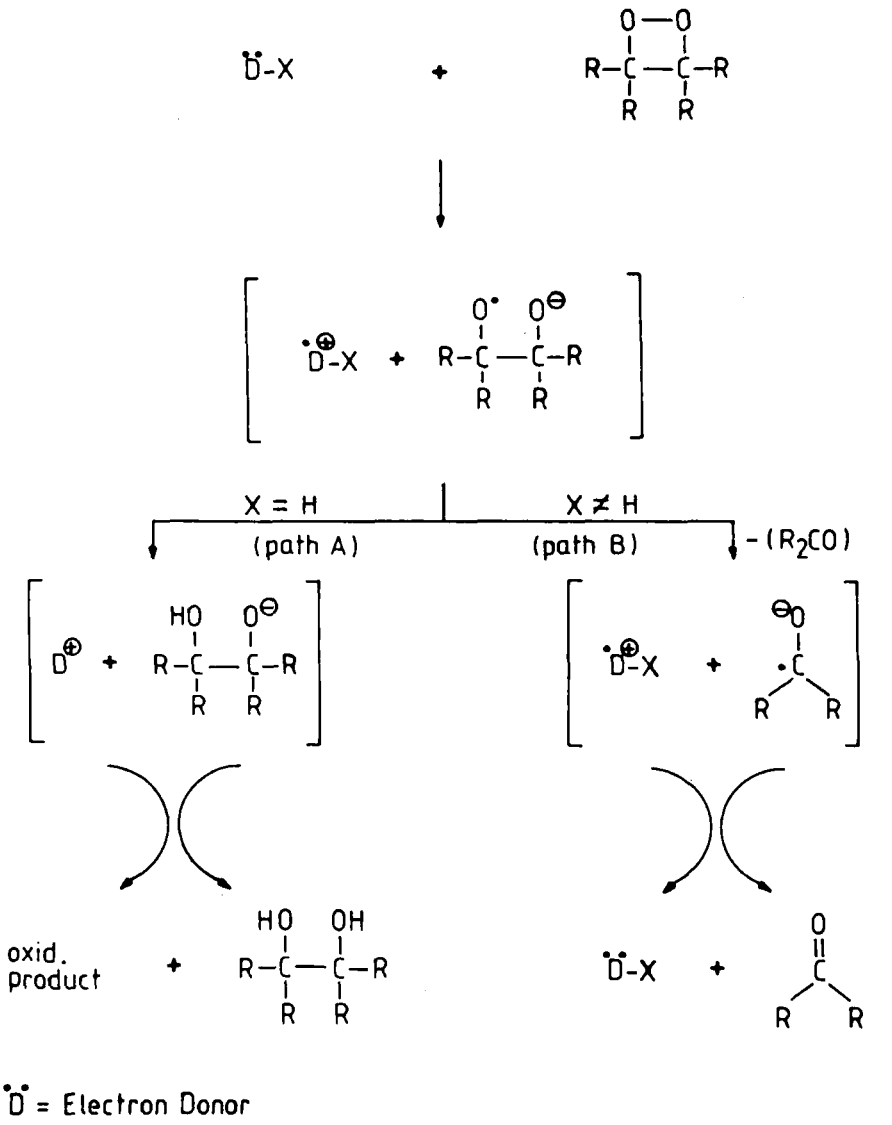
Also ascorbic acid was an effective reductant, leading to 70% conversion of dioxetane **1b** into its triol in H_2O at 15°C, itself being oxidized to dehydroascorbic acid. There appear no reports in the literature that ascorbic acid can serve as reductant for cyclic peroxides. Furthermore, it is significant to mention that ascorbic acid caused ca. 30% catalytic decomposition of the dioxetane **1b** into its carbonyl products, again hinting at SET behaviour. Preliminary results show similar action by tocopherol. In this context it is important to state that a qualitative correlation between the oxidation potentials of these reductants and the rate of reaction with the dioxetane is observed.

DISCUSSION

The formation of the radical cations of phenothiazine (**2a**) and chlorpromazine (**2b**) in the reduction of the dioxetane **1b** to its triol and/or catalytic decomposition into ketone fragments unequestionably manifests SET behaviour[7] in these reactions. The fact that the same type of chemistry (reduction and/or catalytic decomposition) is also exhibited in the reaction of dioxetanes **1** with NADH, the dihydronicotinamide and ascorbic acid, and that a qualitative correlation between the oxidation potentials of these reductants and their rate of reaction with dioxetanes is observed, strongly implies electron transfer. In these redox reactions the dioxetane serves the part of the electron acceptor and the biological substrate the electron donor, provided the latter has a relatively low oxidation potential ($E_{ox} < 0.8$ V).

The SET mechanism, that we propose for this redox process, is displayed for the general case in Scheme 1. If the resulting radical cation can also provide a hydrogen atom and a proton, then the chemical fate of the dioxetane is formation of the vic-diol (path A in Scheme 1) and the biological substrate is oxidized, e.g. NADH to NAD or ascorbic acid to dehydroascorbic acid. In case hydrogen and/or proton transfer by the electron donor is difficult, the radical anion of the dioxetane cleaves (path B in Scheme 1) into the ketyl radical and ketone fragments and electron back transfer regenerates the biological susbstrate (thus acting as *bona fide* catalyst). The overall result is catalytic decomposition of the dioxetane into ketone products.[8,9]

We conclude that substances with low oxidation potentials, which also can readily provide protons and/or hydrogen atoms, qualify as efficient reductants of 1,2-dioxetanes, and cyclic peroxides in general, via single-electron-transfer. Since the living cell



SCHEME 1

is well equipped with such reductant systems, it should no longer be surprising that the photobiological action of dioxetanes in cellular studies is efficiently counteracted via detoxification by such reduction.

Acknowledgements

We thank the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich No. 172 "Molekulare Mechanismen Kanzerogener Primärveränderungen"), the Fritz-Thyssen Stiftung, and the Fonds der Chemischen Industrie for generous financial support. F. Vargas thanks the DAAD for a doctoral fellowship.

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Accepted by Prof. H. Sies.