Superoxide Anion Radical-induced Dioxygenolysis of Quercetin as a Mimic of Quercetinase

Kenji Kano,* Toshiro Mabuchi, Bunji Uno, Yukihiro Esaka, Toshiyuki Tanaka and Munekazu Iinuma*

Gifu Pharmaceutical University, 5-6-1 Mitahora-Higashi, Gifu 502, Japan

Reaction of quercetin with superoxide anion radical is triggered by proton abstraction from quercetin to yield dismutated products of superoxide and deprotonated quercetin, which allows quercetinase-like dioxygenation to give the corresponding depside in a high yield.

Flavonols occur widely in a variety of plants as aglycones (including quercetin **1**) and glycosides. During the past two decades, increasing interest has been paid to their antioxidant activities. Flavonols are considered to scavenge reactive oxygen species such as superoxide anion radical ($O_2^{-\bullet}$), singlet oxygen ($^1O_2$), and the hydroxyl radical.[1–4] Although there exists evidence for $O_2^{-\bullet}$-suppression or $O_2^{-\bullet}$-induced degradation of flavonols,[2,3] the mechanism is not well understood. At present, a direct single-electron transfer (SET) to $O_2^{-\bullet}$ is widely believed to induce the $O_2^{-\bullet}$-dependent oxidation of flavonols,[2,3] however, this begs the question whether $O_2^{-\bullet}$ can oxidize flavonols.

The superoxide anion radical is easily generated by electrochemical reduction of dioxygen (O_2) and is stable in some aprotic solvents, such as DMF. The interaction between **1** and $O_2^{-\bullet}$ in DMF has been electrochemically analysed by us.

Dioxygen is reversibly reduced to $O_2^{-\bullet}$ (at −0.70 V *vs.* SCE), which is subsequently reduced irreversibly to HO_2^- around −1.6 V [Fig. 1(*a*)]. The first oxidation of **1** occurs at +0.91 V, while the reduction is observed at −1.58 V [Fig. 1(*b*)]. The first oxidation involves a two-electron transfer resulting in its quinoid form. In the presence of **1**, $O_2^{-\bullet}$ is completely quenched: the first reduction wave of O_2 becomes irreversible [Fig. 1(*c*)]. Rotating disk voltammetric experiments have revealed that the limiting current of the first reduction wave of O_2 increases with the bulk concentration of **1** (C_q) and then levels off to be doubled when $D_q^{2/3}C_q$ exceeds $D_o^{2/3}C_o$ (D_q, D_o, and C_o are the diffusion coefficients of **1** and O_2, and the bulk concentration of O_2).† These results clearly indicate a net two-electron reduction of O_2 in the presence of **1** with a reaction stoichiometry of 1:1. Chronoamperometric analysis suggested a second-order rate constant of $>3 \times 10^6$ dm^3 mol^{-1} s^{-1}.

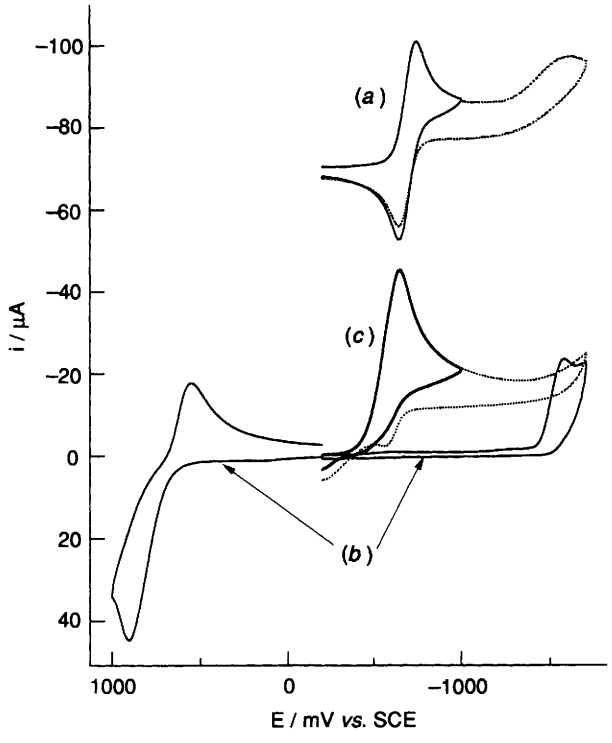


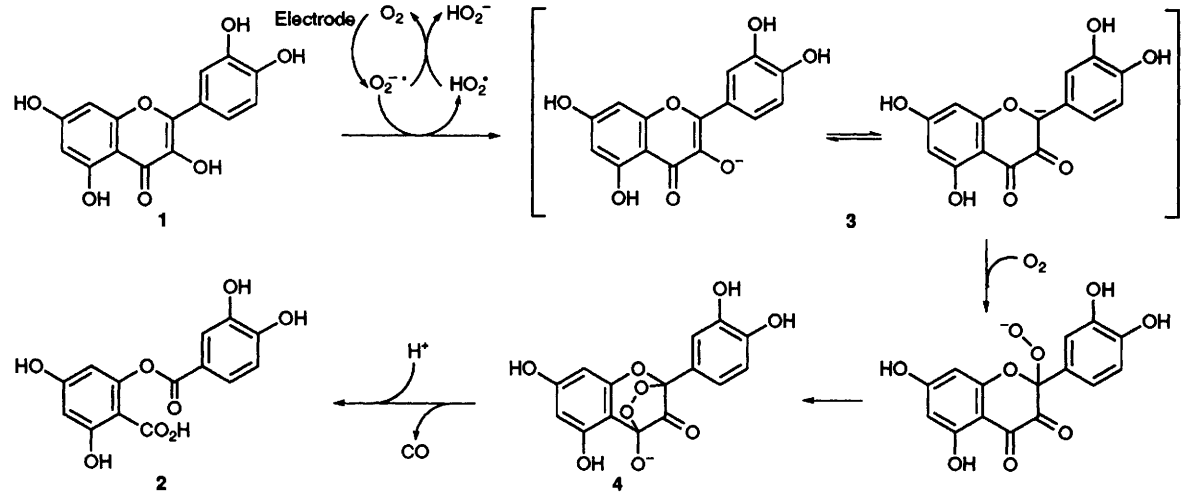
Fig. 1 Cyclic voltammograms of (*a*) 1.2 mmol dm^{-3} O_2, (*b*) 3.0 mmol dm^{-3} **1** and (*c*) 1.2 mmol dm^{-3} O_2 plus 3.0 mmol dm^{-3} **1** at an Au electrode with a scan rate of 50 mV s^{-1}, all in DMF containing 0.1 mol dm^{-3} tetraethylammonium perchlorate

Controlled-potential electrolysis was performed at −1.0 V in the presence of **1** under O_2-bubbling. Generation of one predominant product **2** with concomitant disappearance of **1** in the course of the electrolysis was proved by reversed phase HPLC experiments of the electrolysis solution with eluent composed of MeCN–0.2 mol dm^{-3} phosphate buffer [27:73 (v/v), pH 2.0]. The main product **2** was extracted with ethyl acetate and recrystallized from methanol–0.1 mol dm^{-3} HCl. **2** was characterized as a depside, 2,4-dihydroxy-6-(3,4-dihydroxybenzoyloxy)benzoic acid.‡ The final yield of **2** was as large as 93 mol% with respect to the starting substrate **1**.

The reduction potential of $O_2^{-\bullet}$ is sufficiently negative by *ca.* 2.5 V compared with the oxidation potential of **1**. This fact means that a direct electron transfer from **1** to $O_2^{-\bullet}$ is not feasible. Rather, the reaction is triggered by a proton transfer from **1** (most probably at the 3-OH group) to $O_2^{-\bullet}$. The resultant $HO_2^{\bullet}$ is dismutated to give HO_2^- (or H_2O_2) and O_2. The proton-induced dismutative propensity must enhance the effective basicity of $O_2^{-\bullet}$.[5] A proposed mechanism is illustrated in Scheme 1. O_2 (not $O_2^{-\bullet}$) appears to be incorporated into the deprotonated form of **1** (**3**) yielding a cyclic peroxide **4**. Decarbonylation of **4** leads to **2**. This scheme satisfies the net two-electron reduction of O_2 and the 1:1 stoichiometry between **1** and $O_2^{-\bullet}$. The overall reaction mimics quercetinase-catalysed dioxygenation, although involvement of some activated oxygen was suggested in the enzymatic mechanism.[6] Similar depside formations occur using cobalt Schiff base complexes as catalysts,[7] or $^1O_2$ as a primary reactant.[4]

Considering the proposed mechanism, **1** is expected to undergo base-induced dioxygenolysis to **2**.[8] This could also be shown by addition of one equivalent of 1 mol dm^{-3} NaOH to a solution of **1** in DMF under O_2, yielding **2** (90 mol%) after 6 h. Involvement of the O_2 absorption is rationalised by the fact that under anaerobic conditions **1** is all recovered after neutralisation. These results support strongly our proposal that $O_2^{-\bullet}$ acts as a Brönsted base. Use of larger amounts of NaOH accelerates the dioxygenolysis of **1**, but concomitantly gives rise to a significant decrease in the yields of **2** due to base-catalysed hydrolysis of **2**. Only 3,4-dihydroxybenzoic acid **5** was detected by capillary zone electrophoresis analysis as a main product in alkaline hydrolysates of **2** with 1 mol dm^{-3} NaOH for 2 h at 50°C. Another expected hydrolysate 2,4,6-trihydroxybenzoic acid **6** was decomposed under the present conditions.

On the other hand, in $O_2^{-\bullet}$ (or base-) dependent oxidation of flavonols (or polyphenols), generation of semiquinone-type radicals has been reported.[3] This might be considered to support a SET reaction from flavonols to $O_2^{-\bullet}$. However, deprotonated polyphenols should be easily oxidized by O_2 to yield the corresponding radicals. Indeed, **2**, **5** and **6** all generate the corresponding semiquinone-type radicals in



Scheme 1 Proposed mechanism

aerated alkaline conditions, while **2** gives two distinct radicals and the ESR intensity in the case of **6** is very weak.§ Therefore, the reported ESR spectral results does not necessarily indicate a SET-driven mechanism, although the mechanism might not be completely ruled out because $O_2^{-\bullet}$ is a better oxidant in water than in DMF.

Extrapolating our mechanistic concept to the quercetinase system, the enzyme seems simply to enhance an anionic nature of **1** by coordination of the Cu^{II} cofactor. On the other hand, the present findings suggest that flavonols play a role as superoxide dismutase in hydrophobic surroundings like cellular lipid bilayer.

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Footnotes

† In the case of diffusion-layer titration by rotating disk voltammetry, the efficient concentration of the reactant is given by $D^{2/3}C$.

‡ Satisfactory analytical and spectral data were obtained for **2**.

§ Hyperfine coupling constants of alkaline-generated radical compounds: **2** (major): 0.50 G (1H), **2** (minor): 4.83 G (1H) and 0.86 G (1H), **5**: 1.42 G (1H) and 0.48 G (1H).

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