Hydrogen abstraction from neurotransmitters by active oxygen species facilitated by intramolecular hydrogen bonding in the radical intermediates

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The reactivity of neurotransmitters toward hydrogen abstraction by an active oxygen species (the cumylperoxyl radical) is comparable to that of a strong antioxidant such as catechin due to the strong intramolecular hydrogen bonding, which has been successfully detected by ESR.

Neurotransmitters are small molecules with pivotal biological importance. Norepinephrine, epinephrine, serotonin, and dopamine have neurotransmitter function in the human organism.[1,2] Since human beings consume more than 20% of their oxygen in the brain, neurotransmitters are exposed to considerable amounts of active oxygen species. Active oxygen species such as superoxide or peroxide and their metabolic products may play a key role in the etiology of certain disorders of the central nervous system.[3–7] The patients of schizophrenia are also known to be quite sensitive to the mental and physiological stress that usually increases the concentration of active oxygen species.[8] Under such conditions, the catecholamine moiety of neurotransmitters may be easily oxidized to the corresponding quinone, which might disturb neurotransmission by sending abnormal signals to the receptors or by behaving as an agonist like methamphetamine. Despite such importance in understanding the possible roles of the active oxygen species in disorders of the central nervous system, the reactivities of neurotransmitters toward active oxygen species have yet to be reported.

We report herein the determination of the rates of a series of neurotransmitters (epinephrine, dopamine, L-DOPA, norepinephrine, tyrosine and serotonin, which are shown in Chart 1) with an active oxygen species (the cumylperoxyl radical), in order to evaluate the reactivity of neurotransmitters toward active oxygen species for the first time. The radical intermediates, formed by hydrogen abstraction from the neurotransmitters, have been successfully detected by electron spin resonance (ESR) spectroscopy at low temperature in solution.

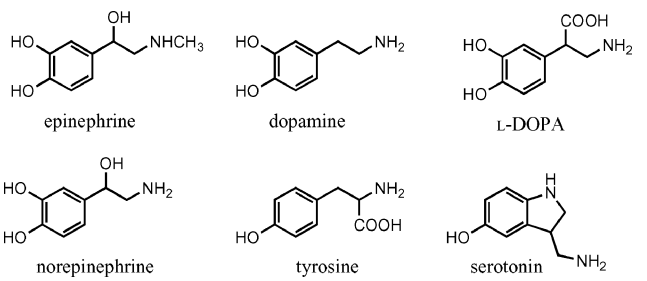
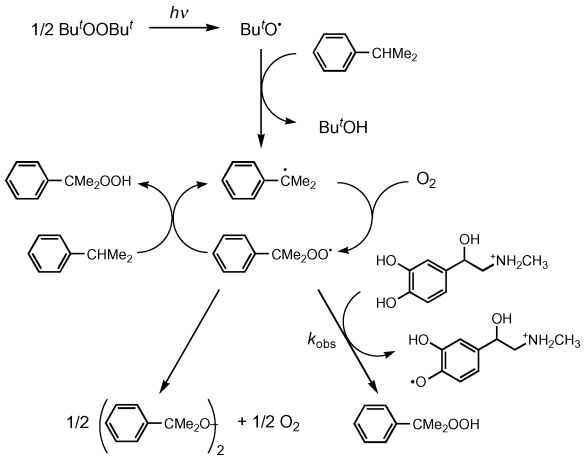


Chart 1

The rates of the hydrogen transfer reactions of a series of neurotransmitters with the cumylperoxyl radical to produce phenoxyl radical species were measured using ESR. The cumylperoxyl radical is formed *via* a photoinduced radical chain process shown in Scheme 1. Under photoirradiation of an oxygen-saturated propionitrile (EtCN) solution of cumene and di-*t*-butyl peroxide, the O–O bond of di-*t*-butyl peroxide is cleaved to produce the *t*-butoxyl radical,[9,10] which abstracts a hydrogen atom from cumene to produce the cumyl radical. The cumyl radical is readily trapped by oxygen to produce the cumylperoxyl radical. The ESR signal of the cumylperoxyl radical has the typical *g* value of a peroxyl radical as shown in Fig. 1a.[11] The cumylperoxyl radical can abstract a hydrogen atom from cumene in the propagation step to yield cumene hydroperoxide, accompanied by regeneration of the cumyl radical (Scheme 1).[10] In the termination step, cumylperoxyl radicals decay *via* a bimolecular reaction to yield the corresponding peroxide and oxygen (Scheme 1).[10] When the illumination is cut off, the ESR signal intensity of the cumylperoxyl radical (Fig. 1a) decays obeying second-order kinetics due to the bimolecular reaction (Fig. 1b).



Scheme 1

In the presence of epinephrine, the decay rate of the cumylperoxyl radical after the light cutoff becomes much greater than that in the absence of a neurotransmitter. The decay rate in the presence of a neurotransmitter (*e.g.*, epinephrine) obeys pseudo-first-order kinetics rather than second-order kinetics. In such a case, the decay of the ESR signal due to the cumylperoxyl

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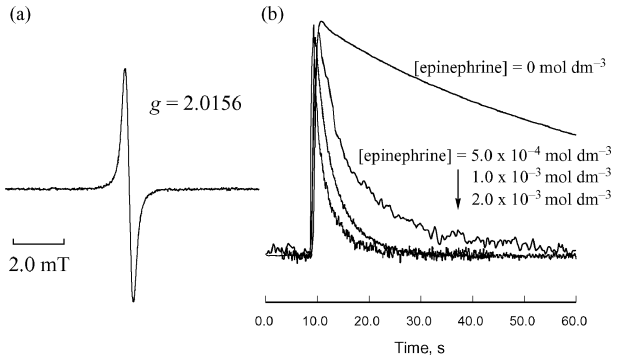

Fig. 1 (a) ESR spectrum of the cumylperoxyl radical in EtCN at −70 °C generated by the photoirradiation of an O_2-saturated EtCN solution containing di-*t*-butyl peroxide (1.0 mol dm^{-3}) and cumene (1.0 mol dm^{-3}) with a 1000 W high-pressure mercury lamp. (b) Time dependence of the ESR signal intensity of the cumylperoxyl radical in the presence of the various concentrations of epinephrine in O_2-saturated EtCN at 193 K.

radical is ascribed to the hydrogen atom transfer from epinephrine to the cumylperoxyl radical (Scheme 1). The pseudo-first-order rate constant (k) exhibits first-order dependence with respect to the concentration of epinephrine. The slope gives the second-order rate constant (k_{obs}) for the hydrogen transfer process of epinephrine. The rate constant of the hydrogen transfer from epinephrine to the cumylperoxyl radical was determined as 2.9×10^2 mol^{-1} dm^3 s^{-1}. This value is the largest among the examined rate constants and is comparable to the value (6.0×10^2 mol^{-1} dm^3 s^{-1}) of catechin, which is one of the strongest antioxidants.[12,13] The rate constants of the hydrogen transfer reactions of other neurotransmitters have also been determined and the results are summarized in Table 1, together with the energy difference values (D_{HT}) between the phenoxyl radicals and the phenols determined by the density functional theory (DFT) calculations.[14] All the neurotransmitters afford significantly larger hydrogen transfer rate constants than tyrosine (<5 × 10 mol^{-1} dm^3 s^{-1}),[15,16] which is a precursor of neurotransmitters. The k_{obs} values of serotonin and 4-methylphenol were also small, and this is consistent with the large D_{HT} values. The k_{obs} value increases with the decrease of the calculated D_{HT} value. In the case of catechin, the hydrogen abstraction from catechin by the cumylperoxyl radical occurs *via* electron transfer from catechin to the cumylperoxyl radical, because catechin has a much higher HOMO level than the neurotransmitters (see Table 1 where the HOMO energies are given) and the one-electron oxidation potential is more negative than that of the cumylperoxyl radical.[12] In contrast to the case of catechin, electron transfer from the neurotransmitters, which have much lower HOMO levels, to the cumylperoxyl radical is energetically not feasible and thus, the hydrogen abstraction from the neurotransmitters by the cumylperoxyl radical occurs by a one-step hydrogen transfer, when the k_{obs} value increases with decrease of the calculated D_{HT} value (Table 1).

Table 1 Rate constants (k_{obs}) for hydrogen abstraction from neurotransmitters by the cumylperoxyl radical in O_2-saturated EtCN at 193 K, energy difference values (D_{HT}) between phenoxyl radicals and phenols in reference to epinephrine and energies of the HOMO level determined by DFT calculations

Neurotransmitter	k_{obs}/mol^{-1} dm^3 s^{-1} [a]	D_{HT}/kJ mol^{-1} [d]	HOMO/eV
Catechin	6.0×10^2 [b]	−25	−5.73
Dopamine	2.0×10^2	−2.9	−8.53
L-DOPA	3.5×10^2	−2.1	−8.61
Epinephrine	2.9×10^2	0[e]	−8.68
Norepinephrine	1.8×10^2	1.7	−8.76
4-Methylphenol	1.1×10 [c]	7.9	−5.74
Serotonin	$<5 \times 10$	7.9	−8.09
Tyrosine	$<5 \times 10$	46	−8.99

[a] Determined in MeCN. [b] ref. 12. [c] ref. 15. [d] Values were determined by ROHF formalism with the B3LYP/6-31G* basis set. [e] 1665.5 kJ mol^{-1}.

A radical intermediate, the phenoxyl radical species in Scheme 1, was detected by ESR in the hydrogen abstraction from epinephrine with the *t*-butoxyl radical. Photoirradiation of an EtCN solution containing di-*t*-butyl peroxide and epinephrine with a 1000 W mercury lamp at 193 K affords the corresponding phenoxyl radical. Under photoirradiation, the O–O bond of di-*t*-butyl peroxide is cleaved to produce the *t*-butoxyl radical which can abstract hydrogen from epinephrine to produce the phenoxyl radical. The ESR spectrum of the phenoxyl radical thus produced under photoirradiation is shown in Fig. 2a. The well-resolved

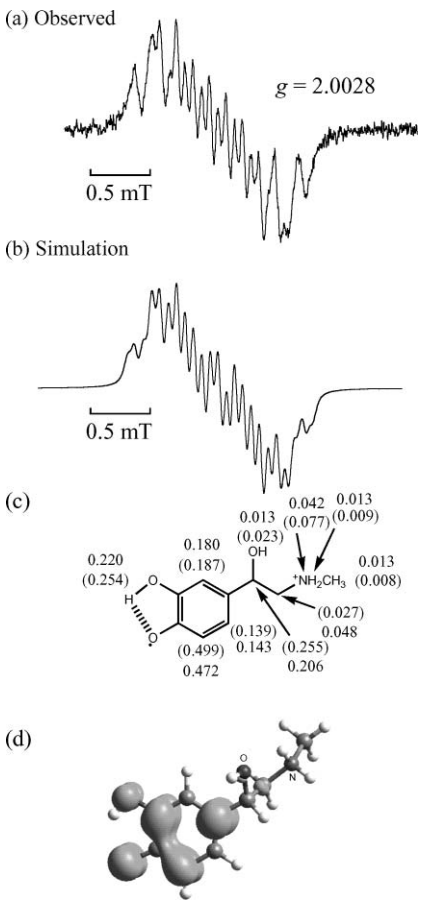

Fig. 2 (a) ESR spectrum of a phenoxyl radical observed under photoirradiation of a deaerated EtCN solution containing epinephrine (0.1 mol dm^{-3}) and Bu^{t}OOBu^t (0.1 mol dm^{-3}) at 193 K. (b) Computer simulation spectrum; the maximum slope linewidth (ΔH_{msl}) = 0.033 mT. (c) *hfc* values (in mT) together with the calculated values given in parentheses with the chemical structure of the phenoxyl radical. (d) DFT optimized structure with spin distribution of the phenoxyl radical, calculated using the UB3LYP/6-311+G(d,p) basis set.

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ESR spectrum in Fig. 2a allows us to determine the hyperfine coupling constants (*hfc*) from the simulation spectrum as shown in Fig. 2b. The computer simulation spectrum using these *hfc* values (Fig. 2b) agrees well with the observed ESR spectrum (Fig. 2a). The *hfc* assignment is also supported by the DFT calculation using B3LYP/6-311+G(d,p) (see the calculated *hfc* values in parentheses in Fig. 2c).[14,17] The ESR spectrum of the hydrogen bonded radical in Fig. 2a is quite different from that reported for the free epinephrine semiquinone radical anion.[18] The existence of strong intramolecular hydrogen bonding in the phenoxyl radical is clearly indicated by the *a*(H) value (0.220 mT) at the phenol proton (Fig. 2c). This is supported by the DFT calculation (Fig. 2d).[19] The high hydrogen abstraction reactivity of epinephrine may be ascribed to the strong intramolecular hydrogen bonding, which stabilizes the hydrogen abstracted radical.[20]

In conclusion, neurotransmitters are susceptible to hydrogen abstraction by an active oxygen species (the cumylperoxyl radical). In particular, the hydrogen abstraction reactivity of catechol amines is found to be comparable to that of a strong antioxidant such as catechin due to the strong intramolecular hydrogen bonding in the phenoxyl radical, which has been successfully detected by ESR.

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