Chemical behavior of oxygen-radical: quenching process of cumyloxyl radical by nicotinamide derivatives

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Abstract

The chemical behavior of the cumyloxyl radical was studied by nanosecond laser flash photolysis of dicumylperoxide. By using nicotinamide derivatives as model compounds of Nicaraven, the quenching of the cumyloxyl radical was examined. Pyridine, formamides, acetoamides, benzamides, 2-propanol, *n*-butylamine were also used as quenchers. The rate constants of the reactions of the cumyloxyl radical with the quenchers were determined. The quenching rate constants for the nicotinamides were dependent on the alkylamide group presumed by hydrogen abstraction by cumyloxyl radical and the alkylamide group of the nicotinamides was concluded to be the key functional group for the quenching of the cumyloxyl radical. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

It is known that *N,N′*-propylenedinicotinamide (Nicaraven, Scheme 1), a dimer of alkylnicotinamide, is an effective treatment for thrombus when administered in vivo [1–3]. Though Nicaraven is supposed to be an oxygen-radical scavenger in vivo, the details of the mechanism are not clear. It is necessary to know what kind of functional group effectively reacts with the oxygen-radical along with getting details of the reaction mechanism, in order to develop a more effective medicine for oxygen-radical scavenging. Thus, a reaction involving the cumyloxyl radical (COR, Scheme 1) as one of the typical oxygen-radicals with alkylnicotinamide derivatives (Scheme 1) having the basic structure of Nicaraven, was examined in this study, because Nicaraven itself is sparingly soluble in solvents other than water.

Chemical behavior of the oxygen-radical has long attracted much attention [4,5]. Inorganic oxygen-radicals such as the hydroxyl radical and the superoxide radical are known to be very reactive, and thus, the reaction with Nicaraven shows no selectivity, occuring on either the pyridine ring, alkyl group, or amide group. Such highly reactive oxygen-radicals do not seem to be suitable for examining the details of the reaction with the Nicaraven model molecule. Direct observation of inorganic oxygen-radicals is sometimes difficult, whereas more detailed and direct observation of organic oxygen-radicals has become possible in recent years. The arylcarbonyloxy radical is reported to have a strong absorption in the visible region as determined by nanosecond laser flash photolysis studies [6–8]. The strong absorption by the oxygen-radical in the visible region is thought due to an intramolecular charge transfer band [9]. The COR generated by laser flash photolysis does not exhibit radical recombination, because the oxygen–oxygen bond is rather unstable. The deactivation of COR is mainly induced by β-fission of the C–C bond to form acetophenone, and hydrogen abstraction leading to cumyl alcohol. Neville et al. [11] observed the primary process of forming acetophenone from COR through β-fission in various solvents using time resolved infra-red spectroscopy [10]. The rate constant of β-fission was reported to increase with increasing solvent polarity and solvent acceptor number [12]. Various reports observing the quenching rate constant of hydrogen abstraction from hydrocarbons by COR have appeared [13,14], but other quenching reactions, such as addition to aromatic ring or electron transfer from electron donors, have rarely been studied in detail.

In this study, the reactivity of COR with alkylnicotinamides, pyridine, alcohols, and amide derivatives, representing the important functional groups in Nicaraven, was studied.

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Nicaraven

N-alkylnicotinamide

COR
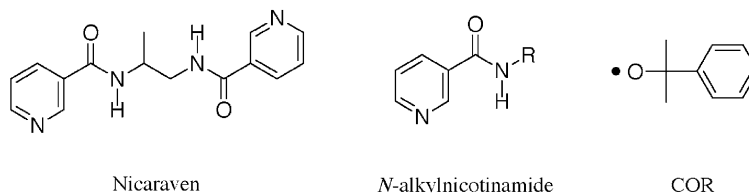
Scheme 1.

2. Experimental

2.1. Materials

Dicumylperoxide (DCP) was purchased from the Nacarai Chemical Co. and was purified by recrystallization from ethanol/water. The compounds 2-propanol, formamide, pyridine, *n*-butylamine were refluxed over calcium oxide or calcium hydride, then fractionally distilled under nitrogen atmosphere, respectively. *N*-isopropylnicotinamide (IPNA) was kindly supplied by Dr. Akimoto at Chugai Seiyaku Co. and was used as received. Nicotinamide (NA) and *N*-methylnicotinamide (MNA) purchased from Kanto Kagaku were purified by recrystallization from a benzene/ethanol mixture. *N*-ethylnicotinamide (ENA) was purchased from Tokyo Kasei and was purified by column chromatography (silica gel/acetonitrile). Benzamide, acetoamide and *N*,*N*-dimethylbenzamide purchased from Kanto Kagaku were purified by recrystallization from a benzene. *N*,*N*-dimethylformamide and *N*,*N*-dimethylacetoamide purchased from Kanto Kagaku were distilled under nitrogen atmosphere. Benzene was washed with concentrated sulfuric acid, then with water, a dilute sodium hydroxide aqueous solution, and water followed by drying with calcium chloride. It was further refluxed over benzophenone kethyl and fractionally distilled under nitrogen atmosphere. Acetonitrile was refluxed over calcium hydride, fractionally distilled, then refluxed over phosphorous pentoxide, and distilled under nitrogen atmosphere.

2.2. Measurements

Nanosecond laser flash photolysis was performed with a XeCl excimer laser (LUMONICS Hyper EX-300, 308 nm, 12 ns FWHM) and a pulsed xenon flash lamp (Tokyo Instruments XF-80) used as a monitoring light source equipped with a monochrometer (RITSU OYO KOGAKU MC-30, 1200 G/mm), and a photomultiplier tube (HAMAMATSU PHOTONICS R-636). The amplified signal was recorded on a digital storage oscilloscope (GOULD DSO4072, 400 Ms/s, 100 MHz). Transient absorption spectra was obtained with a spectrometric multichannel analyzer (SMA: Princeton Instruments IRY-512) equipped with a polychrometer (Jarrell-Ash Monospec27). The timing was controlled by a digital time delay (STANFORD DG535).

3. Results and discussion

3.1. Laser flash photolysis of dicumylperoxide (DCP)

Laser flash photolysis of an acetonitrile solution of DCP (0.1 M) was carried out with an excimer laser (308 nm, 12 ns) under an aerated condition. The transient absorption was measured by SMA. The transient intermediate decayed with first-order kinetics. The similar transient absorption spectrum was obtained under a degassed condition Fig. 1(a), and the decay rate constant ($3.2 \times 10^5 \, \mathrm{s}^{-1}$) under air was almost equal to that ($4.4 \times 10^5 \, \mathrm{s}^{-1}$) under a degassed condition. This strongly suggests that the transient is an oxygen-centered radical species, since the lifetime was insensitive to molecular oxygen; COR is assumed to be formed upon laser photolysis of DCP. The spectrum of the intermediate actually agrees well with that of COR reported by Banks and Scaiano [6] and Ingold and coworkers [7].

In benzene, however, the transient absorption of COR has not yet been reported. Possible reaction of COR with benzene ring might be concerned to complicate the transient behavior. The laser flash photolysis of DCP (0.1 M) under degassed condition gave a somewhat different transient absorption in the visible region as shown in Fig. 1(b). An appreciable absorption in the shorter wavelength region was observed in addition to one in the visible region. The transient absorption, however, monotonically decreased at every
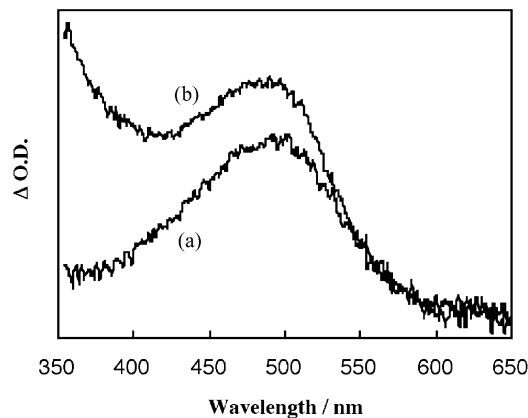

Fig. 1. Transient absorption spectra upon 308 nm excitation of dicumylperoxide solutions in (a) acetonitrile, (b) benzene at 80 ns after excitation under the degassed condition.

wavelength with a rate constant of $3.3 \times 10^5 \, s^{-1}$ under air and $3.7 \times 10^5 \, s^{-1}$ under the degassed condition. The transient absorption in Fig. 1.b was thus safely assigned as COR in benzene. Almost the similar lifetime of COR in benzene with that in acetonitrile indicates that COR has negligible reactivity against aromatic nuclei.

3.2. Effect of various quenchers on the transient decay

The reactivity of COR with various quenchers was estimated by observing the transient decay in the laser flash photolysis experiment. Alkylnicotinamide derivatives (NA, MNA, ENA, and IPNA) were used as model compounds of Nicaraven. To obtain information about what functional group within Nicaraven reacts with COR, pyridine, 2-propanol, *n*-butylamine, formamide, *N,N*-dimethylformamide, acetoamide, *N,N*-dimethylacetoamide, benzamide, and *N,N*-dimethylbenzamide were also used as model compounds. The decay profiles of COR were analyzed as a single exponential decay. When alkyl nicotinamides were added as quenchers in acetonitrile under air, the laser flash photolysis showed the appearance of a transient absorption other than COR at around 370 nm just after the laser pulse (Fig. 2). Transient absorption was thus observed by the laser flash photolysis of alkyl nicotinamides themselves in acetonitrile, because they have small absorbances around 0.29–0.72 at 308 nm and can be excited by the excimer laser. In each case a similar transient absorption at around 370 nm was observed as expected: NA; $\lambda_{max} = 378$ nm, $\tau = 0.14 \, \mu s$, MNA; $\lambda_{max} = 372$ nm, $\tau = 0.16 \, \mu s$, IPNA; $\lambda_{max} = 362$ nm, $\tau = 0.13 \, \mu s$. Subtraction of the normalized transient absorption by alkylnicotinamides themselves from those observed in the laser flash photolysis of DCP and alkyl nicotinamides gave a spectrum which agrees well with that of COR in Fig. 1(a). These indicate that th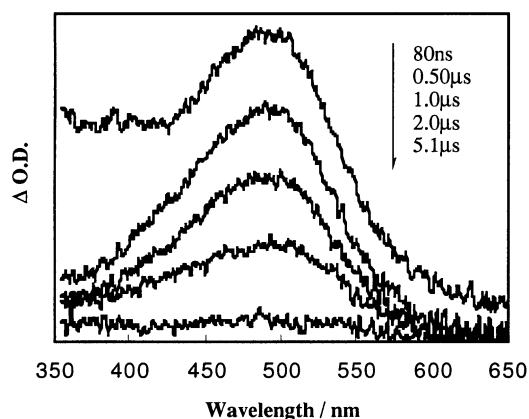e transient absorption of alkyl nicotinamides is simply superimposed on COR absorption. The decay signal of COR was thus analyzed as the single exponential curve after a time interval of several μs where the transients from the alkyl nicotinamides with short lifetimes had already disappeared.

The decay behavior of COR in the presence of various quenchers was observed and the obtained decay rate constant was plotted against the concentration of the quenchers as shown in Fig. 3. The rate constant of the reaction of COR with the quenchers, k_q, was determined from the slope of the plot, because the decay rate constant can be expressed as the following equation:

$$k_d = k_{d0} + k_q \text{(quencher)}$$

where k_d, k_{d0} and k_q denote the rate constants of the deactivation of COR, the inherent deactivation of COR, and the quenching by the quencher, respectively. The concentration of DCP in the experiment for formamide and nicotinamide was 0.1 M, while [DCP] for *N*-ethylnicotinamide

Fig. 2. Transient absorbtion spectra of 0.1 M DCP in the presence of nicotinamide (0.05 M) as a quencher in acetonitrile upon 308 nm excimer laser excitation.
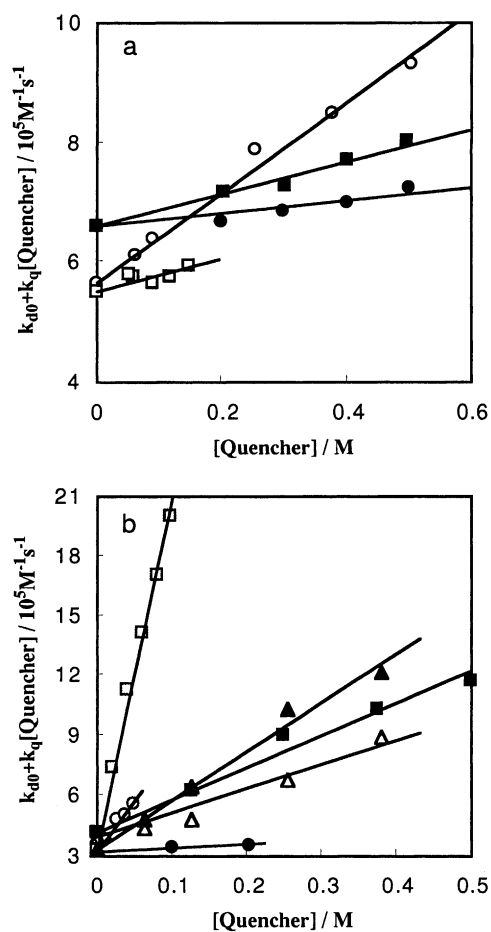
Fig. 3. Quenching of cumyloxyradical in (a) acetonitrile by (○) formamide; (□) nicotinamide; (●) *N*-methylnicotinamide; (■) *N*-ethylnicotinamide and in (b) benzene by (○) 2-propanol; (□) *n*-butylamine; (●) *N*-isopropylnicotinamide; (■) *N,N*-dimethylformamide; (△) *N,N*-dimethylbenzamide; (▲) *N,N*-dimethylacetoamide.

Table 1
The rate constants for the quenching of cumyloxyl radical

Quencher	Solvent	k_q ($M^{-1} s^{-1}$)
n-Butylamine	Benzene	1.8×10^7
2-Propanol	Benzene	4.6×10^6
Pyridine	Benzene	$<10^4$
Formamide	Acetonitrile	7.6×10^5
N,N-dimethylformamide	Benzene	1.5×10^6
Acetoamide	Acetonitrile	$<10^4$
N,N-dimethylacetoamide	Benzene	1.1×10^6
Benzamide	Acetonitrile	$<10^4$
N,N-dimethylbenzamide	Benzene	2.4×10^6
Nicotinamide	Acetonitrile	2.6×10^5
N-methylnicotinamide	Acetonitrile	1.1×10^5
N-ethylnicotinamide	Acetonitrile	2.8×10^5
N-isopropylnicotinamide	Benzene	3.6×10^5

Fig. 4. Correlation between the observed quenching rate constant and the oxidation potential of the qunchers.

and *N*-methylnicotinamide was increased to be $0.2 M$ to avoid the inner filtering effect by the quencher upon 308 nm light excitation. The inherent decay rate constant k_{d0} depended on [DCP] owing to the reaction of COR with DCP itself in accordance with Ingold's report [11]. The obtained quenching constant is listed in Table 1. Formamide, NA, MNA, and ENA were examined in acetonitrile due to their poor solubility in benzene. Alkyl nicotinamides exhibit moderate reactivities with COR with rate constants around 1.1×10^5–$3.6 \times 10^5 M^{-1} s^{-1}$ (Table 1). Possible mode of reaction involved in the quenching of the oxygen-centered radical, COR, by organic compounds would be (1) electron transfer to COR, (2) addition of COR to aromatic nuclei, and (3) hydrogen abstraction by COR, respectively. Nicaraven has functional group of pyridine nuclei, amide group, and alkyl group within the molecule. Among the compounds examined here, the observed quenching rate constant had obviously no correlation with the oxidation potential of the qunchers as plotted in Fig. 4 [19–23], indicating the electron transfer quenching is most unlikely to occur except the case of butylamine. The electron transfer from COR to pyridine is largely endoergic [15,16]. Addition of COR to aromatic nuclei is also questionable for the quenching mechanism, since pyridine and benzamide showed almost no reactivity against COR. Even in benzene COR has an appreciable lifetime of $2.6 \mu s$, strongly supporting the idea. The rate constants for every series of nicotine amides, formamides, acetoamides, and benzamides strongly suggest that the *N*-alkyl group of the amide causes the quenching reaction. The rate constant increased in the order of methyl ($1.1 \times 10^{-5} M^{-1} s^{-1}$) < ethyl ($2.8 \times 10^{-5} M^{-1} s^{-1}$) < isopropyl ($3.6 \times 10^{-5} M^{-1} s^{-1}$) for the series of *N*-alkylnicotinamides, though the reactivity was smaller than that of 2-propanol as a standard quencher for hydrogen abstraction. The dimethylation of formamide, acetoamide, and benzamide all increased the quenching reactivity. These results strongly suggest that hydrogen abstraction from the alkyl group substituted on the nitrogen atom of the amide compounds is one of the key reactions for the quench-
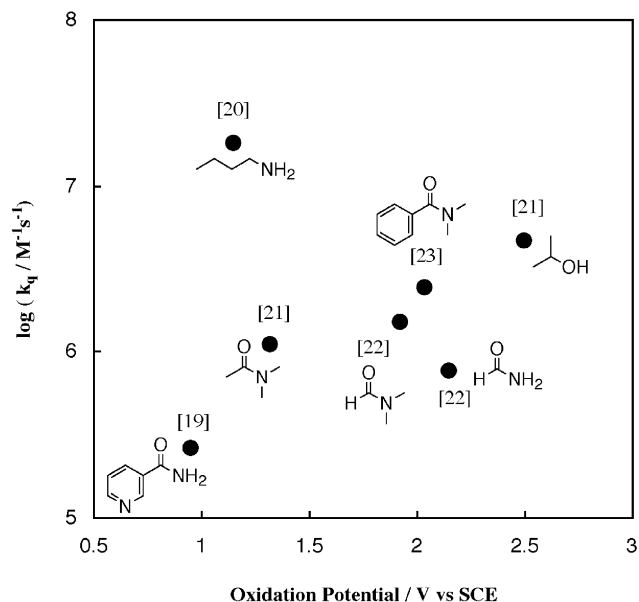
ing of COR. As regards the amide group, very interesting results were obtained; nicotinamide and formamide which have no alkyl group showed appreciable reactivity against COR. These also indicate that the amide group itself plays an important role in the quenching of COR. Amide hydrogen could be the reactive site for hydrogen abstraction by COR in the case. Benzamide and acetoamide, however, had almost no reactivity. The puzzling results may be explained by different stability among the resultant amidyl radical derived from nicotinamide, benzamide, and acetoamide. Larger electron withdrawing effect by pyridyl group of nicotinamide than phenyl and methyl groups in benzamide and acetoamide would stabilize more the amidyl radical to exhibit the appreciable reactivity against COR. The rather higher reactivity of formamide also suggests that the aldehyde HC=O group also contributes to the reaction with COR in the case. The low reactivity of COR as compared with other ordinary oxygen-radicals like the hydroxyl radical [17] is understood as the electron donating effect of cumyl group substantially reducing the electrophilicity of the oxygen-radical [18]. The very low reactivity of COR enables one to distinguish the amide group from the other functional groups.

4. Conclusion

In order to clarify what functional group within Nicaraven selectively reacts with the oxygen-radical, the reactions of COR with various quenchers were examined. The measurement of the rate constants between COR and various quenchers revealed that the alkyl and the amide group were the key functional groups for the reaction with the oxygen-radical.

Acknowledgements

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