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CLEAVAGE OF DISULFIDE DEPENDENT ON SUPEROXIDE

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Summary: Disulfide bond was cleaved by superoxide in aprotic media and by a hydroxyl radical derived from superoxide in aqueous medium. The reaction mechanism was examined in detail.

Superoxide is an important species which plays various roles in biological systems. Superoxide is known to participate in lipid peroxidation,[1] inflamation,[2] carcinogenesis,[3] enzymatic reactions,[4] etc..

We have reported that the disulfide interchange reaction is initiated by superoxide.[5] The first step of the reaction is the cleavage of disulfide by superoxide. The reactions of superoxide with disulfides are significant since the disulfide linkage plays an important role in the living systems. Here we report the disulfide cleavage reaction dependent on superoxide.

Superoxide was produced in acetonitrile by electrophoresis.[6] DNPSSR (DNP=2,4-dinitrophenyl) is cleaved by nucleophiles or reductants to generate $DNPS^-$. So the rate of DNPSSR cleavage by superoxide was determined by measuring the absorbance spectrum of $DNPS^-$ (λ max = 460 nm in acetonitrile).

The reaction was second order: first order in superoxide and in disulfide, respectively. The second order rate constants of the reaction are shown in Table I. When R was a bulky group such as tert-butyl, the disulfide was not cleaved. The relation between the logarithms of the rate constants

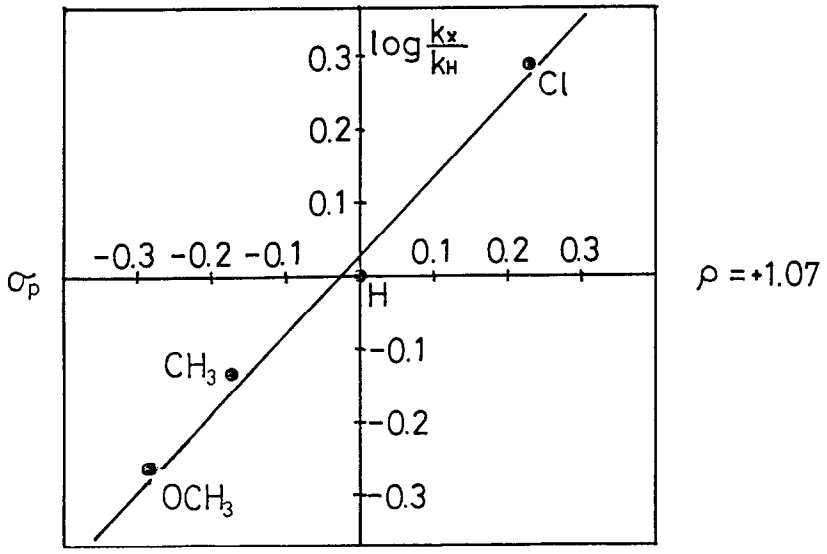
Table I Reaction rate constants** of DNPSSR cleavage by superoxide in acetonitrile (25 ℃)

-R	$10^{-5}k_2$ ($M^{-1}s^{-1}$)	-R	$10^{-5}k_2$ ($M^{-1}s^{-1}$)
CH_2CH_3	4.2	$CH_2CONHCH_3$	8.8
$CH(CH_3)_2$	0.5	$CH_2CH_2CONHCH_3$	3.4
$C(CH_3)_3$	0	$CH_2CH_2CH_2CONHCH_3$	3.4
C_6H_5	5.1	$CH_2CH_2CON(CH_3)_2$	2.9
$p\text{-}ClC_6H_4$	10.0	$CH_2CHCONHCH_3$	6.2
$p\text{-}CH_3C_6H_4$	3.8	$NHCOCH_3$	
$p\text{-}CH_3OC_6H_4$	2.8	$CH_2CH_2COOCH_2CH_3$	5.6

** determined by stopped flow method

and the substituent constants of Hammett (σp) when R was aromatic is illustrated in Fig.1. Aromatic compounds with an electron withdrawing group were cleaved more easily. These facts suggest that superoxide reacts with disulfide in S_N2 like process including one electron transfer to produce the thiolate anion and thiyl radical, which cause the chain interchange reaction of disulfides.

Fig.1 Hammett plots for cleavage of DNPSSR by $O_2^{\cdot-}$ (R=p-X-C_6H_4)



The reactivity of superoxide in aqueous solution was investigated by use of a xanthine-xanthineoxidase system[7] as the superoxide source. In this system, DTNB[8] was cleaved to generate TNB^-, which was detected at 412 nm in visible spectrum. The generation of TNB^- was inhibited by SOD(superoxide dismutase), catalase and the scavengers of hydroxyl radical (DMSO, sodium formate and mannitol are strong scavengers and urea is a weak one) (Table II). This result suggests that the reactant which cleaved the disulfide was hydroxyl radical generated by the Haber-Weiss reaction. It is known that the Haber-Weiss reaction is not so fast[9] and iron effectively catalyzes this reaction to generate hydroxyl radical.[10] The addition of $FeSO_4$ accelerated the generation of TNB^- (Table III). The iron-catalyzed Haber-Weiss reaction is known to be accelerated by EDTA and inhibited by DETAPAC (diethylenetriamine pentaacetic acid).[10] So the effects of the chelating agents were investigated (Fig.2). The generation of TNB^- was effectively inhibited by DETAPAC and accelerated by EDTA. These results indicate that a very small quantity of iron, which may be contained in the reaction mixture,

catalyzed the Haber-Weiss reaction and the hydroxyl radical cleaved the disulfide. The mechanism of TNB^- generation is shown in Scheme 1.

Table II Effects of inhibitors on cleavage of DTNB (25 ℃)

addition		relative rate	addition		relative rate
no addition		100	denat.catalase	5 ng/ml	79
SOD	0.3 μg/ml	31	DMSO	2 mM	48
SOD	0.7 μg/ml	15	DMSO	4 mM	29
SOD	1 μg/ml	6	sodium formate	4 mM	49
catalase	0.6 ng/ml	52	sodium formate	8 mM	31
catalase	1.1 ng/ml	27	mannitol	8 mM	29
catalase	1.7 ng/ml	5	urea	10 mM	92

pH 7.0, 50 mM potassium phosphate, 0.1 mM xanthine, 0.1 mM EDTA, 0.3 mM DTNB, 45 μg xanthine oxidase

Table III Effects of Fe^{2+} on the cleavage of DTNB

addition		relative rate
no addition		100
$FeSO_4$	0.01 mM	146
$FeSO_4$	0.05 mM	195
$FeSO_4$	0.10 mM	221

pH 7.0, 50 mM potassium phosphate, 0.1 mM xanthine, 0.3 mM DTNB, 45 μg xanthine oxidase, 25℃

Scheme 1

$$2O_2^{\cdot-} + 2H^+ \longrightarrow H_2O_2 + O_2$$
$$H_2O_2 + Fe^{2+} \longrightarrow \cdot OH + OH^- + Fe^{3+}$$
$$Fe^{3+} + O_2^{\cdot-} \longrightarrow Fe^{2+} + O_2$$
$$ESSE + \cdot OH \longrightarrow ES\cdot + ESOH$$
$$ES\cdot + O_2^{\cdot-} \longrightarrow ES^- + O_2$$

Superoxide cleaves disulfide in aprotic media. In protic media, superoxide is not so strong a nucleophile as to cleave disulfide, but the hydroxyl radical derived from superoxide cleaves disulfide. The results suggest that a protein denaturation may be caused by the generation of superoxide.

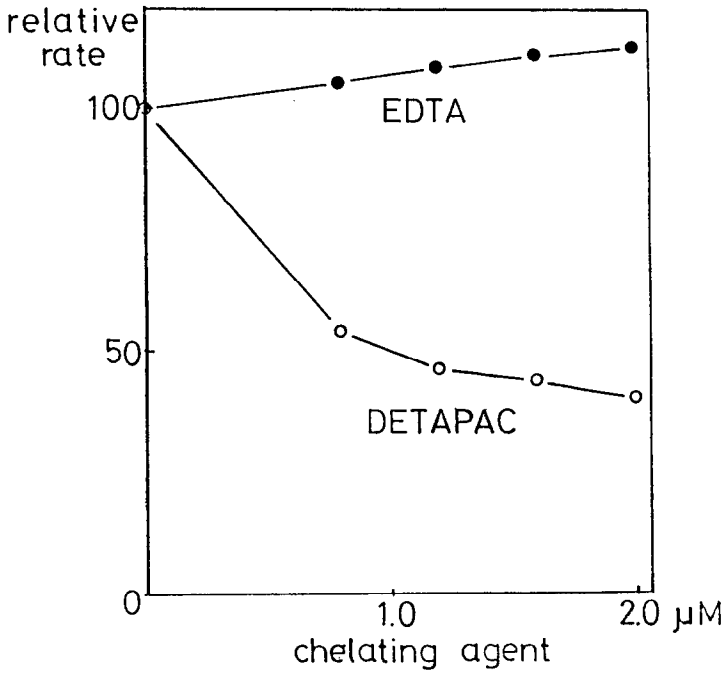


Fig.2 Effects of chelating agents on the cleavage of DTNB
pH 7.0, 0.2 M potassium phosphate
0.1 mM xanthine
0.3 mM DTNB
45 μg xanthine oxidase

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References and Notes

1) E.W. Kellogg III, I. Fridovich, J. Biol. Chem., 250, 8812 (1975)
2) J.M. McCord, Science, 185, 529 (1974)
3) A.M. Michelson, M.F. Buckingham, Biochem. Biophys. Res. Commun., 58, 1079 (1974)
4) F. Hirata, O. Hayaishi, J. Biol. Chem., 250, 5960 (1975)
5) T. Nagano, K. Arakane, M. Hirobe, Tetrahedron Lett., 21, 5021 (1980)
6) supporting electrolyte: tetra-n-butyl ammonium perchlorate (0.1 M), electrode: platinum plate,
The electrolytic reduction was made at constant potential (-0.90 V vs SCE). The concentration of superoxide was determined on the basis of UV absorption (λ max = 250 nm, ε = 1460 cm^{-1} ;T. Ozawa et al, FEBS Lett., 74, 99 (1977)).
7) J.M. McCord, I. Fridovich, J. Biol. Chem., 244, 6049 (1969)
8) DTNB = 5,5′-dithio-bis-(2-nitrobenzoic acid), TNB^- = ES^-
9) J. Weinsten, B.H.J. Bielski, J. Am. Chem. Soc., 101, 58 (1979)
10) B. Halliwell, FEBS Lett., 92, 321 (1978)

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