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DISULFIDE INTERCHANGE REACTIONS BY SUPEROXIDE

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ABSTRACT: Superoxide, which was produced by an electrochemical method, catalyzes instantaneously the interchange reaction of disulfides. The relationship between the half-wave potentials of disulfides and the amounts of superoxide required to complete the reaction were examined.

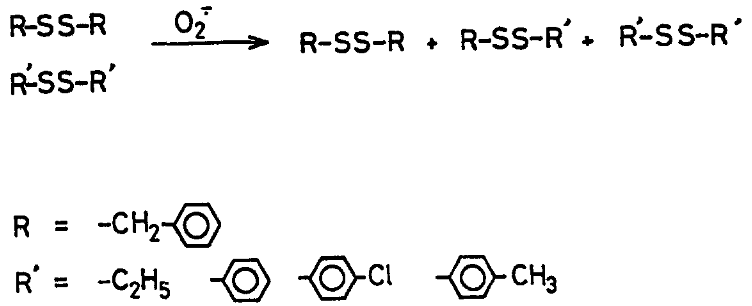
The significant roles of superoxide $O_2^{\overline{\cdot}}$ in biological systems are becoming increasingly made clear in recent years[1)-3)] with considerable attention also being paid to the chemistry of superoxide.[4)-6)] However little studies have been reported on the reactions of superoxide with biological substances.[7)-10)]

In the present paper we wish to report that superoxide catalyzes an interchange reaction of the S-S bond but does not form $-SO_3^-$ and $-SO_2^-$ in the reaction with disulfides.[11)] On examination of the reaction we used alkyl and aryl disulfides as models for the S-S bond in proteins. Since the disulfide linkage plays an important role in the living systems,[12)] the reaction of superoxide with disulfides is very significant and there are some interesting reports about the interchange reactions of disulfides.[13),14)]

Now, superoxide is available to the chemist from two sources, that is, the alkali metal salts[15)-17)] (KO_2, NaO_2 etc) and electrochemical reduction of O_2 to $O_2^{\overline{\cdot}}$.[18),19)] Because KO_2 or NaO_2 easily results in other activated oxygen species ($\cdot OH$, ^-OOH) in the reaction solutions and also since there are solubility difficulties in organic solvents, the formation of $O_2^{\overline{\cdot}}$ by the electrochemical method is more desirable than by the alkali metal salts-crown ether. We used superoxide produced by electrochemical method for the interchange reactions of disulfides.

The electrolysis of oxygen[18)] was carried out according to the modification of the procedure used by J. M. McCord, I. Fridovich[19)] and T. Ozawa[20)] et.al.. A H-type cell was used in which the cathodic and anodic chambers were separated from each other by a sintered glass disk. Each electrode was a plate of Pt(50 cm^2). The electrode potential was referred to the aqueous saturated calomel electrode (S.C.E) which was connected to the cathodic chamber. The electrolytic

Scheme 1



solution containing 0.1M TBAP(tetra-n-butyl ammonium perchlorate) as a supporting electrolyte was placed in each chamber.(capacity: 40-50 ml) The electrolytic reduction of oxygen was made at constant potential (-0.87 V vs. SCE).

In a typical experiment, the CH_3CN solution of superoxide (2.1×10^{-2} mmol) (The amounts of superoxide were determined on the basis of U.V. spectra; λ_{max} = 258 nm, ε = 1460)[20] was added to the mixture of dibenzyl disulfide ($R{=}CH_2C_6H_5$) (0.5 mmol) and diphenyl disulfide ($R'{=}C_6H_5$)(0.5 mmol) at 25°C.(Scheme 1) Instantaneously, 0.5 mmol of the unsymmetrical disulfide ($C_6H_5SSCH_2C_6H_5$) was produced. The unsymmetrical disulfide was identified with the authentic sample by I.R. and N.M.R. spectra. The yield was determined by HPLC. (Instruments: Waters 9000; monitored by 254 nm; Column: Wakogel LC-5H)

Fig.1 and 2 show the relations between the amounts of disulfides and superoxide. In all cases ($R{=}CH_2C_6H_5$; $R'{=}C_6H_5$, $p\text{-}C_6H_4Cl$, $p\text{-}C_6H_4CH_3$, C_2H_5) the forma-

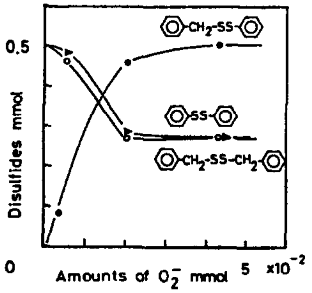


Fig.1 Relationship between amounts of disulfides (●—● $C_6H_5CH_2SSC_6H_5$, ▶—▶ $C_6H_5SSC_6H_5$, ○—○ $C_6H_5CH_2SSCH_2C_6H_5$) and superoxide. The amounts of superoxide and disulfides were determined, respectively, by U.V. spectra and H.P.L.C..

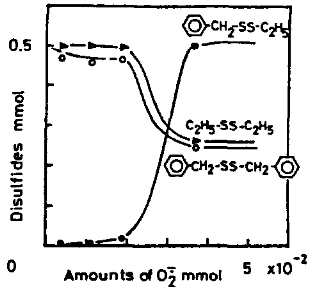


Fig.2 Relationship between amounts of disulfides (●—● $C_6H_5CH_2SS\text{-}C_2H_5$, ▶—▶ $C_2H_5SSC_2H_5$, ○—○ $C_6H_5CH_2SS\text{-}CH_2C_6H_5$) and superoxide. The amounts of superoxide and disulfides were determined, respectively, by U.V. spectra and H.P.L.C..

tion ratio of the three disulfides (compound I, II and III) became 1:2:1 (= I: II:III). The ratio did not change even if the reaction time was longer, and oxidized products were not obtained.

To reach the equilibrium state with this ratio the interchange reaction of $C_6H_5CH_2SSCH_2C_6H_5$ and $C_2H_5SSC_2H_5$ required more amounts (3.6×10^{-2}mmol<) of superoxide than that of $C_6H_5CH_2SSCH_2C_6H_5$ and $C_6H_5SSC_6H_5$ (2.1×10^{-2}mmol<).(Fig.1) The interchange reactions of RSSR and R'SSR' ($R=CH_2C_6H_5$; $R'=ClC_6H_4$ and $CH_3C_6H_4$) required the same amounts of superoxide as that of $C_6H_5CH_2SSCH_2C_6H_5$ and $C_6H_5SSC_6H_5$ in order to attain the equilibrium state. It may be due to the low halfwave potentials ($E_{1/2}$ vs SCE) of $C_2H_5SSC_2H_5$ (-2.0 V) and $C_6H_5CH_2SSCH_2C_6H_5$ (-1.78 V) compared with those of $ClC_6H_4SSC_6H_4Cl$ (-0.74 V), $C_6H_5SSC_6H_5$ (-0.82 V) and $CH_3C_6H_4SSC_6H_4CH_3$ (-0.88 V). (Fig.3)

On the other hand the electrolytic reduction of $C_2H_5SSC_2H_5$ and $C_6H_5CH_2SSCH_2C_6H_5$ in CH_3CN was done under Ar atmosphere (constant potential: -0.87 V vs SCE; halfwave potential of superoxide: -0.87 V vs SCE), but unsymmetrical disulfide was not obtained. This fact demonstrates that it is characteristic of superoxide to be able to transfer the electron to disulfide which has a lower halfwave potential than that of superoxide, even if the reaction of $C_6H_5CH_2SSCH_2C_6H_5$ and $C_2H_5SSC_2H_5$ requires a greater amount of superoxide. It cannot be explained yet why superoxide can reduce $C_2H_5SSC_2H_5$ or $C_6H_5CH_2SSCH_2C_6H_5$

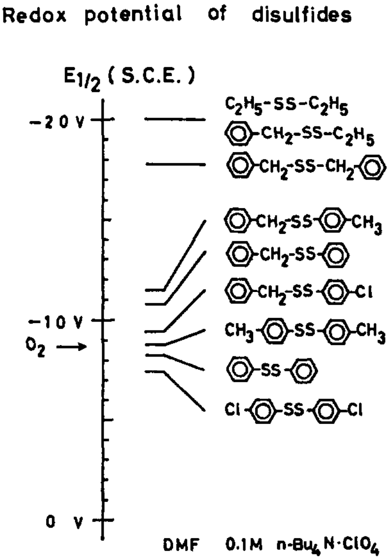

Redox potential of disulfides

Fig.3 Halfwave potentials vs S.C.E. of various disulfides in DMF solution containing 0.1 M tetra-n-butyl ammonium perchlorate as a supporting electrolyte but the above results indicate that superoxide can catalyze the interchange reaction of disulfides having not only higher but also lower halfwave potentials than that of superoxide. This interchange reaction may be expected to cause a protein denaturation.

Further studies on this reaction in biological systems are in progress.

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