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Acceleration of the Copper-catalyzed Autoxidation of Cysteine by Ethylenediaminetetraacetic Acid and Related Polyaminopolycarboxylic Acids

Polyaminopolycarboxylic acid with at least two ligand nitrogens such as ethylenediaminetetraacetic acid accelerates the copper-catalyzed autoxidation of cysteine. The extent of acceleration of the reaction is related to the stability constant of the Cu(II) chelate of the polyaminopolycarboxylic acid. N-Hydroxyethylethylenediaminetriacetic acid, N-hydroxy-1,3-diaminopropanetetraacetic acid and ethyleneglycol-bis-(β-aminoethylether)tetraacetic acid are the most potent accelerators and those chelating agents have the stability constants of approximately $10^{17.5}$. Addition of a sufficient excess of the chelating agent over cysteine retards the oxidation.

Keywords——autoxidation of cysteine; copper-catalyzed autoxidation; acceleration of the reaction; EDTA; polyaminopolycarboxylic acid; stability complex of Cu(II) chelate

A role of the metal ion in the metal ion-catalyzed autoxidation is to participate in electron transfer from the substrate to the metal ion, which has been indicated indirectly by the inhibitory effect of chelating agent. In the copper-catalyzed autoxidation of sulfur-containing substrate, however, the effect of chelating agent appears to be complicated because of the strong affinity of the catalyst for the substrate. Autoxidation of glutathione catalyzed by copper-1,10-phenanthroline is strongly inhibited by addition of ethylenediaminetetraacetic acid (EDTA).[1] The rate of spontaneous oxidation of dithiothreitol, which is probably initiated by a trace of metal ions, such as copper or iron, is strongly inhibited by EDTA.[2] In the copper-catalyzed autoxidation of cysteine in an alkaline medium, on the contrary, the reaction is little prevented by addition of neocuproine or diethyldithiocarbamate, and this fact is ascribable to the strong affinity of Cu(II) for cysteine.[3] The literatures describing the copper-catalyzed autoxidation of thiols have indicated that the addition of EDTA to the reaction medium generally inhibits the oxidation. No indication of the acceleration of autoxidation has yet been reported. The present paper describes experiments which demonstrate that the addition of EDTA and related polyaminopolycarboxylic acids stimulate the copper-catalyzed autoxidation of cysteine.

Oxidation of cysteine was conducted under oxygen at 20°. Initial concentrations of the reactants and glycylglycine used as a buffering agent were as follows; 1.38×10^{-6} M Cu(II), 2.0×10^{-3} M cysteine and 1.6×10^{-2} M glycylglycine. Routinely, the kinetic run was made at pH 7.4. Plot of the concentration of cysteine oxidized against the reaction time gave a straight line for over 50% reaction. The initial rate was calculated from the slope of the straight line.

The rate of autoxidation of cysteine was varied relative to the concentration of EDTA added. The relationship between the rate of autoxidation and the concentration of EDTA is presented in Fig. 1. In Fig. 1, the rate is expressed as a relative value, v/v_0, where v and v_0 indicate the initial rates obtained in the presence and in the absence of EDTA respectively. Apparently, the addition of EDTA stimulates the autoxidation. The rate was augmented with an increase of the concentration of EDTA and reached a maximum at approximately 2×10^{-5} M. Further addition of a sufficient excess of EDTA over 2×10^{-5} M retards the reaction. The extent of acceleration of the autoxidation by EDTA depends on the pH value of the medium, giving a maximum at pH 7.5.

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2) H.P. Misra, *J. Biol. Chem.*, **249**, 2151 (1974).
3) D. Cavallini, C. DeMarco, S. Dupré and G. Rotilio, *Biochim. Biophy. Acta*, **130**, 354 (1969).

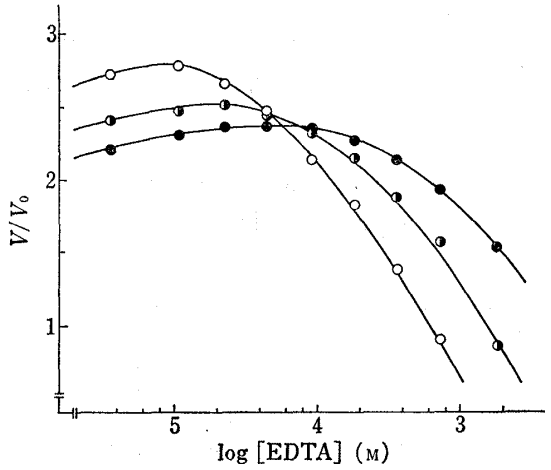

Fig. 1. Effect of Varying Concentrations of EDTA on the Rate of Autoxidation of Cysteine

[Cu(II)] = 1.38×10^{-6}M,
[glycylglycine] = 1.6×10^{-2}M,
[cysteine] = 1.0×10^{-3}M (○), 2.0×10^{-3}M (◑), or 4.0×10^{-3}M (●).

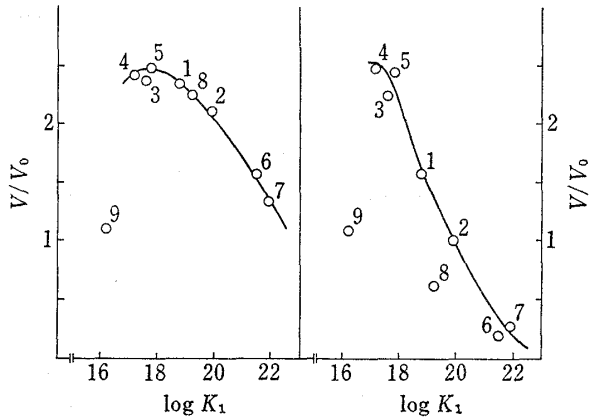

Fig. 2. Plot of the Relative Rate of Autoxidation against the Stability Constant of Cu(II)-Polyaminopolycarboxylic Acid

[Cu(II)] = 1.38×10^{-6}M, [cysteine] = 2.0×10^{-3}M,
[glycylglycine] = 1.6×10^{-2}M,
[polyaminopolycarboxylic acid] = 1.0×10^{-4}M (left) and 8.0×10^{-4}M (right).

1: EDTA[a].
2: propylenediaminetetraacetic acid (PDTA)[b].
3: N-hydroxyethylethylenediaminetriacetic acid (HEDTA)[c].
4: 2-hydroxy-1,3-diaminopropanetetraacetic acid (HDPTA)[d].
5: ethyleneglycol-bis (β-aminoethylether) tetraacetic acid (EGTA)[e].
6: diethylenetriaminepentaacetic acid (DTPA)[f].
7: cyclohexanediaminetetraacetic acid (CDTA)[g].
8: triethylenetetraminehexaacetic acid (TTHA)[h].
9: ethylenediamine-N,N′-diacetic acid (EDDA)[i].

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b) H. M. N. H. Irving and J. P. Conesa, *J. Inorg. Nucl. Chem.*, 26, 1945 (1964).
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d) L.C. Thompson and S.K. Kundra, *J. Inorg. Nucl. Chem.*, 28, 2945 (1966).
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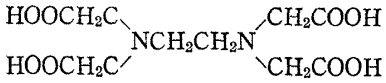
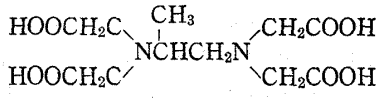
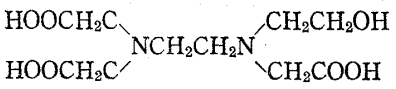
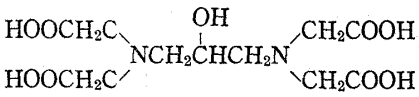
g) G. Anderegg, *Helv. Chim. Acta*, 46, 1833 (1963).
h) L. Harju, *Anal. Chim. Acta*, 50, 475 (1970).
i) S. Chaberek and A.E. Martell, *J. Am. Chem. Soc.*, 74, 6228 (1952).

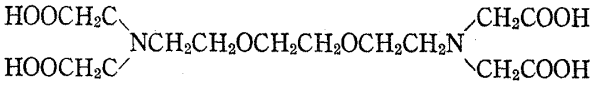
Addition of other related polyaminopolycarboxylic acids modify the rate of autoxidation, and the extent of modification depends apparently upon the stability constant, K_1, of the Cu(II) chelate of the polyaminopolycarboxylic acid in the solution. All the polyaminopolycarboxylic acids possessing more than two ligand nitrogens, except for ethylenediaminediacetic acid (EDDA), accelerate the autoxidation. Among those chelating agents, N-hydroxyethylethylenediaminetriacetic acid (HEDTA), 2-hydroxy-1,3-diaminopropanetetraacetic acid (HDPTA) and ethyleneglycol-bis-(β-aminoethylether)tetraacetic acid (EGTA) are the most potent accelerators and in addition those chelating agents do not inhibit the autoxidation at equimolar concentration to the substrate. Of much interest is that the stability constants of Cu(II) chelates of those chelating agents are in a range of the magnitude from 10^{17} to 10^{18}. Concerning other polyaminopolycarboxylic acids with log K_1 of more than 18, the relative rate is reduced as log K_1 increases. The relationship between the rate of autoxidation and the stability constant of the Cu(II) chelate is shown in Fig. 2. The polyaminopolycarboxylic acids with one ligand nitrogen, as well as EDDA, do not affect the rate of autoxidation under the experimental condition, and all have the K_1 values of less than 10^{17}. This fact indicates that those chelating agents do not disturb the Cu(II)-cysteine interaction. Therefore, it may be concluded that the polyaminopolycarboxylic acid accelerating the copper-catalyzed autoxidation of cysteine has an ability to form a Cu(II) chelate which is fairly stable as compared with the Cu(II)-cysteine. Studies on the reaction mechanism are now in progress.

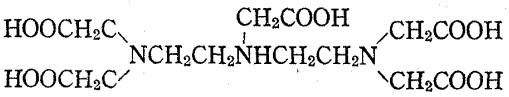
EDTA: 

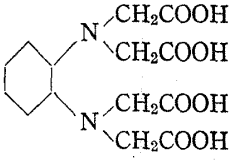
PDTA: 

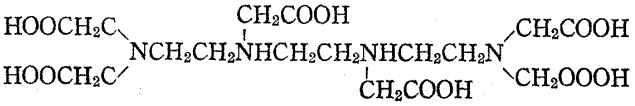
HEDTA: 

HDPTA: 

EGTA: 

DTPA: 

CDTA: 

TTHA: 

EDDA: $HOOCH_2C\text{-}NHCH_2CH_2NH\text{-}CH_2COOH$

Chart 1

National Institute of Radiological Sciences Anagawa, Chiba 280, Japan

Akira Hanaki
Hiroko Kamide

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