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Short Communications

Polarographic Determination of Stability Constants and Rate Constants of Iron Chelates

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In a previous note [1] the stability constants of the di- and monoprotonated Fe(II)-EDTA complexes (EDTA ~ ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid) were calculated from oxidation experiments. This note presents the stability constants of the protonated Fe(II)-EDTA and Fe(II)-CyDTA complexes (CyDTA ~ *trans*-1,2-cyclohexanediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid) and the stability constants of Fe(III)-EDTA and Fe(III)-CyDTA obtained by polarographic measurements of the dependence of the half-wave potential on pH in buffered aqueous solution. The rate constants of the oxidation of Fe(II)-CyDTA by hydrogen peroxide are also given.

The reduction of Fe(III)-EDTA or Fe(III)-CyDTA in the pH-range 1 – 6 can be written as follows:

$$Fe(III)Y^- + zH^+ + e \rightarrow Fe(II)H_zY^{(2-z)-}$$

where Y^{4-} denotes the anion of EDTA or CyDTA and z is the number of protons taking part in the reduction. Using the general expressions [2] of the reduction of a complexed metal ion and assuming that the diffusion coefficients of the different iron species are equal, the following equation is obtained giving the dependence of the half-wave potential ($E_{\frac{1}{2}}$) on the hydrogen ion concentration:

$$E_{\frac{1}{2}} = E_0 - \frac{RT}{F}\{\ln(1+[Y^{4-}]K_{Fe(III)Y^-}) - \ln(1+[Y^{4-}]K_{Fe(II)Y^{2-}}(\sum_{i=0}^{i=z}(\prod_{j=0}^{j=i}K_j)[H^+]^i))\}$$

where E_0 is the standard potential of the Fe^{3+}/Fe^{2+} couple,[3] $K_{Fe(III)Y^-}$ the stability constant of Fe(III)-EDTA or Fe(III)-CyDTA, and $K_{Fe(II)Y^{2-}}$ the stability constant [3–6] of Fe(II)-EDTA or Fe(II)-CyDTA. K_j is the acid dissociation constant of a protonated Fe(II)-EDTA or Fe(II)-CyDTA complex. K_0 is 1.

The calculations were carried out by means of a GIER computer. A program for minimising $\sum(E_{\frac{1}{2}obs}-E_{\frac{1}{2}calc})^2$ as a function of the stability constant $K_{Fe(III)Y^-}$ and the acid dissociation constants of the protonated complexes was written in GIER ALGOL 4 using the LETAGROP procedure.[7]

Table 1 shows the calculated stability constants, enthalpies, and entropies of formation (the acid dissociation constants of the protonated complexes have been converted to the corresponding stability constants). The values in brackets are the stability constants obtained by the oxidation experiments and the agreement is seen to be good. The stability constants of Fe(III)-EDTA, Fe(III)-CyDTA, and monoprotonated Fe(II)-EDTA at 20°C agree quite well with those found in the literature.[6]

Table 2 shows the rate constants, activation energy, and activation entropy of the oxidation of Fe(II)-CyDTA by hydrogen peroxide. The experimental conditions, reaction schemes, and calculations are identical with those previously given.[1]

The rate constant of the unprotonated Fe(II)-CyDTA complex is less than half that of the corresponding Fe(II)-EDTA complex. This may be due to an increased amount of CyDTA being bound sexadentately to Fe(II).

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Table 1. The stability constants at different temperatures together with the enthalpy and entropy of formation. Ionic strength 0.20.

	Temp. C°	$K_{Fe(III)Y^-} \times 10^{-25}$	$K_{Fe(II)HY^-} \times 10^{-6}$	$K_{Fe(II)H_2Y} \times 10^{-3}$
EDTA	10.0	5.6 ± 0.2	8.1 ± 0.3 (8.0 ± 0.2)	2.3 ± 0.4 (2.4 ± 0.4)
	20.0	2.8 ± 0.3	8.0 ± 0.4 (8.0 ± 0.1)	2.1 ± 0.3 (1.8 ± 0.4)
	30.0	1.3 ± 0.2	7.8 ± 0.3 (6.7 ± 0.3)	2.1 ± 0.5 (2.5 ± 0.8)
	40.0	0.70 ± 0.04	7.6 ± 0.3 (7.4 ± 0.1)	2.0 ± 0.4 (2.2 ± 1.2)
	ΔH° kcal	− 12.2 ± 0.4	− 0.41 ± 0.06 (− 0.7 ± 0.3)	− 0.8 ± 0.4
	ΔS° cal/K	75 ± 3	30 ± 2 (29 ± 4)	12 ± 4
	Temp. C°	$K_{Fe(III)Y^-} \times 10^{-29}$	$K_{Fe(II)HY^-} \times 10^{-9}$	$K_{Fe(II)H_2Y} \times 10^{-5}$
CyDTA	18.2	3.1 ± 0.5	2.0 ± 0.4 (2.0 ± 0.3)	2.3 ± 0.9 (3 ± 1)
	28.1	1.4 ± 0.3	2.1 ± 0.2 (2.0 ± 0.4)	2 ± 1 (3 ± 2)
	35.0	0.80 ± 0.04	1.8 ± 0.3 (1.0 ± 0.5)	2.1 ± 0.7 (3 ± 2)
	40.0	0.56 ± 0.03	1.8 ± 0.4 (1.5 ± 0.7)	1.9 ± 0.9 (1 ± 1)
	ΔH° kcal	− 14.2 ± 0.8	− 0.9 ± 0.3	− 1.1 ± 0.9
	ΔS° cal/K	86 ± 6	39 ± 3	20 ± 4

Table 2. The rate constants, the activation energy, and the activation entropy of the oxidation of Fe(II) − CyDTA by hydrogen peroxide at different temperatures. Ionic strength 0.20.

Temp. C°	$k_{Fe(II)Y^{2-}} \times 10^{-3}$ $M^{-1}sec^{-1}$	$k_{Fe(II)HY^-} \times 10^{-3}$ $M^{-1}sec^{-1}$	$k_{Fe(II)H_2Y} \times 10^{-3}$ $M^{-1}sec^{-1}$
18.2	3.20 ± 0.05	2.1 ± 0.2	1.6 ± 0.8
28.1	5.10 ± 0.08	3.3 ± 0.2	1.7 ± 0.9
35.0	7.02 ± 0.05	7.1 ± 0.04	1.9 ± 0.9
40.0	8.96 ± 0.07	7.1 ± 0.7	2 ± 2
E_{act} kcal	8.0 ± 0.2		
$\Delta S^\neq$ cal/K	− 17.3 ± 2		

It is rather peculiar that the rate constants of the protonated Fe(II)-CyDTA complexes – contrary to the rate constants of the corresponding Fe(II)-EDTA complexes [1] – are smaller than that of the unprotonated complex. At present no explanation can be given for this result.

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Formation of Malonate-semialdehyde: Nicotinamide Adenine Dinucleotide (NAD) Oxidoreductase in *Pseudomonas fluorescens* P-2

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Malonate-semialdehyde:nicotinamide adenine dinucleotide (NAD) oxidoreductase (EC 1.2.1.18) catalyses the formation of acetyl-CoA from malonate semialdehyde. The enzyme from *Pseudomonas fluorescens*, when purified about 30-fold, appeared to be specific for malonate semialdehyde.[1] Both NAD and CoA were required for maximal activity of the purified enzyme.

In the present investigation we have studied the formation of malonate-semialdehyde:NAD oxidoreductase during the growth of *Pseudomonas fluorescens* P-2. The enzyme was formed in the presence of β-alanine, pantothenate, isobutyraldehyde, 3-hydroxybutyrate, 3-hydroxyisobutyrate, acetaldehyde, and acetate and slightly in the presence of malonate semialdehyde, propionaldehyde, and 3-aminobutyrate (Fig. 1). The enzyme activity

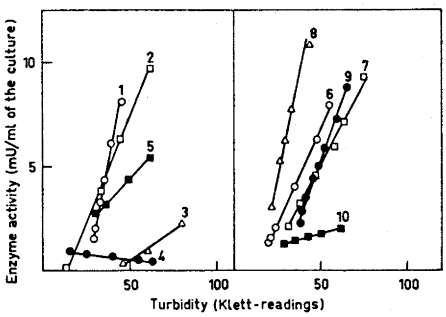

Fig. 1. The formation of malonate-semialdehyde:NAD oxidoreductase on various carbon compounds in *Pseudomonas fluorescens* P-2. Enzyme actvity was determined by the method of Hayaishi *et al.*[1]

1 = β-alanine, 2 = pantothenate, 3 = malonate-semialdehyde, 4 = α-alanine, 5 = acetate, 6 = isobutyraldehyde, 7 = 3-hydroxybutyrate, 8 = 3-hydroxyisobutyrate, 9 = acetaldehyde, 10 = propionaldehyde.

The concentrations of the carbon compounds were 10 mM.

was not found on glyoxylate, succinate, 2-oxoglutarate, 3-oxoglutarate, malonate, pyruvate, propanol, propionate, glycine, *N*-hydroxyethyl-β-alanine, or 4-amino-3-hydroxybutyrate. Although the various compounds mentioned above all caused induction of the enzyme, the real inducer of malonate-semialdehyde:NAD oxidoreductase seems to be β-alanine. 3-Hydroxyisobutyrate and isobutyraldehyde also may function as inducers, because the activity curves obtained on these compounds resembled that obtained on β-alanine, on which activity reached a maximum at the end of the exponential phase of growth. On the other hand, the enzyme activities on acetaldehyde, acetate, and some other compounds reached their maxima during the early logarithmic phase of growth.