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Kinetics of the reduction of Fe(III)-Acetohydroxamic acid Complex by L- Cysteine

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Stopped flow kinetics of the reduction of Fe(III)-Acetohydroxamic acid Complex by L- Cysteine was studied at pH 3.0(formate buffer), 3.50(formate buffer), 4.0(formate buffer), 4.5(acetate buffer) under pseudo first order conditions. Reaction was found to be first order in L-Cysteine. Rate law for the redox reaction was evaluated.

$$\text{Rate} = -d[Fe^{III}(AHA)_n]/ dt = k\,[\text{L-Cysteine}]\,[Fe^{III}(AHA)_n]$$

$$k_{obs} = k[\text{L-Cysteine.}]$$

$$-d[Fe^{III}(AHA)_n]/ dt = k_{obs}\,[Fe^{III}(AHA)_n]$$

Key words: Acetohydroxamic acid, L-Cysteine, Kinetics, Stopped flow, Reduction, Pseudo first order.

Introduction

The amino acids, L-cystine(3,3-Dithiobis(2-Aminopropionic Acid), Dicysteine) and L-cysteine (2-Amino-3-Mercaptopropionic Acid), are substances of great biological importance. The concentration of L-cysteine in plasma has been estimated to lie around 10μM (Brown and Roche, 1983). L-cysteine participates in a number of biological processes that depend directly on the particular reactivity of thiol (Glazer and Smith, 1971 and Harris and Waters, 1976), while the oxidized derivatives of L-cysteine have additional metabolic roles. Cysteine may play a role in the prevention and/or treatment of the following medical conditions: Cysteine and cystine can be used to help prevent or treat alcoholism, heart disease, liver disease (cirrhosis, hepatitis, etc.), Wilson's disease (copper toxicity), and bronchopulmonary diseases such as asthma, chronic bronchitis, cystic fibrosis, pneumonia, and sinusitis. Cysteine also aids collagen production and facilitates proper skin elasticity and texture. Cysteine, cystine, and NAC possess powerful antioxidant properties and work best when taken in combination with selenium and vitamin E (Lininger et al, 2000). They promote liver detoxification by binding toxins and heavy metals such as mercury and lead and facilitating their removal from the body. These amino acids also reduce free radical damage and, in combination with their "liver repair" services, are ideal in treating substance abuse (Midnight Illusions, 2000-2004). Cysteine helps protect the brain and liver from damage from alcohol, drugs etc.

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It has also been found that it may help in strengthening the protective lining of the stomach as well as intestines, which may help prevent damage caused by aspirin and similar drugs. Cysteine is also critical to the metabolism of a number of essential biochemicals including coenzyme A, heparin, biotin, lipoid acid, and glutathione.

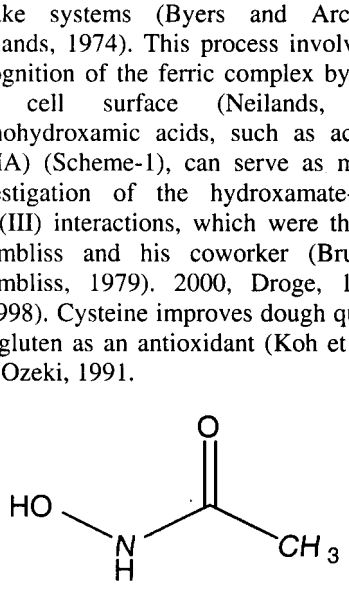
General dosage is not known but as supplement cysteine is used at 200 mg two to three times per day. People suffering from AIDS/HIV may benefit from cysteine in proper amounts, as low levels are normally reported in people with this problem

As a key constituent of glutathione, cysteine has many important physiological functions. Glutathione is a,potent antioxidant, protecting fatty tissues from the damaging effects of free radicals (Breuille and Obled

Food sources of cysteine include poultry, yogurt, egg yolks, red peppers, garlic, onions, broccoli, Brussel sprouts, oats, and wheat germ.

Although iron is a very abundant metal, its insolubility posses a problem for the iron supply of the living organism. Low solubility of iron (Fatemi, Kadir Williamson and Moore, 1991) and generation of harmful free radicals makes it toxic, to overcome these problems a variety of iron capture transport and storage systems have evolved. Many microbes (bacteria, certain yeast and other fungi, as well as some species of plants) have developed the strategy of secreting specific iron chelators, called

siderophores, which have a high affinity for Fe(III) (Winkelmann, Van der Helm and Neilands, 1987). The siderophores are typically multidentate, nitrogen and oxygen-donor ligands that usually have hydroxamate, catecholate, or carboxylate moieties. Although they are designed to have an extremely high affinity for Fe+3, siderophores can bind other "hard" ions, such as Al(III), Zn(II), Ga(III), Cr(III), and, Pu(III,IV). (Hard metal ions have high charge to ionic radius ratios and form strong inner sphere complexes with ligands containing "hard" donor atoms, such as oxygen.) Specific transport systems enable the cells to take up the iron siderophore complexes to satisfy their iron demands (Braun, Gunter and Hantke, 1991). Sdiderophores solublize ferric ion and enable it to be transported into cells via specific membrane bound uptake systems (Byers and Arceneaux, 1977 and Neilands, 1974). This process involves a highly specific recognition of the ferric complex by receptor proteins at the cell surface (Neilands, 1982). Synthetic monohydroxamic acids, such as acetohydroxamic acid (AHA) (Scheme-1), can serve as model ligand for the investigation of the hydroxamate-based siderophore-iron(III) interactions, which were thoroughly studied by Crumbliss and his coworker (Bruce , Monzyk and Crumbliss, 1979). 2000, Droge, 1999 and Droge et al.1998). Cysteine improves dough quality by working on the gluten as an antioxidant (Koh et al. 1996 and Araki and Ozeki, 1991.



Acetohydroxamic acid

Scheme -1

The biological importance of hydroxamic acids is well established (Kehl, 1982). They are known as constituents of antibiotics, growth factors, food additives, tumour inhibitors and cell division factors. Hydroxamic acids have been shown to possess diverse biological activities, many of which are due to their complexing properties towards transition metal ions (Raymond, 1990, Crumbliss, 1991 and Raymond, 1994). Iron(III) complexes of naturally occurring hydroxamic acids, called siderophores, are involved in the processes of iron transport from the environment into the living organisms (Albrecht-Gary and Crumbliss, 1998 and Neilands, 1995). Natural trihydroxamic acid, desferrioxamine B, (Desferrioxamine (Desferal®), for instance, can be used to treat iron overload since the drug binds iron with a large preference over other metal ions such as calcium (Kd=10^{-31} M for iron, Kd=10^{-9} M for calcium) (Keber 1964) has been widely used in the treatment of ir overload associated with β-thalassemia (Cooley anaemia(Liu and Hider, 2002 and Martell, Anderse and Badman,1981). The same compound has also be employed in chelation therapy to remove aluminiu from patients suffering from dialysis, encephalopat and Alzheimer's disease (McLachlan, Farnell, Gal Karlik, Eichorn, and DeBoni, 1983). Data have been published on the inhibitory activity of hydroxa acid derivatives of amino acids and peptides metalloproteinases(Yatabe, Kawai, Oku Tanaka,1998 and Mock and Cheng, 2000). mechanism of inhibition appears to involve chelatio metals at their active sites. Some aminohydroxa acids have been investigated with the aim of desig metal chelates as suitable sources of various t elements essential in animal nutrition (Brown Roche, 1983). With regard to the strong ability hydroxamic acids to form chelates, clarification of t interactions with metal ions is of particular importa in terms of biological effects (Nigović, Kujunc Sanković and Vikić-Topić, 2002).

Materials and Methods

All the reagents used were of A.R grade. Disti deionized water was boiled to free from CO_2 Analy grade reagents and distilled water was used preparation of the solutions.

Preparation of Solutions

Iron(III) solution

It was prepared by dissolving $Fe(NO_3)_3.9H_2O$ in 0.0 HNO_3 Standardization with Fe-Orthophenonthro method (Jaffery, Bassett, Mendham and Denny, 19 was used to determine actual concentration which found approximately 0.01M with 6% error. T solution served as stock solution.

Acetohydroxamic acid solution

It was prepared as per requirement. acetohydroxamic acid solution was prepared dissolving calculated amount of AHA in deioni distilled water.

L-Cysteine solution

L-Cysteine solution was freshly prepared by dissolv calculated and accurately weighed amount of Cysteine hydrochloride in degassed buffer solutions desired pH. Different dilutions of the solution w prepared accordingly. Nitrogen gas was purged for 15minutes to completely evolve oxygen gas.

Buffer solutions

Formate buffers of pH 2.5, 3.0, 3.5, 4.0 and acetate buffer of pH 4.5 all having μ = 0.1M were prepared using standard sodium hydroxide and formic acid/acetic cid solutions. The ionic strength was maintained by adding weighed amount of solid NaCl.

Formation of Fe(III)-AHA complex

Complex solution was prepared by mixing Fe(III) and AHA solution of known concentration. The concentration of AHA was kept 5times over the concentration of Fe(III). Solutions were made up to mark with required buffer.

Instrumentation

λ_{max} of the complex at different pH was determined by monitoring absorption spectra at particular pH on Shimadzu spectrophotometer UV-160A.The molar extinction coefficients (ϵ) of Fe(III) - AHA were calculated.(Table 1)

For all pH measurements Orion pH – meter model SA - 720 was used.

Absorbance change in visible region was monitored on Spectronic21.

The output of Spectronic21 was read in to Pentium I computer interfaced through "Labpro" compatible with "Logger Pro" program distributed by "Vernier".

Labpro interface and the Logger Pro program together allowed us to save the records of individual kinetic runs in files with voltage output of Spectronic21.

Kinetic study was followed by stopped flow method. SFA – 11 is a fast kinetic accessory, which eliminates the problem of long mixing time. Its mixing time is less than 20 milli seconds. This means it can measure half-life down to about 0.05 seconds. The cell is thermo stated.

Table 1. λ_{max} and molar absorptivity of Fe(III)-AHA complex at different pH. μ = 0.1M , Temperature = 303K

S.No.	pH	λ_{max} nm	ϵ $M^{-1}cm^{-1}$
1	3.0	465	1581.32
2	3.5	456	1795.89
3	4.0	450	2020.62
4	4.5	425	2576.32

Table 2: Values of k_{obs} at different pH. [Fe(III)-AHA] = 5.0092E-4Mμ = 0.1M , Temperature = 303K

S.No.		k_{obs} s^{-1}			
	[L-Cys] M	pH 3.0	pH 3.5	pH 4.0	pH 4.5
1	0.25	0.763	0.905	0.932	-------
2	0.20	0.616	0.731	0.835	-------
3	0.15	0.456	0.601	0.563	-------
4	0.125	0.386	0.451	0.492	0.825
5	0.100	0.339	0.342	0.383	0.680
6	0.075	0.276	0.272	0.250	0.462
7	0,050	-------	0.145	0.139	0.273
8	0.025	-------	-------	0.0517	0.114
9	0.0125	-------	-------	0.0278	-------
10	0,0063	-------	-------	0.00948	-------
11	0.0050	-------	-------	-------	0.0185

Table 3: Values of k at different pH. [Fe(III)-AHA] = 5.0092E-4M
μ = 0.1M , Temperature = 303K

S.No.	pH	k_{obs} s^{-1}
1	3.0	2.82
2	3.5	3.78
3	4.0	4.06
4	4.5	6.97

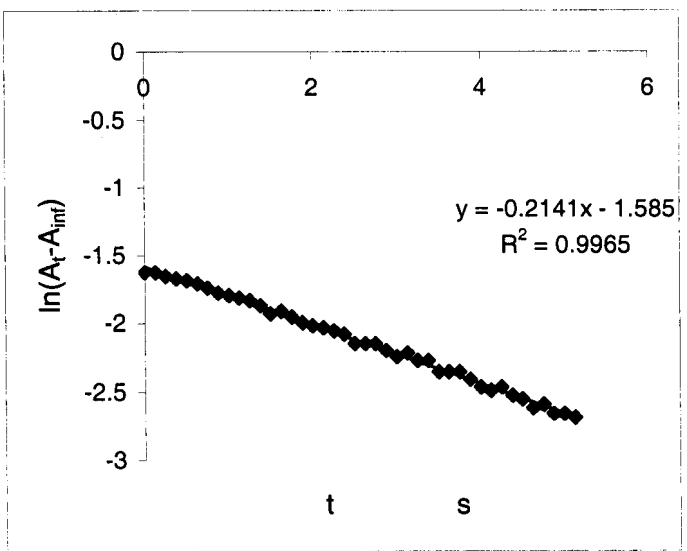


Fig. 2: [Fe(III)-AHA] = 5.0092E-4M[L-Cysteine]=0.051MpH=3, μ = 0.1M ,λ_{max} = 465nm, T=303K

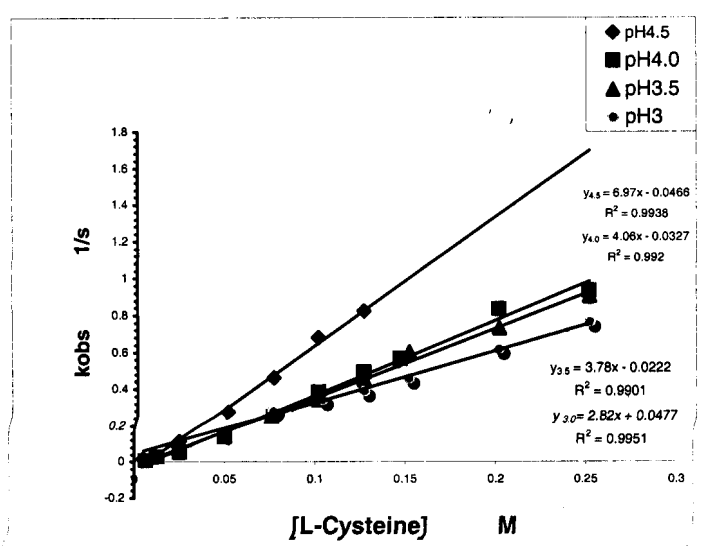


Fig.3: Plot of k_{obs} vs [L-Cysteine] at different pH.[Fe(III)-AHA] = 5.0092E-4M,T=303K,μ =0.1M

Results and Discussion

It has been observed that the λ_{max} decreases with increasing pH (Table 1).

$$Fe(III) + CH_3CONHOH \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} CH_3CONHO^- \rightarrow [(CH_3CONHO)nFe]$$

n =1,2 or 3. n depends upon pH.

Possibility of maximum complexation i.e. 1:3 increases with increase in pH. Fe(III)-AHA is reduced to Fe(II) - AHA on addition of reducing agent such as L-cysteine. Fe(II)-AHA is a colorless species(G. Schwarzenbach and, K. Schwarzenbach, 1963).

The plots of ln(At-A$_\infty$) against time turns out to be straight lines over many half-lives. This indicates that the system is first order with respect to both L-Cysteine and Fe(III)-AHA complex. The slope of this line is the pseudo first order rate constant (k_{obs}). For each concentration of L-Cysteine ten kinetic runs were performed to determine k_{obs}. A sample graph for the kinetic run is shown in Fig. 2. The values of rate constants are given in table2.

On the basis of these observations, the rate equation for the given redox reaction comes out to be as follows:

$d[Fe^{III}(AHA)_n]/ dt = k$ [L-Cysteine]
;

$[Fe^{III}(AHA)_n]$

since [L-Cysteine] is in large excess, we can define k_{obs} as

since [L-Cysteine] is in large excess, we can define k_{obs} as

$k_{obs} = k$ [L-Cysteine]

When k_{obs} values are plotted against the concentration of L-Cysteine a straight line graph at each pH is obtained (Fig. 3 & table 3). The slopes of the straight lines correspond to k in the rate equation.

On the basis of these observations we can propose the following mechanism

$$Fe(III) + CH_3CONHOH \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} CH_3CONHO^- \rightarrow [(\ CH_3CONHO)nFe]$$

$$RSH \rightleftharpoons RS^- + H^+$$

$$RS^- \rightarrow RS^\circ + e^-$$

$$2RS^\circ \rightarrow RSSR$$

$$RSH + Fe^{(III)}(AHA)_n \xrightarrow{RDS} RS^\circ + Fe^{(II)}(AHA)_n$$

$$2RS^\circ \xrightarrow{Fast} RSSR$$

Where

RSH = L-Cysteine

Rate = $-d[Fe^{III}(AHA)_n]/ dt = k$ [L-Cysteine] $[Fe^{III}(AHA)_n]$

Rate = k_{obs} $[Fe^{III}(AHA)_n]$

Where
$k_{obs} = k$ [L-Cysteine]

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