

Reaction of Ascorbate with Different Species of Iron(III)- 3,4,5-Trihydroxy Benzoic Acid Complex

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Summary: Different species of Fe(III)-galic (FeHL, FeL₂, FeL₃) acid complexes are formed at different pH values. These species have different rates of reduction with ascorbate. By measuring the overall rates of reduction of Fe(III) to Fe(II) at different pH and using the calculated species distribution at the corresponding pH a graphical procedure was applied to obtain the bimolecular rate constants for reduction of individual complex species. These were $k_{FeHL} = 87.9$, $k_{FeL} = 11.5$ and $k_{FeL2} = 4$ all in units of $M^{-1}S^{-1}$.

Introduction

Ascorbic acid is known to enhance iron absorption both by reducing iron to the more soluble ferrous form and by the formation of soluble iron ascorbate chelators [1]. Iron metabolism in R.E.S. (reticulo endothelial system) is apparently ascorbate dependent. It is found that in scorbutic rodent, spleen storage iron is greatly increased and consists largely of hemosiderin, whereas in the liver storage iron is in fact decreased, most of such decrease being due to lowered ferritin levels [2].

Some enzymes proline hydroxylase for example have an absolute requirement for both, ferrous iron and ascorbate [3]. However ascorbate is not stoichiometrically consumed in the reaction, and the enzyme system can operate for a few cycle without ascorbate before becoming inactive. It is suspected that the ascorbate is necessary to maintain the iron in the ferrous state.

In the light of above information, the assumption, that if gallic acid, in any form, (beverages hot and cold) consumed by the human body, will result in deficiency of iron, has reasonable weight. A detailed study of stoichiometry and stability of the different species formed between Fe(III) and 3,4,5, trihydroxy benzoic acid has been reported elsewhere [4].

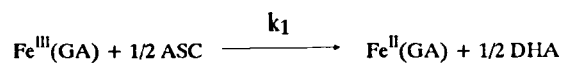
The question how the iron can be released from this grip ? is also important. Since as seen above (Table 1 [5,6,7], siderophores have a marked preferences for Fe(III) over Fe(II), reduction may be a viable mechanism of iron release [8,9].

Reduction of many Fe(III)-siderophore complexes has been studied i.e., ferrichrome, tris aceto hydroxamate and many others [10,11,12]. In the present study reduction of complex of gallic acid has been undertaken with the objectives outlined above.

Results and Discussion

The reaction was carried out under pseudo 1st order conditions with reductant concentration taken in excess over the complex concentration. The reduction of the complex with ascorbate was found to be first order in the complex concentration as was evident from the linear plot of $\ln(A_t - A_\infty)$ versus t . Observed rate constants (k_{obs}) were determined for different concentrations of ascorbic acid at constant temperature. Plots of k_{obs} vs [Ascorbate] at a given pH and constant temperature were also linear indicating that the reaction was also first order in ascorbate concentration.

These reactions were repeated at pH 4.2, 4.4, 4.6, 4.8, 5, 5.2 and 5.4. A plot of k_{obs} versus concentration of ascorbate was found to be linear with zero intercept (Fig. 1 Table 1). The slope of this plot gave k (rate constant), we call it $k_{1,2}$ at pH 5 and above because this reduction is for a mixture of complex species [ML] and [ML₂] [13].



ASC = Ascorbate

DHA = Dehydroascorbate

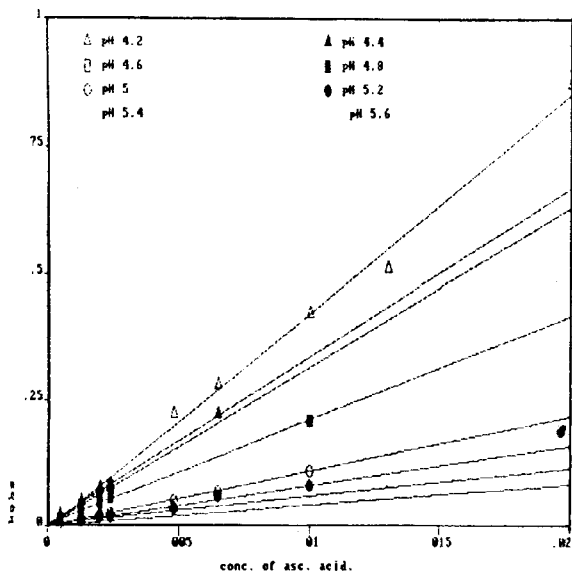
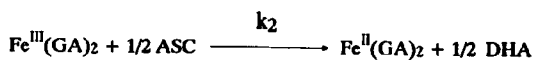


Fig. 1: Reduction of FeGA at different pH by ascorbate.

Table 1: Rate constant of FeGA at different pH with ascorbate T = 30°C.

pH	k M ⁻¹ S ⁻¹
4.2	40
4.4	36
4.6	30
4.8	22
5.0	10
5.2	8
5.4	6
5.6	4



$$\text{rate} = k_1 [\text{ML}] [\text{ASC}] + k_2 [\text{ML}_2] [\text{ASC}]$$

$$= \{k_1[\text{ML}] + k_2 [\text{ML}_2]\} [\text{ASC}]$$

Since the concentration of ascorbate is constant during the reaction then

$$k_{1,2} = k_1 [\text{ML}] + k_2 [\text{ML}_2]$$

The values of $k_{1,2}$ at pH 5, 5.2, 5.4 and 5.6 were calculated at 30°C, and it was found that rate constant had an inverse relationship with pH [14].

Selected pH ranges were those which had only ML and ML_2 . If the concentrations of these

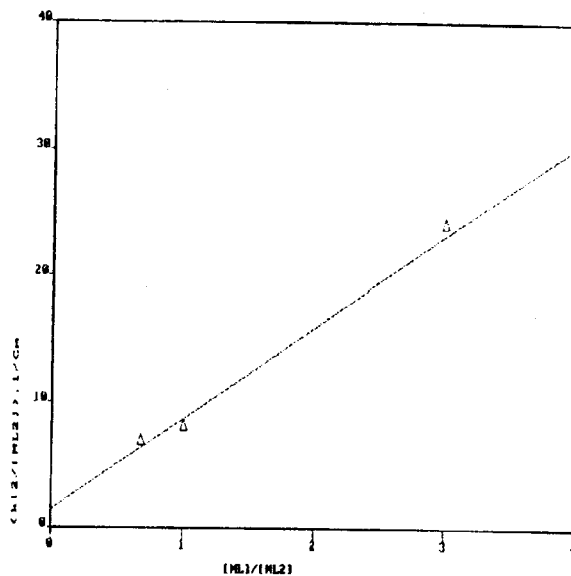


Fig.2: Determination of k_1 and k_2

Table 2: Determination of k_1 and k_2 at 30°C.

pH	$k_{1,2} \text{ M}^{-1} \text{ S}^{-1}$	$[\text{ML}_2]/\text{Cm}$	$[\text{ML}]/\text{Cm}$	$[\text{ML}]/[\text{ML}_2]$
5.0	10	0.05	0.9	18
5.2	8	0.22	0.78	4
5.4	6	0.25	0.75	3
5.6	5	0.50	0.50	1
5.8	4	0.60	0.40	0.68

species at these given pH's are known, as it is possible to calculate from the equilibrium studies cited in [4], we can determine k_1 and k_2 values separately.

$$k_{1,2}/[\text{ML}_2] = k_1 \frac{[\text{ML}]}{[\text{ML}_2]} + k_2$$

However the E° values of ascorbate is also pH dependent and increases but this change is negligible in this pH range.

The graph between $k_{1,2}/[\text{ML}_2]$ vs $[\text{ML}]/[\text{ML}_2]$ gives slope = k_1 and intercept = k_2 (Table 2, Fig. 2). These result showed the k_1 value 13 and k_2 4.

From the stability constant data it is now known that below pH 5 the species are ML and MHL.



Therefore rate = $k_{obs} [\text{comp}]_{\text{total}}$

$$\text{rate} = (k_{11}[\text{MHL}] + k_1[\text{ML}]) [\text{ASC}]$$

$$= k_1 + k_{11}[\text{H}] [\text{ML}]$$

$$= \frac{k_1 + k_{11}[\text{H}]}{1 + K[\text{H}]} = k_{1,11}$$

$$\text{or } k_{1,11} (1 + K[\text{H}]) = k_1 + k_{11} K[\text{H}]$$

This graph between $k_{1,11}(1 + K[\text{H}])$ and $K[\text{H}]$ gave the slope equal to k_{11} and intercept equal to k_1 (Table 3, Fig. 3). This showed intercept (k_1) 10.10 and slope (k_{11}) 87.90. The k_{11} was found to be much greater than k_1 . It might be due to conversion of charged species $[\text{Fe}(\text{HGA})(\text{H})]^+$ at pH below 4 into a neutral species $[\text{Fe}(\text{HGA})]$ at higher pH.

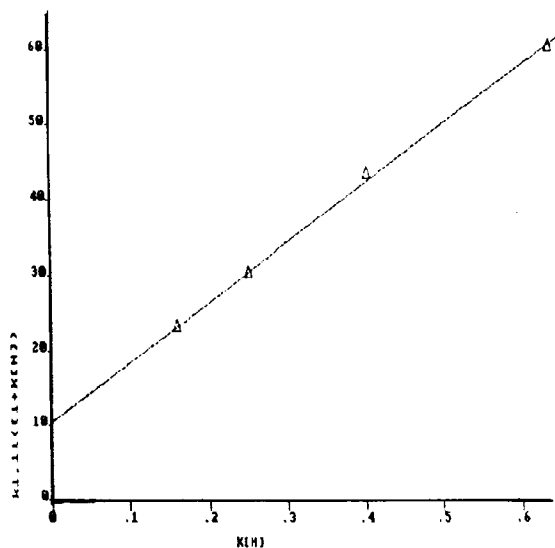


Fig.3: Determination of k_1 and k_2

Table 3: Determination of k_1 and k_2 at 30°C.

pH	$k_{1,11}$	$1 + K[\text{H}]$	$k_{1,11}(1 + K[\text{H}])$	$K[\text{H}]$
4.2	40	1.63	70	0.63
4.4	36	1.40	51	0.40
4.6	30	1.25	38	0.25
4.8	24	1.16	30	0.16

Experimental

The kinetics of reduction of the complex was followed at the wavelength of maximum absorbance (500 nm). The concentration of the complex were in the range of 1×10^{-4} . The reductant concentration was kept in excess and spanned as wide a range as experimentally possible. At least three reproducible kinetic runs were performed with each reductant solution. A minimum of thirty minutes was allowed to attain temperature equilibration of the working solutions.

The rate law for electron transfer reactions is first order in each reactant:

$$-d[\text{ox}]/dt = k[\text{ox}][\text{red}]$$

where $[\text{ox}]$ represent the oxidant concentration and $[\text{red}]$ represent the reductant concentration. When $[\text{red}] > [\text{ox}]$, pseudo first order conditions, the $[\text{red}]$ does not significantly change during the reaction. Therefore

$$k_{obs} = k[\text{red}]$$

and

$$-d[\text{ox}]/dt = k_{obs}[\text{ox}]$$

The change in concentration of oxidant is monitored as change in absorbance, in terms of photo multiplier voltage output, as concentration is linearly proportional to absorbance. Thus

$$A_t - A \propto [\text{ox}]$$

and

$$\ln(A_t - A) = -k_{obs}t + c$$

A plot of $\ln(A_t - A)$ vs t gives a straight line with a slope of $-k_{obs}$.

Rates were monitored by decrease in absorbance which was recorded with Beckmann recorder attached to Beckman model 25 spectrophotometer at 560 nm. Each concentration was repeated three times.

These experiments were repeated at pH 4, 4.2, 4.4, 4.6, 4.8, 5, 5.2, 5.4 and 5.6 at 30°C.

The ionic strength was kept constant 0.1 in all these experiments.

Data was treated with computer programme "rate 2" and "rate 3" and for very slow reactions Guggenheim method was used and data was treated with computer program "Gugg". For all calculations r (correlation coefficient) = 9900, at least, was assumed to be reliable.

Conclusion

The variation of the rate constant with pH may be ascribed to two factors.

(a) The reduction ability of ascorbate increases with the increase in pH. The first pK_a of ascorbate is 3.95 which is more than one unit apart from the mean pH of this study. Thus although an increase in the rate of reduction by ascorbate would be expected, this effect would be a minor one in this pH range (15). The change in E° of ascorbate in this range is about 0.04 and

$$\Delta \log k_{1,2} = -8.45 (\Delta E^\circ).$$

However since the effect of pH on the reducing ability of ascorbate can not be ignored, it is not feasible to attempt to isolate the rate constants into acid dependent and acid independent term for the reduction of the two monogallate species respectively.

(b) The concentration of the different species of the complex $[ML]$, $[ML_2]$ and $[ML_3]$ vary with pH and each species has its own specific rate constant of reduction. ML species predominate at lower pH having a fast rate of reduction, as the pH increases the ML gets converted into ML_2 , which has a slower rate of reduction as compared to ML . Above pH 7 only ML_3 species exist which is not reduced by ascorbate.

The equilibrium between these species plays an important role on the rate of reduction of iron chelated by Gallic acid, a bidentate ligand.

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