Kinetics of Oxidation of Glutathione by Micelle Trapped Tris(benzhydroxamato)iron(III)

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Abstract: Kinetics of the oxidation of glutathione (GSH) by micelle Sodium dodecyl sulphate (SDS) trapped tris(benzhydroxamato)iron(III) (TBH) at pH 3.3 and at 298 K has been described. The reaction is first order in TBS and GSH. Increase in pH decreased the rate. Order in H^+ is one. The reaction between TBH and GSH on micellar surface (Stern layer) differs from that occurring in bulk aqueous phase in absence of micelle (SDS). Reactive FeL_3 species on micellar surface is FeL^{2+} .

Keywords: Kinetics, Micellized tris(benzhydroxamato)iron(III), Sodium dodecyl sulphate, Glutathione.

Introduction

Tris(benzhydroxamato)iron(III), TBH, has been forwarded as a siderophore model compound¹⁻³-a microbial iron transport compound¹⁻⁸. Recently the micelle trapped TBH on sodium dodecyl sulphate, SDS, has been advocated to simulate the dipolar double layer siderophores that is not present in simple soluble hydroxamato complexes. It has been reported that TBH at pH 7.0 is reduced smoothly by sodium dithionite and ascorbic acid⁹. Ascorbic acid and glutathione, both are present in cells of plants, which are known to produce these siderophores (macromolecules containing catecholate and hydroxamate groups for uptake of iron deficiency). Reduction of glutathione, GSH, by the model compound is expected to throw light on the mechanism of iron uptake by siderophores whether it is (i) the ligand exchange with the complex and its subsequent release of iron or (ii) dissociation of the complex, ligation by GSH and subsequent electron transfer or (iii) An associative interchange mechanism with GSH and subsequent electron transfer leading to the release of iron(II).

These studies have a bearing in the field of bio-inorganic chemistry, particularly when it has been found that glutathione competes effectively with enterobactin¹⁰ and also reduces iron (III) readily at pH < 6.0. This is said to offer a realistic explanation for in vivo removal of iron from enterobactin without necessitating its destruction. It is also pertinent to point out that GSH has a high affinity for iron(II) and renders it likely to be low molecular weight intracellular iron transport compound. Such a role is not likely to be restricted to bacteria¹¹ and indeed offers a realistic reason for almost universal distribution of glutathione. It should be noted that evidence for removal of iron by reduction has been previously reported for hydroxamate containing siderophores¹²⁻¹⁴ and catecholate containing siderophore, agrobactin and excreted micrococcus denitrificans¹⁵. The kinetics of the reduction of GSH by micellised TBH was undertaken at pH 3.3 where the reaction rate is amenable to conventional kinetic analysis.

Experimental

Chemicals were of AnalaR specifications. Bezhydroxamic acid was prepared by the reported method¹⁶ and used after recrystallization and melting point determination. Ferric complex was prepared¹⁷ using AnalaR ferric nitrate and recrystallized from aqueous ethanol. Glutathione (reduced) (Serva feinbiochemica, Heidelberg) was used as such.

TBH is sparingly soluble in water¹⁸. It dissolves in water to which sufficient SDS has been added both to exceed the critical micelle concentration (CMC) and to ensure that the concentration of micelle each containing about 60 molecules¹⁹ exceeds the total iron(III) concentration. CMC of SDS in pure water^{19,20} is $8 \times 10^{-3} \text{ mol dm}^{-3}$ but lower $2.3 \times 10^{-3} \text{ mol dm}^{-3}$ for finite ion concentration^{19,21} $\{ [\text{NaCl}] = 0.05 \text{ mol dm}^{-3} \}$ ²⁰. Micelle concentration was always more than 10 times [TBH] in every reaction mixture.

Kinetic measurements

Reaction of micellized TBH with GSH (reduced) was carried out in a thermostatic cell holder spectrophotometrically using Shimadzu make UV-Visible spectrophotometer (Graphicord). The decrease in the concentration of TBH was followed at 485 nm at pH 3.3 and 298 K. Double distilled water was used to prepare stock solutions and reaction mixtures. GSH solution at pH 3.3 and TBH solution in SDS at pH 3.3 were thermostated separately for half an hour prior to mixing. HClO_4 was used to adjust the pH. GSH solutions were freshly prepared every day before use. The reactions were carried out under pseudo-first order conditions *i.e.* $[\text{GSH}] > 10 [\text{TBH}]$. Practically no change in the pH was observed after completion of the reaction.

Results and Discussion

The rate is first order with respect to TBH when $[\text{Fe (III)}] < 10^{-4} \text{ mol dm}^{-3}$. The second order rate constant at pH 3.3 and 298 K is $(1.05 \pm 0.04) \times 10^{-2} \text{ dm}^3 \text{ per mol}$ when [TBH] varies from 1.16 to $2.58 \times 10^{-5} \text{ mol dm}^{-3}$ and $[\text{GSH}] = [\text{SDS}] = 0.02 \text{ mol dm}^{-3}$. The rate is strictly first order with respect to [GSH] from 1.0 to $8.0 \times 10^{-2} \text{ mol dm}^{-3}$ with $k_2 = k_1/[\text{GSH}] = (0.98 \pm 0.04) \times 10^{-2} \text{ dm}^3 \text{ per mol}$ (Table 1). The rate in aqueous solution in absence of SDS, however, increases asymptotically with increase in [GSH] and within the concentration range $\{[\text{GSH}] = 1.0 - 8.0 \times 10^{-3} \text{ mol dm}^{-3}\}$ observed first order rate constant in aqueous solution, k_{obs} , follows the relation²²:

$$k_{\text{obs}} = 0.55[\text{GSH}] + 67.5[\text{GSH}]^2$$

Increase in pH [2.0—3.6] decreases the rate (Table 2). The order in H^+ is one in absence of any externally added HL. In aqueous solution in absence of SDS, increase in pH increases the rate²². This is contrary to what has been observed in the micellar pseudo phase (Table 2). The rate is inversely proportional to the concentration of hydroxamic acid [HL] added (Table 2) and levels off at higher [HL] (Figure 1). All these features suggest that the process proceeds *via* ligand dissociation as per equilibria:

Table 1. Effect of GSH concentration on rate.

[TBH] $\times 10^5 = 2.58 \text{ mol dm}^{-3}$; [SDS] = 0.02 mol dm^{-3} ; pH = 3.3; Temp. = 298 K

[GSH] $\times 10^2$ (mol dm^{-3})	1.0	2.0	3.0	4.0	6.0	7.0	8.0
$k_{\text{obs}} \times 10^4$ (s^{-1})	1.0	2.0	2.9	3.9	5.85	6.8	7.8
$k_2 \times 10^2 = k_{\text{obs}} / [\text{GSH}]$ ($\text{dm}^{-3} / \text{mol s}$)	1.0	2.0	0.966	0.975	0.975	0.971	0.975

Table 2. Effect of added [HL] and [H⁺] on the rate of reaction and on mole fraction of FeL_3 species.

[TBH] = $2.58 \times 10^{-5} \text{ mol dm}^{-3}$; [GSH] = [SDS] = 0.02 mol dm^{-3} ; pH = 3.3;
Temp. = 298 K

[HL] $\times 10^2$	1.0	2.0	3.0	4.0	5.0	9.0
$k_{\text{obs}} \times 10^5 \text{ s}^{-1}$	5.9	2.25	1.4	1.2	0.65	0.50
I	0.760	0.932	0.948	0.960	0.968	0.984
II	0.239	0.077	0.072	0.052	0.040	0.012
pH	2.0	2.5	3.0	3.3	3.6	
$k_{\text{obs}} \times 10^4 \text{ s}^{-1}$	26.5	9.4	3.6	2.0	1.1	
I	-	-	-	0.0024	0.007	
II	0.0001	0.065	0.217	0.364	0.535	
III	0.589	0.783	0.731	0.612	0.449	
IV	0.411	0.152	0.051	0.021	0.008	

I, II, III and IV are mole fractions of $[\text{FeL}_3]$, $[\text{FeL}^{2+}]$, $[\text{FeL}^{2+}]$ and $[\text{Fe}^{3+}]$ respectively calculated using K values at pH 3.0 (ref.18).

At pH 3.3, using the values of the formation constants in aqueous solution¹⁸ one can calculate the mole fraction of each species existing in equilibrium with each other in absence and presence of added HL. The mole fraction of species (I) to (IV) at pH 3.3, T=298 K and $[\text{Fe}(\text{III})] = 2.58 \times 10^{-5} \text{ M}$ approximates to 0.006, 0.415, 0.524 and 0.055 respectively but are in right order. The values are approximate as the K values used are the values reported¹⁸ at pH 3.0. Thus the reactive species is controlled by equilibrium (2). Recently, Lindman *et al.*²³, from ESR measurements on vanadyl ion, VO^{2+} , in aqueous solution of SDS have concluded

that virtually all VO^{2+} ions become attached to SDS at concentrations only slightly above the CMC. At pH 3.3 the micelle trapped²³ species is FeL^{2+} at $[\text{SDS}] = 0.02 \text{ M}$ which is much above its CMC²⁰. It is very likely that a very strong binding for bivalent ions like VO^{2+} is a general phenomenon regardless of the nature of micelles, provided that the charge sign of the solute is opposite to that on the micellar surface²⁴. This is also supported by the fact that pK_a values of weak acids, like HL, on the micellar surface are higher than those in aqueous solution²⁵. This favors formation of FeL^{2+} .

Figure 1. Plot of k_{obs} vs added $[\text{HL}]$

Since bivalent ions are bound strongly to the micelles (*vide infra*) it seems fair to presume that the complex will be anchored to micelle through its chelating ring penetrating, to some extent, beyond Stern layer into the micellar core. This leads to a situation that encounters between FeL^{2+} in the micelle phase and GSH in aqueous phase are not at all suitable for the operation of an inner sphere path much less even I (a) process—interchange associative. The reactions between Fe (III) complexes is well known to proceed through I (a) mechanism with rate controlling ligation of the entering group—the GSH here. This rules out the formation of even the ternary complex involving leaving and entering group with metal ion (so called Coordination Foothold)²⁶. Whatever be the process, the micellar induced operation of the outer sphere path is ruled out. The rates reported for the process be taken as the rate of reaction in the micellar phase between anchored FeL^{2+} and anchored GSH in specific orientation for a facile removal of an electron from SH group.

The difference in the rate dependence of the reaction on $[\text{GSH}]$ and $[\text{H}^+]$ in aqueous and micellar phases does suggest that a complex reaction in aqueous system can be rendered simpler in micellar phase (the Stern Layer) and that the electron transfer process in micellar pseudo phase may involve appropriate positioning of the electron donor and acceptor species.

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