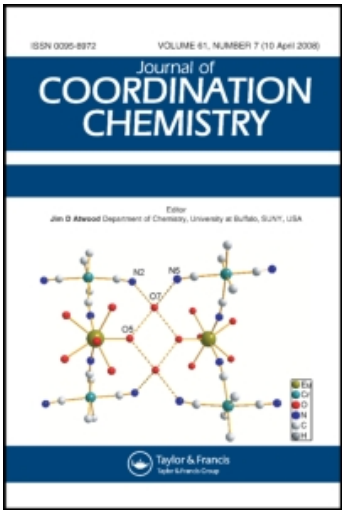

Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

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Online publication date: 15 September 2010

To cite this Article El-Ayaan, Usama and Linert, Wolfgang(2002) 'A Kinetic Study of the Reaction Between Glutathione and Iron(III) in the pH Range from 1 to 3', Journal of Coordination Chemistry, 55: 11, 1309 — 1314
To link to this Article: DOI: 10.1080/0095897021000036398
URL: http://dx.doi.org/10.1080/0095897021000036398

J. Coord. Chem., 2002, Vol. 55(11), pp. 1309–1314

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A KINETIC STUDY OF THE REACTION BETWEEN GLUTATHIONE AND IRON(III) IN THE pH RANGE FROM 1 TO 3

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(Received 31 October 2001; In final form 12 March 2002)

Anaerobic reactions of iron(III) with glutathione (GSH) have been studied kinetically using stopped-flow spectrophotometry. One mixing reduced GSH with iron(III) a very rapid increase in absorbance with a broad peak centred at 620 nm was recorded. The rapid formation of the blue complex was followed by a decomposition step at a slower rate, yielding a colourless product. GSH reduces iron(III) readily in aqueous solution to yield the iron(II) GSH complex.

Keywords: Glutathione; Stopped-flow kinetics; Iron(III) complexes

INTRODUCTION

Glutathione *N*-(*N*-L-γ-glutamyl-L-cysteinylglycine) is a widely distributed biological species with sulphur-containing metal-binding sites. It is considered to be an essential constituent of living cells [1,2]. Since the discovery of glutathione (GSH) by Hopkins and the recognition that this thiol could play a role in electron transfer [3,4], there have been many studies of the autocatalytic oxidation of this biologically important compound and other related thiols [5,6]. The coordination chemistry of GSH with metal ions has considerable interest [7–13]. Since our interest is focused on the interaction between iron and the catecholamines [14,15], because of their involvement in the progression of Parkinson's disease, we were encouraged to carry out a study of GSH and its possible interaction with iron.

The GSH structure (Fig. 1) possesses eight potential binding sites, two carboxylic acid groups, an amino group, a sulfhydryl group and two amide functions.

As all the binding sites cannot be simultaneously coordinated to a single metal ion, the coordination chemistry of the GSH is characterised by the formation of protonated and polynuclear complexes [2,6].

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ISSN 0095-8972 print: ISSN 1029-0389 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/0095897021000036398

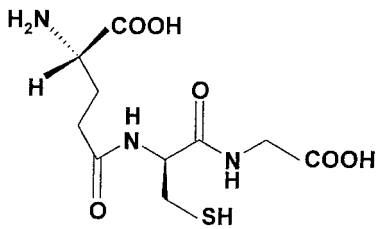


FIGURE 1 Structure of glutathione

EXPERIMENTAL

Glutathione, $C_{10}H_{17}N_3O_6S$ [reduced form], was supplied by Aldrich and used without further purification. Stock solutions of iron(III) (as nitrate nonahydrate, Merck) were made up from deoxygenated solutions that contained calculated amounts of HNO_3 and KNO_3 to maintain a final ionic strength of 0.10 mol dm^{-3}. The pH range covered in the present work ranges from 1.0 to 3.0. The pH was measured immediately after each kinetic run with a WTW pH 521 pH meter and $[H^+]$ was calculated by using the empirical relationship [16], $[H^+] = 10^{-[pH-0.131/0.984]}$. Since the iron concentration was kept low, the change of pH due to H^+ released on complex formation was negligible.

The experimental procedure involves the potentiometric titration of solutions against 0.01 mol dm^{-3} KOH made up of 2.5 cm^3 of hydrochloric acid (1×10^{-3} mol dm^{-3}) plus 2.5 cm^3 of aqueous potassium nitrate (2.00 mol dm^{-3}). To this solution was added, in turn, 5 cm^3 of 0.01 mol dm^{-3} aqueous GSH solution, and 0.5 cm^3 of 0.01 mol dm^{-3} iron(III) nitrate solution. The total volume was then adjusted to 25 mL. After adding each increment of titrant, the solution was stirred for about two minutes and the pH reading recorded.

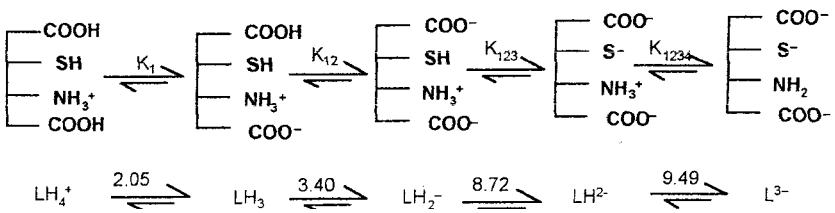
Stopped-flow experiments

The appearance and disappearance of the blue complex was followed at 620 nm with a Bio-sequential SX-17MV sequential stopped-flow ASVD spectrofluorimeter and yielded observed *psuedo*-first-order rate constants, k_1^{obs} and k_2^{obs}, respectively. All kinetic runs were performed with GSH in large excess over iron(III) in order to maintain *pseudo*-first-order kinetics. All measurements were carried out at 25°C in solutions of ionic strength 0.100 mol dm^{-3} (KNO_3). Triplicate runs gave reproducibilities of ± 5% for k^{obs}.

RESULTS AND DISCUSSION

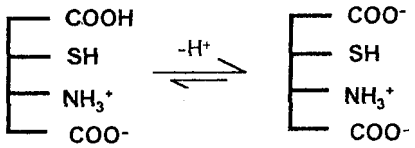
Potentiometric Titrations

The successive deprotonation of GSH was reported as follows.



When GSH is dissolved in water (1 mM) the initial pH is 3.3 which is above the first pK_a of 2.05. This suggests that the GSH species present at pH = 3 is LH_3.

From the titration of GSH in the absence of metal ion up to pH 6 one proton is titrated per GSH.



When GSH–iron(III) mixtures were titrated, an extra proton was released in addition to that released by GSH alone. Iron(III) when dissolved in water releases about one proton and when reacted with GSH another proton must be released to form GSSG according to the following equation:

$$[Fe^{II}(H_2O)_xGSH]^{2+} + (6-x)(H_2O) \leftrightarrow H^+ + [Fe^{II}(H_2O)_6]^{2+} + (1/2)GSSG$$

Due to the presence of iron(III) originally, there must be another proton in solution which was titrated to the first end point along with the proton on the GSH carboxylate group.

This proton seems to originate from the thiol group, which pK_a is lowered by metal coordination. However, it is still associated with the complex until the first end point is reached.

Stopped-flow Kinetics

Formation of the Fe(III)–GSH Complex

The formation and decomposition rate constants of the blue complex formed between Fe(III) and GSH were monitored at 620 nm (the wavelength at which the maximum absorption of the blue complex was obtained).

GSH solutions absorb at 240 nm, while the maximum absorption of Fe(III) in very acidic media is 310 nm. Therefore no interference from these compounds in the kinetic study of complex formation is to be expected. The observed rate of formation of the complex is faster than the rate of its decomposition by a factor of about 500. Experimentally, this reaction follows the equation rate (1).

$$\frac{d[\text{complex}]}{dt} = k_1^{obs}[Fe^{III}] \tag{1}$$

At constant pH, a plot of k_1^{obs} *versus* [GSH] gives a straight line with gradient k (Fig. 2), {kinetic data related to k_1^{obs} and k_2^{obs} are summarised in (Table I)}. This leads to the following relation (2).

$$k_1^{obs} = k_1[GSH] \tag{2}$$

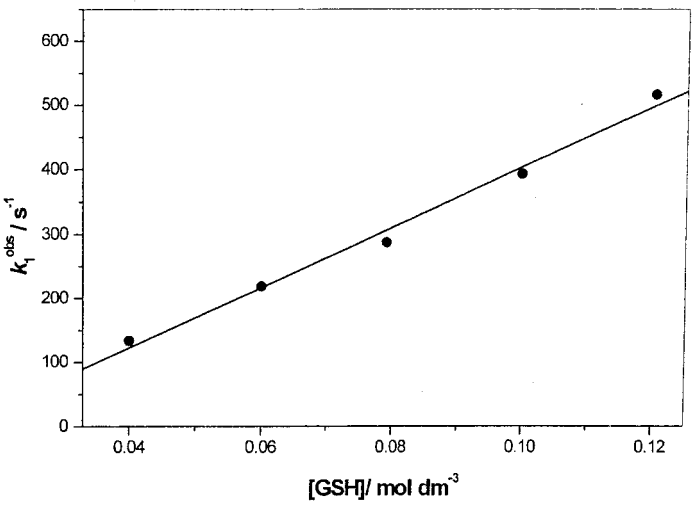


FIGURE 2 Dependence of k_1^{obs} on [GSH].

TABLE I Typical values of k_1^{obs} and k_2^{obs} $\{[Fe]_T = (0.5-2)\times 10^{-3}\,mol\,dm^{-3}\}$

pH	[GSH](mol dm^{-3})	k_1^{obs}(s^{-1})	$10^3\,k_2^{obs}$(s^{-1})
1.69	0.04	109	6.15
1.68	0.04	115	7.74
2.9	0.02	184	53.9
2.9	0.02	180	79.3
2.9	0.02	178	86.0
1.62	0.08	121	9.53
2.20	0.08	225	3.81
2.37	0.08	249	60.2
2.57	0.08	265	120.0
1.73	0.04	120	7.79
1.88	0.04	141	16.0
2.22	0.04	168	28.4
2.50	0.04	219	51.7
2.20	0.10	394	33.6
2.20	0.12	523	43.7
2.20	0.06	219	14.4
1.54	0.04	97	2.60
1.66	0.06	134	4.91
1.80	0.08	168	28.5
1.97	0.10	228	79.5
1.77	0.04	121	6.21
1.80	0.06	153	11.8
2.03	0.08	210	445
2.21	0.10	327	866

From Fig. 2, a value of k_1 was obtained as $4.6\times10^3\,dm^3\,mol^{-1}\,s^{-1}$ and from the intercept k_{-1} was obtained as $46.0\,s^{-1}$. This led us to consider the following simple relation for complex formation (3).

$$Fe(OH)^{2+} + GSH \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Complex \qquad (3)$$

Comparing Eqs. (1) and (2) yields the rate equation (4);

$$\frac{d[\text{complex}]}{dt} = k_1[\text{Fe}^{\text{III}}][\text{GSH}] \tag{4}$$

this relation shows clearly that the overall reaction is second order with a rate constant of $4.6\times10^3\,\text{dm}^3\,\text{mol}^{-1}\,\text{s}^{-1}$ at 25°C.

Decomposition Step (Decay of the Blue Complex)

The overall reaction can be considered as follows (5).

$$\text{Fe(OH)}^{2+} + \text{GSH} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Complex} \xrightarrow{k_d} \text{Fe(II)} + (1/2)\text{GSSG} \tag{5}$$

GSH and other thiols were found to reduce iron(III) to iron(II) anaerobically in acidic solutions [17].

$$\begin{aligned}\frac{-d[\text{coloured complex}]}{dt} &= \left(\frac{k_2 obs}{[\text{GSH}]^\circ}\right)[\text{Fe}]_\text{T}[\text{GSH}]_\text{T} \\ &= k_2[\text{Fe}]_\text{T}[\text{GSH}]_\text{T}\end{aligned} \tag{6}$$

since $k_2 = k_2^{\text{obs}}/[\text{GSH}]^\circ$

In order to show the dependence of k_2^{obs} on $[\text{H}^+]$, the function $[\text{GSH}]^\circ/k_2^{\text{obs}}$ is plotted against $[\text{H}^+]$. A straight line is obtained (Fig. 3) with an intercept of $1.77\times10^2\,\text{mol}\,\text{dm}^{-3}\,\text{s}^{-1}$, and a slope of 0.21 s as in (7).

$$\frac{[\text{GSH}]^\circ}{k_2^{\text{obs}}} = a[\text{H}^+] + b \tag{7}$$

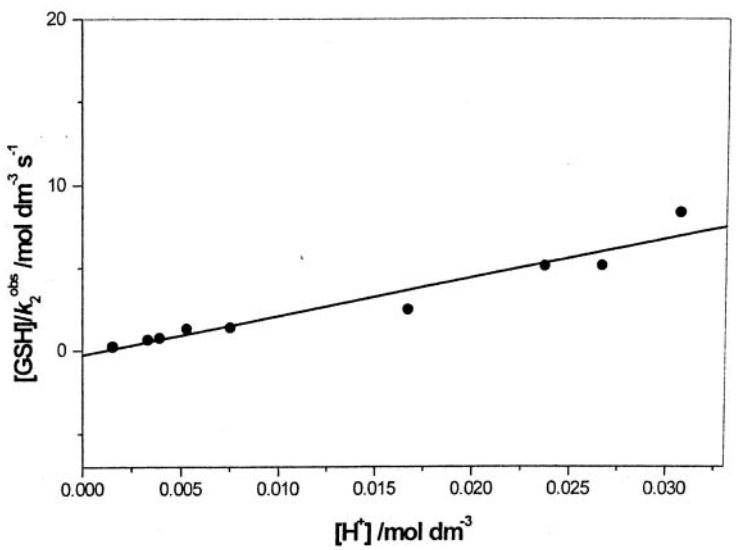
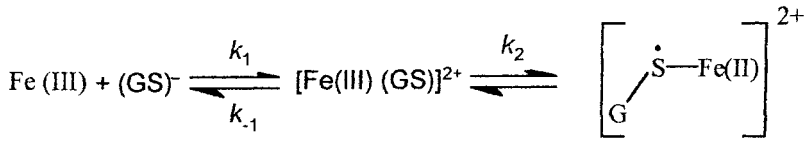


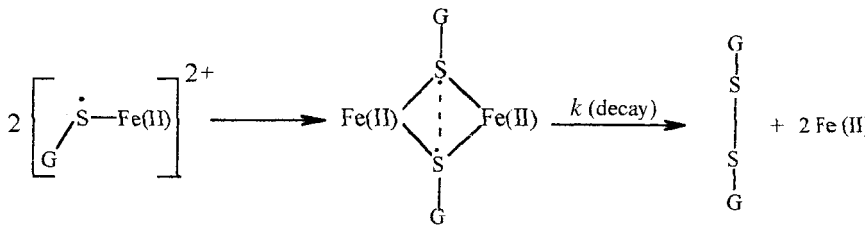
FIGURE 3 Dependence of k_2^{obs} on $[\text{H}^+]$.

The blue complex appears to be formed according to the following mechanism:



(8)

This scheme shows that Fe(III) reacts with reduced GSH in a 1 : 1 ratio to form an intermediate, followed by a very rapid electron transfer to form the blue complex containing iron(III). Two blue complex molecules may dimerise and then decompose to give GSSG and Fe(II) (9).



(9)

As the decay of the blue complex involves the production of GSSG, dimerisation of GSH must be involved.

Acknowledgements

Thanks are due to the Hochschuljubiläumstiftung der Stadt Wien (Project H-65/2000) and support of the project *via* the EU COST project D8/13 and the Japan Society of the Promotion of Sciences.

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