Kinetics and Mechanism of the Oxidation of Glutathione by 12-Tungstocobaltoate(III) in Aqueous Solutions. A Preliminary Report

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Investigations of the redox reactions of biologically important thiols have aroused considerable interest of late. As a result of these studies, several proposals which enhance the understanding of their roles as electron transfer enzymes are now documented [1–6]. In general, the oxidation of thiols to disulphides occurs through either of two pathways. In the first, electron transfer is preceded by the formation of intermediate complexes with significant thermodynamic stabilities as in the reactions of Cr(VI) with L-cysteine [1], glutathione and penicillamine [2], and ferricytochrome c with glutathione [3]. In some cases, these metal–thiol complexes are linked with the effectiveness of the thiol in removing unwanted metal ions [4, 5] from biological systems. Alternatively, simple electron transfer occurs without prior complex formation. This class of thiol reactions is exemplified by the oxidations of glutathione and cysteine by Mo(VI) [6] and the reactions of cysteine, mercaptoethylamine and mercaptoacetic acid with 12-tungstocobaltoate(III) [7].

Reports of the use of various heteropoly molybdates and tungstates as electron acceptors in biological processes such as photosynthesis [8, 9] have been made. 12-Tungstocobaltoate(III) ion, $Co^{III}O_4W_{12}O_{36}^{5-}$ (hereafter designated [Co(III)]), and 12-tungstocobaltoate(II) ion, $Co^{II}O_4W_{12}O_{36}^{6-}$ (hereafter referred to as [Co(II)]) are examples of heteropoly tungstates in which the cobalt atoms are surrounded by several WO_6 units. These anions have been established to be substitution inert [10, 11]. As part of our continued interest in the reactions of a wide spectrum of thiols with [Co(III)], we studied the title reaction in the hope of relating our findings to the points raised above.

Experimental

Materials

Potassium salts of 12-tungstocobaltoate(III) and 12-tungstocobaltoate(II) ions were prepared, characterized and standardized as described by McAuley and his coworkers [11]. Reduced glutathione (Aldrich chemical), perchloric acid (B.D.H.) and sodium perchlorate, B.D.H. (A.R. grade) were used without further purification.

Stoichiometry

The reaction stoichiometry was determined by spectrophotometric titrations. Spectra of solutions containing various concentrations of the thiol ($0.8–9.6 \times 10^{-4} M$), at constant oxidant concentration of $2.0 \times 10^{-4} M$, and $[H^+] = 0.05\ M$ were measured after the reactions had been completed. Plots of absorbances at 390 nm *versus* thiol concentration gave the titration curves from which the stoichiometry was evaluated.

Kinetics

Kinetic investigations were carried out using pseudo-first-order conditions (excess glutathione). Under these conditions, reaction rates conformed to the conventional range. Consequently, decreases in absorbance due to [Co(III)] were studied at 390 nm on a Unicam SP 8000 spectrophotometer with fully thermostatted cell compartments.

Pseudo-first-order plots were linear to more than 90% reaction. The slopes of such plots gave k_{obs} at different initial concentrations of glutathione and hydrogen ion*. Replicated measurements agreed to ±3%.

Results and Discussion

Stoichiometric studies showed that 1.03 ± 0.2 mol of glutathione was oxidized per mol of the oxidant, consistent with the reaction:

$$2[Co(III)] + 2GSH \rightarrow 2[Co(II)] + GSSG + 2H^+ \quad (1)$$

where glutathione is represented as GSH and GSSG is the disulphide product.

Observed rate constants, k_{obs}, show a linear dependence on glutathione concentration at constant $[H^+]$ (Fig. 1). The linearities of the pseudo-first-order plots as well as the latter plot indicate that the reaction is first order in both glutathione and [Co(III)]. However, the decrease in the rate constant as a func-

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*A table of k_{obs} at different thiol and acid concentrations at 23.8 ± 0.2 °C is available on request.

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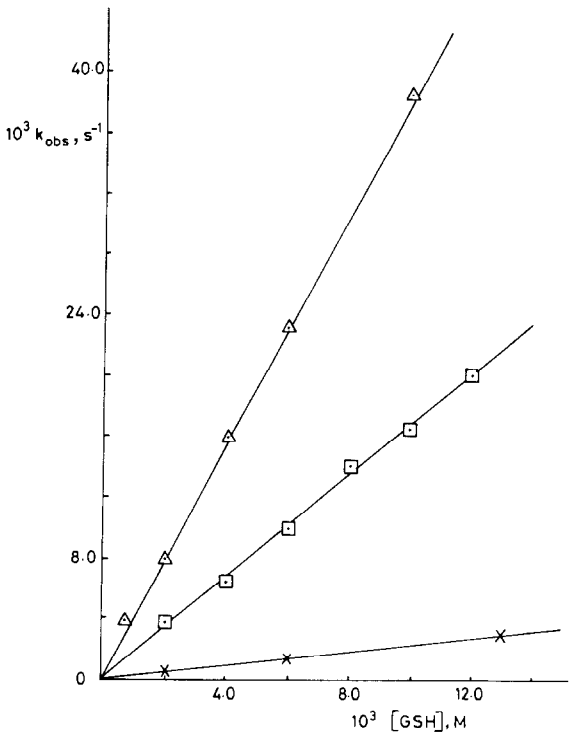

Fig. 1. The dependence of observed rate constant (k_{obs}) on the concentration of glutathione at 23.8 ± 0.2 °C. [Co(III)] = 2.0×10^{-4} *M*; I = 1.0 *M* ($NaClO_4$); [H^+] = 0.02 *M* (△); [H^+] = 0.06 *M* (□); [H^+] = 0.50 *M* (×).

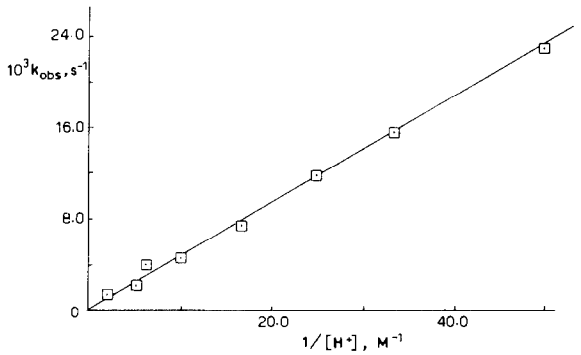

Fig. 2. The dependence of observed rate constant (k_{obs}) on 1/[H^+]. [Co(III)] = 2.0×10^{-4} *M*; [GSH] = 6.0×10^{-3}*M*.

tion of [H^+] (Fig. 2) strongly suggests a preequilibrium step involving the release of a proton. One obvious rationalization of this is the deprotonation of the sulphydryl group in the thiol as in the reactions of the oxidant with L-cysteine, mercaptoacetic acid and mercaptoethylamine [7] as well as the oxidation of glutathione by ferricytochrome c [3]. This view is upheld by the observation that the reactions of [Co(III)] with thiols like methionine [12] and thiourea [13] (which have no sulphydryl groups) were acid independent.

Hence, from the experimental data the following scheme can be proposed for the title reaction:

$$GSH \rightleftharpoons^{K_a} GS^- + H^+ \quad (2)$$

$$[Co(III)] + GS^- \xrightarrow{k_1} \text{Products} \quad (3)$$

where K_a is the acid dissociation constant of the -SH group in glutathione. This scheme leads to the expression for the observed rate constant shown in eqn. 4:

$$k_{obs} = \frac{k_1 K_a [GSH]}{[H^+] + K_a} \quad (4)$$

Under conditions where $[H^+] \gg K_a$ [14] eqn. 4 can be simplified as

$$k_{obs} = \frac{k_1 K_a GSH]}{[H^+]} \quad (5)$$

which is in good agreement with the plots of k_{obs} against GSH and 1/[H^+] (Figs. 1 and 2) observed in this study.

Since [Co(III)] and glutathione are one-electron reactants, the most likely feature of the rate-determining step is a simple electron-transfer from the ligand to the oxidant, reminiscent of the outer-sphere mechanism. When the absorbances of solutions of the oxidant alone and those of glutathione, acid and the oxidant were compared at 390 nm, there was no difference. Similarly, the spectra of [Co(III)] and those of solutions containing [Co(III)], acid and glutathione showed a clear shift in the λ_{max} of [Co(III)] on addition of the substrate. These results suggest that no complex of significant stability was formed between [Co(III)] and glutathione at the time of mixing. Although they are not by themselves sufficiently convincing to rule out the operation of the inner-sphere mechanism, when they are supplemented by the substitution inertness of the oxidant, it could be inferred that the most plausible mechanistic option open to this reaction is the outer-sphere mechanism. Thus, the oxidation of glutathione by [Co(III)] can be placed alongside those of the oxidant with L-cysteine, mercaptoacetic acid and mercaptoethylamine [7] as well as the reduction of Mo(VI) by glutathione [6] in which the thiols were oxidized to the corresponding disulphides without the formation of complexes of significant thermodynamic stabilities. Further studies on this and similar reactions are presently in progress.

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References

1 J. P. McCann and A. McAuley, *J. Chem. Soc. Dalton,* 783 (1975).
2 M. A. Olatunji and A. McAuley, *Can. J. Chem., 55,* 3335 (1977).
3 (a) N. Kujundzic and J. Everse, *Biochem. Biophys. Res. Commun., 82,* 1211 (1978). (b) J. Everse and N. Kujundzic, *Biochemistry, 18,* 2688 (1979).
4 Y. Sugiura, Y. Hojo and H. Tanaka, *Chem. Pharm. Bull., 20,* 1362 (1972).
5 J. M. Walshe, *Chim. Sci., 20,* 401 (1964).
6 J. F. Martin and J. Spance, *J. Phys. Chem., 74,* 2863 (1970).
7 G. A. Ayoko and M. A. Olatunji, submitted for publication.
8 G. Girault and J. M. Galmiche, *Biochim. Biophys. Acta, 333,* 314 (1975).
9 B. A. Zilinskas and Govindjee, *Biochim. Biophys. Res. Commun., 387,* 306 (1975).
10 C. H. Brubaker and P. Rasmussen, *Inorg. Chem.,* 977 (1964).
11 Z. Amjad, J. C. Brodovitch and A. McAuley, *Can. J. Chem., 55,* 3581 (1977).
12 O. Ishola and M. A. Olatunji, unpublished results.
13 M. A. Olatunji and A. McAuley, unpublished results.
14 R. E. Benesh and R. Benesh, *J. Am. Chem. Soc., 77,* 5877 (1955).