Reaction of the Vanadate Ion with Chlorpromazine and the Chlorpromazine Free Radical with the Vanadyl Ion

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In a recent issue of this journal, Sakurai *et al.* [1] suggested that the oxidation of chlorpromazine 2-chloro-10-[3-dimethylaminopropyl] phenothiazine) by the vanadate ion takes place over the pH range 5–8 to produce a chlorpromazine free radical and a vanadyl ion.

$$V(V) + ClPz \rightleftharpoons + V(IV) + ClPz^{\ddagger\bullet} \quad (1)$$

ESR spectrometry was used to measure the vanadyl ion and the chlorpromazine free radical was determined using optical absorption spectrophotometry. This was an extension of earlier work by Vyskocil *et al.* [2] using only ESR and detecting only V(IV). In both cases, the experimental procedure involved mixing the vanadate and chlorpromazine in a sodium phosphate buffer and then acidifying a sample of the reaction mixture with HCl. The acidification was believed to stabilize the $ClPz^{\ddagger}$.

It should be noted, however, that the one electron reduction potential of chlorpromazine has been reported as 0.78 V, independent of pH, in the acid region [3]. The potential of the V(V)/V(IV) couple is 1.0 V in 1 M acid, but decreases rapidly as the pH is raised [4]. At pH 2, the potentials of $ClPz^{\ddagger}$/ClPz and V(V)/V(IV) are about equal. This would suggest that V(V) could oxidize chlorpromazine only in solutions more acid than pH 2. In more basic solutions, the reaction would be reversed. We have, therefore, carried out measurements on both the forward and reverse reactions at several acid concentrations and have confirmed that this is indeed the case and that the results reported previously [1, 2] were due to the experimental procedure employed.

Experimental

Kinetic measurements on the V(V) oxidation of chlorpromazine were carried out using a Durrum D-100 stopped-flow spectrophotometer, interfaced via a transient analyzer to a microcomputer. The kinetics of the chlorpromazine radical reaction with V(IV) were measured using the pulsed radiolysis apparatus described previously [5]. In both cases, the chlorpromazine radical absorbance at 525 nm was monitored.

Chlorpromazine hydrochloride was from Sigma and $VOSO_4$ and Na_3VO_4 were from Alfa. Water was purified by a Millipore Multi-Q system (for pulsed radiolysis) or a Gelman Water-I system (for stopped-flow). Stock solutions of V(IV) were prepared one day in advance due to slow dissolution of $VOSO_4$. Stock solutions of V(V) in acid were stable and were diluted as needed. Solutions used in the stopped flow experiments were deoxygenated by bubbling with N_2 and solutions used in pulsed radiolysis were bubbled with N_2O.

TABLE I. Rate of Reaction of V(V) with Chlorpromazine.[a]

T (°C)	ClPz (mM)	V(V) (mM)	V(IV) (mM)	k_1 (s^{-1})
$[H^+]$ = 1.0 M, μ = 1.0 M				
25.1	0.0015	25.9		0.15
	0.0076	5.18		0.045
	0.0076	12.95		0.082
	0.0076	25.9		0.14
	0.025	25.9		0.16
	0.043	25.9		0.15
	0.124	25.9		0.19
31.3	0.0076	5.18		0.055
	0.0076	12.95		0.13
	0.0076	25.9		0.21
38.7	0.0076	5.18		0.085
	0.0076	12.95		0.18
	0.0076	25.9		0.30
24.8	0.0076	12.95		0.10
	0.0076	12.95	24.35	0.16
$[H^+]$ = 0.1 M, μ = 0.5 M				
25.0	0.009	0.81		0.021
	0.009	0.78	2.0	0.0076
35.4	0.009	0.81		0.012
	0.009	0.78	2.0	(0.13)[b]

[a]Measured by the stopped-flow technique. [b]Rate constant for the reverse reaction, see text.

Results and Discussion

The reaction of the vanadate ion with chlorpromazine was measured using the stopped-flow apparatus in strongly acid solutions. The results of this study are presented in Table I. Only the first

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order rate constants, derived from a non-linear least squares treatment of the data, are presented since the apparent second order rate constants depended on both the concentration of chlorpromazine and vanadate. This suggests that even in 1 M acid, the reverse reaction, due to the accumulation of vanadyl and the chlorpromazine free radical, is important. This is confirmed by the strong influence of added vanadyl ion on the measured rate constant. At 0.1 M acid and 35 °C, the addition of vanadyl ions to the vanadate solution prior to mixing not only eliminated the production of the chlorpromazine free radical but also bleached the small ambient concentration of the radical in the chlorpromazine solution. The rate of this reaction is given in the table.

In order to produce the chlorpromazine free radical for rate measurements, solutions of 0.5–1 mM ClPz and 0.3 M KBr, saturated with N_2O, were pulse irradiated. Under these conditions, the following reactions take place within 5 μs after the pulse [6, 7]:

$$H_2O \longrightarrow\longrightarrow e_{aq}^-, H, OH \quad (2)$$

$$e_{aq}^- + N_2O \longrightarrow N_2 + OH^- + OH \quad (3)$$

$$OH + 2Br^- \longrightarrow OH^- + Br_2^- \quad (4)$$

$$Br_2^- + ClPz \longrightarrow 2Br^- + ClPz^{\ddagger} \quad (5)$$

The chlorpromazine free radical produced by pulse radiolysis was found to absorb at 525 nm with an absorptivity of $\sim 1.2 \times 10^4$ M^{-1} cm^{-1}, in agreement with the absorptivity reported in strong acid [3] as well as pH 3–6 [6, 7]. The radical showed no decay over the reaction times investigated (up to 200 ms).

Upon the addition of V(IV) , the decay of the chlorpromazine radical was notable. The rate of decay was dependent on the concentration of V(IV) and strongly dependent on pH. The second order rate constants for the reverse reaction (1) are: pH 3.9, $\sim 3 \times 10^2$; pH 4.9, 5×10^5; pH 5.1, 2×10^6; pH 6.7, 6×10^6 M^{-1} s^{-1}. Attempts to measure a rate constant at higher pH, or over a wide range of V(IV) concentration, were prevented by the tendency of the solution to become cloudy. Also, additions of V(IV) to the solutions changed the pH. Although this change was not large, the strong dependence of the rate on pH contributed to an uncertainty in the derived second order rate constant which we estimate to be ~25%. It is clear, however, that at pH values above 4, the chlorpromazine radical oxidizes the vanadyl ion.

The results we report here explain the phenomena reported in references [1, 2]. Mixing vanadate and chlorpromazine in a phosphate buffer gives a thermodynamically stable mixture – there is no reaction. Acidification leads to a thermodynamically unstable mixture, which proceeds to react. This is shown clearly in Fig. 2 of ref. [1], where the rate of increase in the chlorpromazine radical absorbance after acidification corresponds to a rate constant of about 5–10 M^{-1} s^{-1}, in accord with our results.

The interest in the chlorpromazine–vanadium system arises from the importance of chlorpromazine and related phenothiazine derivatives in treating manic-depressive illness and the possibility that vanadate may play a role in that illness [8]. The present results indicate that the chlorpromazine free radical is sufficiently stable at physiological pH to play a role and that the chlorpromazine free radical can oxidize vanadyl to vanadate, contrary to previous conclusions [1, 2].

The work reported in references [1, 2] was prompted by the observation that phenothiazines like chlorpromazine catalyze the reduction of vanadate by NADH (reduced nicotinamide adenine dinucleotide) at pH 7.2 in a system also containing FAD (flavin adenine dinucleotide) [9]. It is known that the chlorpromazine free radical can oxidize NADH to the NAD free radical [10] and the NAD radical appears to have a sufficiently negative reduction potential (–0.93 V [11, 12]) that it could be oxidized by vanadate, by FAD, and by oxygen (which appears to have been present in the mixture). The formation of $ClPz^{\ddagger}$ by the equilibrium reaction 1 is sufficient to explain these results. Although reaction 1 is driven to the left at pH 7, the small equilibrium concentration of $ClPz^{\ddagger}$ rapidly reacts with NADH and is replenished by the excess V(V) in the rate determining step.

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