Kinetics and Mechanism of the Reduction of Chlorpromazine Radical by Ascorbic Acid

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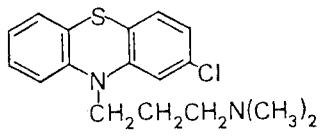
Abstract: The stoichiometry of the ascorbic acid (H_2A) reduction of the radical cation (ClP^+) generated by oxidation of chlorpromazine [2-chloro-*N*-(3-dimethylaminopropyl)phenothiazine], ClP, has been experimentally determined to be $H_2A + 2ClP^+ \rightarrow A + 2ClP + 2H^+$. The kinetics of the reaction in acidic media have been monitored by stopped-flow techniques ($[H^+]$ ranging from 0.25 to 1.00 M, μ = 1.00, 25.0 °C). Observed pseudo-first-order rate constants, k_{obsd}, depend upon the concentration of the reagent in excess, ascorbic acid, in accordance with the expression $k_{obsd} = kK_c[H_2A]/(1 + K_c[H_2A])$, where k represents the first-order rate constant for electron transfer within a reversible precursor complex described by equilibrium quotient K_c. Both k_{obsd} and K_c are $[H^+]$ dependent, k_{obsd} exhibiting contributions from both $[H^+]$ dependent and independent terms; $k = k_1 + k_2[H^+]$ where k_1 and k_2 were found to have values of 17.5 ± 3.2 s^{-1} and 109 ± 5 M^{-1} s^{-1}, respectively. K_c varies in accordance with the expression $K_c = K[H^+]^{-2}$, leading to a value of 326 ± 38 M for K.

Introduction

Chlorpromazine [2-chloro-*N*-(3-dimethylaminopropyl)-phenothiazine] (I) has been the subject of a large number of



Chlorpromazine (I)

studies employing a broad spectrum of oxidants which have been chosen to examine the course of electron transfer reactions involving this and other phenothiazine derivatives.[1-5] Ce(IV),[2] Br_2,[2] Fe(III),[3] and Co(III)[3] have all been shown to oxidize chlorpromazine via a one-electron pathway which produces a paramagnetic cation characterized by an intense visible absorption band (λ_{max} 523 nm) and complex ESR spectrum.[2,6] This chlorpromazine radical cation (ClP^+) is subject to disproportionation to chlorpromazine (ClP) and a two-electron oxidized product (ClP^{2+}), the latter having been shown to ultimately lead to a 5-sulfoxide derivative.[1,2,7]

Reduction of ClP^+ to ClP by Fe(II), ascorbic acid, reduced glutathione, and cysteine has been demonstrated,[2] but no definitive exploration of the mechanism of reduction of the radical cation has been carried out. This paper summarizes the results of a series of experiments designed to explore possible pathways for electron transfer from ascorbic acid to chlorpromazine radical cation in acidic media. The results suggest the formation of a precursor complex which subsequently undergoes electron transfer leading to chlorpromazine and dehydroascorbic acid.

Experimental Section

Chlorpromazine hydrochloride and ascorbic acid, obtained from Sigma, were used without further purification. Chlorpromazine radical cation (ClP^+) was prepared by 10.0-mA controlled current electrolysis of 3.84×10^{-4} M chlorpromazine in 0.80 M HCl, 0.20 M KBr (for determination of molar absorptivity), or in the media employed in kinetics studies ($[Cl^-]$ = 1.00 M, $[Li^+] + [H^+]$ = 1.00 M, $[H^+]$ ranging from 0.25 to 1.00 M). Typical initial $[ClP^+]$ in kinetics studies was $\leq 5 \times 10^{-6}$ M, corresponding to <2% conversion of available [ClP]. Routine spectrophotometric determinations were made using Cary 14 and Perkin-Elmer 124 spectrophotometers. Molar absorptivity of ClP^+ at μ = 1.00 M, determined by least-squares analysis of absorbance at 523 nm vs. electrolysis time, was found to be 9560 ± 410 M^{-1} cm^{-1}.[2]

Reaction stoichiometry was determined by monitoring the decrease in absorbance at 523 nm following addition of less than equivalent amounts of ascorbic acid to solutions containing ClP^+. The stoichiometric coefficient, expressed as $\Delta[ClP^+]/\Delta$[ascorbic acid], was found to be 2.13 ± 0.09.

Kinetics studies were carried out in a stopped-flow spectrophotometer consisting of a two-jet Lucite mixer with quartz windows and 2.5-cm light path, Beckman DU monochromator, 1P28 photomultiplier, Biomation 610B transient recorder, Fluidyne 722 digital interface, and Wang 2200S 8K × 8 microcomputer equipped with Wang 2250 parallel interface. Nominal mixing time for this spectrophotometer is 5 ms. Values of k_{obsd}, the pseudo-first-order rate constants for reduction of ClP^+ by ascorbic acid, were obtained by least-squares analysis of absorbance vs. time data in accordance with the function $-\ln[(A_t - A_\infty)/(A_0 - A_\infty)] = k_{obsd}t$ immediately after acquisition of data. A_t, A_0, and A_∞ represent absorbance at time = t, initial absorbance, and absorbance at effective infinite time, respectively. Of the 255 values of absorbance vs. time obtained in the course of an individual experiment, the last 15 were used to compute A_∞. Weights for each data point at time t were proportional to the excess absorbance $(A_t - A_\infty)$ at each respective point. Many of the kinetics runs were conducted in the presence of air, which was shown to have no detectable effect on the rate constants. All runs were carried out at least five times.

Results and Discussion

When acidic solutions of chlorpromazine radical cation (ClP^+) and ascorbic acid are mixed, the intense pink color of ClP^+ disappears rapidly leaving a colorless solution of chlorpromazine[2] and dehydroascorbic acid. The disappearance of color is too rapid to study by conventional spectrophotometric methods, requiring the use of stopped-flow techniques. All kinetics studies were carried out under conditions in which [ascorbic acid] $\geq 10 \times [ClP^+]$. The pseudo-first-order rate constants for reduction of ClP^+ by ascorbic acid are presented in Table I. Each kinetics experiment was observed to exhibit a first-order dependence on $[ClP^+]$. However, the data do not fit a second-order rate law, exhibiting saturation of the pseudo-first-order rate constants as [ascorbic acid] increases. For example, at $[H^+]$ = 0.50 M, k_{obsd} is seen to increase by a factor of only 9 for a 30-fold increase in reductant concentration, Figure 1. In addition, plots of $1/k_{obsd}$ vs. 1/[ascorbic acid] were found to be linear at each $[H^+]$ employed, indicating that the rate law for electron transfer can be expressed as $k_{obsd} = kK_c[H_2A]/(1 + K_c[H_2A])$, where k represents a first-order rate constant for disappearance of an intermediate complex described by the apparent equilibrium quotient K_c and $[H_2A]$ represents total concentration of ascorbic acid.[8-10]

The data in Table I have been analyzed by a nonlinear least-squares program which minimizes the sum of the squared deviations of the parameters a and b for the function $y = abx/(1 + bx)$ where a, b, x, and y represent k, K_c, [ascorbic

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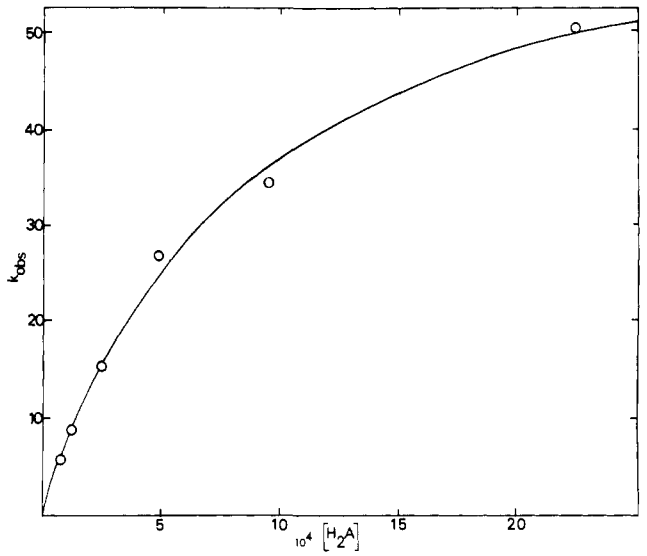

Figure 1. Plot of k_{obsd} vs. [ascorbic acid] for reduction of ClP^+. $[H^+]$ = 0.50, 25.0 °C, μ = 1.00. Least-squares line.

Table I. Rate Constants for the Reduction of Chlorpromazine Radical Cation by Ascorbic Acid, 25 °C, μ = 1.00 (Cl^-)

$[H^+]$, M	$10^4[H_2A]$, M	k_{obsd}, s^{-1} [a]	k_{calcd}, s^{-1}
1.00	0.748	3.14	3.15
	2.48	9.12	9.85
	4.90	17.0	18.1
	9.62	32.1	31.3
	22.7	54.8	55.4
0.75	0.740	3.72	3.81
	1.23	6.23	6.19
	4.85	20.7	20.7
	22.5	55.0	55.0
0.50	0.740	5.54	5.52
	1.23	8.74	8.73
	2.45	15.4	15.4
	4.85	26.7	25.1
	9.51	34.5	36.5
	22.5	50.8	50.1
0.25	0.247	5.30	5.99
	0.740	14.0	14.3
	1.23	20.4	19.7
	4.90	34.5	34.6

[a] Each entry represents the average of at least five individual determinations.

acid], and k_{obsd}, respectively. Adequacy of fit is demonstrated by the excellent agreement between k_{obsd} and k_{calcd} (Table I). Values of k and K_c, evaluated at each $[H^+]$, are presented in Table II.

A plausible mechanism for the reduction of chlorpromazine radical cation by ascorbic acid involves rapid complexation between reactants, followed by intramolecular transfer of one electron, generating chlorpromazine and ascorbate radical.[11]

$$H_2A + ClP^+ \rightleftharpoons \text{complex}$$

$$\text{complex} \rightarrow A^{-}\cdot + ClP$$

Formulation of a mechanism which is consistent with the rate law must not only explain the saturation of the observed rate constants at high [ascorbic acid], but must also take into account the $[H^+]$ dependence of both the rate constant and equilibrium constant for precursor formation. The values of k and K_c (Table II) are both found to vary with changing $[H^+]$. The apparent equilibrium constant increases with decreasing $[H^+]$, the value increasing ca. 17-fold for a 4-fold decrease in $[H^+]$. Indeed, a plot of log K_c vs. log $[H^+]$ is linear, with slope = -2.1 ± 0.1, indicating that the observed precursor equilibrium constant actually exhibits an inverse dependence on $[H^+]^2$. Thus, the values of the apparent equilibrium constants K_c measured at each $[H^+]$ are *conditional* constants, reflecting an equilibrium which can be represented as

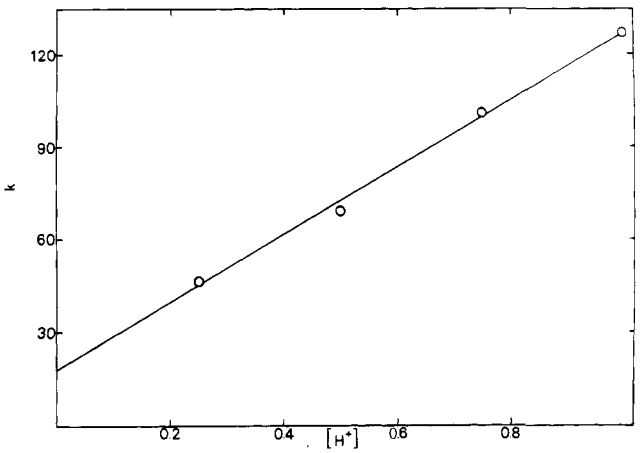

Figure 2. Plot of k vs. $[H^+]$ for the ascorbic acid reduction of ClP^+. 25.0 °C, μ = 1.00.

Table II. Kinetics Parameters for Electron Transfer between Ascorbic Acid and Chlorpromazine Radical Cation, 25 °C, μ = 1.00 (Cl^-)

$[H^+]$, M	k, s^{-1} [a]	K_c, M^{-1}	K, M [b]
1.00	127 ± 10	339 ± 42	339
0.75	101 ± 1.5	530 ± 5	298
0.50	69.1 ± 3.5	1174 ± 130	293
0.25	46.4 ± 1.8	6000 ± 550	375
		K = 326 ± 38	

[a] Errors are 1σ. [b] K is defined as $K_c[H^+]^2$.

$$ClP^+ + H_2A \rightleftharpoons \text{complex} + 2H^+$$

for which $K = [\text{complex}][H^+]^2/[ClP^+][H_2A]$ and $K_c = [\text{complex}]/[ClP^+][H_2A] = K/[H^+]^2$. Values of K, calculated as $K_c[H^+]^2$, are also presented in Table II. The average value of K so obtained is 326 ± 38 M.

The first-order rate constants for electron transfer are observed to increase with increasing $[H^+]$. A plot of k vs. $[H^+]$ is found to be linear, Figure 2, with intercept and slope of 17.5 ± 3.2 s^{-1} and 109 ± 5 M^{-1} s^{-1}, respectively. Thus k is actually a composite rate constant reflecting contributions from parallel reaction pathways ($k = k_1 + k_2[H^+]$), one of which (k_1) corresponds to a $[H^+]$ independent route for electron transfer, and the other (k_2) reflecting a $[H^+]$ dependent pathway to products.

The most plausible reaction scheme which is consistent with both rate law and stoichiometry includes[12,13] the initial complexation step

$$ClP^+ + H_2A \rightleftharpoons ClPA^- + 2H^+ \text{ (rapid)}$$

followed by parallel rate-limiting electron transfer events

$$ClPA^- \xrightarrow{k_1} ClP + A^{-}\cdot$$

$$H^+ + ClPA^- \xrightarrow{k_2} ClP + A^{-}\cdot + H^+$$

and the ultimate disappearance of $A^{-}\cdot$ via either oxidation by ClP^+

$$A^{-}\cdot + ClP^+ \rightarrow ClP + A$$

or by disproportionation,[11]

$$2\,H^+ + 2\,A^{-}\cdot \rightarrow H_2A + A$$

$A^{-}\cdot$ and A representing ascorbate radical and dehydroascorbic acid, respectively. Discrimination between the proposed fates of $A^{-}\cdot$ cannot be made with the available data.

There are alternate reaction schemes which are consistent with the rate law and stoichiometry. For example, formation of a nonreactive ascorbic acid dimer at high $[H_2A]$ would lead to saturation of the observed rate constant. A change in the rate-determining step from bimolecular complex formation to unimolecular product formation will also exhibit saturation, as will the bimolecular reaction of uncomplexed reactants in the presence of a nonreactive complex. Since the $ClPA^-$ complex is expected to be highly unstable with respect to intramolecular electron transfer, and in light of previous demonstrations of complex formation between both ClP and ClP^+ and various electron donors (see below), the reaction sequence expressed above appears to most accurately reflect the course of the reaction.

With the available data, it is impossible to determine either the exact site of protonation in the k_2 electron transfer path or the source of the hydrogen ions lost in the precursor equilibrium. In the strongly acidic media employed in this study, the principal form of ascorbic acid is the neutral H_2A form. Since chlorpromazine itself is protonated at the N terminus in such media, it is to be expected that ClP^+ is protonated as well. Loss of one hydrogen ion each from H_2A and ClP^+, or loss of two hydrogen ions from H_2A upon complex formation, would account for the $[H^+]^{-2}$ dependence of K_c.

Chlorpromazine has been shown to undergo charge transfer interactions with several neural transmitter molecules. Thus, chlorpromazine hydrochloride forms 1:2 and 1:1 charge transfer complexes with acetylcholine in aqueous media[14] while the free base exhibits a 1:1 interaction with acetylcholine in dimethyl sulfoxide.[14] Furthermore, conductometric evidence indicating the existence of a 1:2 interaction between ClP^+ and acetylcholine has also been observed. In addition, both serotonin and 6-hydroxydopamine have been reported to engage in charge transfer interactions with chlorpromazine, and the stoichiometric relationships have been determined. We assume that a similar charge transfer interaction is operative in the present system.

Unfortunately, the aforementioned studies have not been carried out under conditions which allow quantitative determination of either the equilibrium constants for binding or the rate parameters for electron transfer in such systems, and only loose comparisons can be drawn to the present study. In spite of the widespread use of ClP in the treatment of schizophrenia, there is a surprising paucity of information regarding the kinetics and mechanism of electron transfer reactions involving phenothiazine derivatives and relatively mild, multielectron donors such as epinephrine, *l*-Dopa, catechol, and ascorbic acid. Such experiments, which are expected to shed light on the mechanism of psychopharmacological activity of these phenothiazine derivatives, are in progress in this laboratory.

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References and Notes

(1) T. Iwaoka and M. Kondo, *Bull Chem. Soc. Jpn.*, **47**, 980 (1974).
(2) D. C. Borg and G. C. Cotzias, *Proc. Natl. Acad. Sci. U.S.A.*, **48**, 623 (1962).
(3) D. C. Borg and G. C. Cotzias, *Proc. Natl. Acad. Sci. U.S.A.*, **48**, 617 (1962).
(4) A. Szent-Györgyi, "Introduction to a Submolecular Biology", Academic Press, New York, N.Y., 1960.
(5) R. M. Julien, "A Primer of Drug Action", W. H. Freeman, San Francisco, Calif., 1975.
(6) L. H. Piette and I. S. Forrest, *Biochim. Biophys. Acta*, **57**, 419 (1962).
(7) J. P. Billon, *Bull. Soc. Chim. Fr.*, 1923 (1961).
(8) W. G. Movius and R. Linck, *J. Am. Chem. Soc.*, **92**, 2677 (1970).
(9) A. W. Adamson and E. Gonick, *Inorg. Chem.*, **2**, 129 (1963).
(10) K. Kustin and D. L. Toppen, *Inorg. Chem.*, **12**, 1404 (1973).
(11) B. H. J. Bielski, H. W. Richter, and P. C. Chaw, *Ann. N.Y. Acad. Sci.*, **258**, 231 (1975).
(12) An alternate mechanism which satisfies the stoichiometry involves reaction between ClP^{2+} and H_2A.

$$2ClP^+ \rightleftharpoons ClP + ClP^{2+} \qquad K$$

$$H_2A + ClP^{2+} \rightleftharpoons ClP + A + 2H^+ \qquad k$$

Although such a mechanism could show saturation at high $[H_2A]$, the observed rate expression should exhibit pseudo-second-order kinetic behavior corresponding to the second-order formation of ClP^{2+} from ClP^+ via disproportionation. Within the limits of our ability to determine the reaction order with respect to disappearance of ClP^+ (the 6-bit analog to digital converter in the transient recorder is limiting factor) the reaction appears to exhibit pseudo-first-order kinetics, and this scheme does not seem to apply. The absence of a [ClP] dependence is also in accord with this premise.
(13) F. L. Harris and D. L. Toppen, *Inorg. Chem.*, **17**, 74 (1978).
(14) F. Gutmann, L. C. Smith, and M. A. Slifkin, *Adv. Biochem. Psychopharmacol.*, **9**, 15 (1974).