Inorganica Chimica Acta, *91* (1984) 39–42

Reaction of Vanadate Ion with Chlorpromazine, Formation of Vanadyl Ion and Chlorpromazine Free Radical

HIROMU SAKURAI*, TETSUKO GIDA, SHIGERU SHIMOMURA

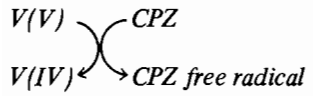
Faculty of Pharmaceutical Sciences, University of Tokushima, Sho-machi 1, Tokushima 770, Japan

and KAZUHIKO ISHIZU

Faculty of Sciences, Ehime University, Matsuyama, Ehime 780, Japan

Received June 10, 1983

The red colored product, which was identified as a chlorpromazine (CPZ) free radical, was observed in the reaction of CPZ with the vanadate ion (+5 oxidation state). The product and the mechanism for the reaction were characterized from optical and EPR spectrometries. Optimal conditions for generation of the free radical were determined as reaction time within one minute at pH 6 and free radical stabilizing time of 30 minutes by acidifying with HCl. Under these conditions, the stoichiometry for the reaction was found to be 1:1, indicating the involvement of one electron transfer from CPZ to the vanadate ion to form the free radical and vanadyl ion (+4 oxidation state). A possible reaction scheme was proposed:



The implications of this reaction were discussed with regard to the pharmacological action of the vanadate ion and CPZ.

Introduction

It is known that the vanadium ion is an essential trace element for mammalians. It is found to be present in many tissues and the concentrations in human blood and plasma are reported to be in the range of 0.005–8.4 μM (M = mol dm^{-1}) [1]. Since the discovery by Cantley *et al.* that the vanadate ion (+5 oxidation state) is a strong inhibitor of Na^+, K^+–ATPase and that the vanadyl ion (+4 oxidation state) is less inhibitive of the enzyme [2], considerable interest in the biological function of the vanadium ion has been shown [3]. Clinical studies showed that the vanadate ion may be involved in the etiology of manic-depressive illness [4], against which phenothiazine and imipramine derivatives have a therapeutic effect. On the contrary, it has been reported that the free radical form of CPZ (chlorpromazine) may be involved in its action on microsomal Na^+, K^+-ATPase from rat brain [5].

In view of the importance of these physiological functions of vanadium ion and CPZ, both of which may be associated with the enzyme activity of Na^+, K^+–ATPase and thus the therapeutic effect on manic-depressive illness, we have attempted to obtain information at a molecular level about the interaction of the vanadate ion with therapeutic drugs. We found that the vanadate ion was able to oxidize CPZ at physiological pH, detecting both CPZ free radical and reduced vanadyl ion by optical and EPR spectrometries. Herein we report the redox reaction between CPZ and vanadate ion, and propose a possible reaction mechanism.

Experimental

Chlorpromazine hydrochloride (CPZ) was a special gift from Shionogi Pharmaceuticals KK. Other chemicals used were of reagent grade. Optical absorption spectra were recorded with a Hitachi 330 spectrometer. EPR spectra were measured with a JEOL FE2XG X-band spectrometer with 100 KHz magnetic field modulation at room temperature and liquid nitrogen temperature (77 K). The magnetic field was calibrated by the splitting of Mn(II) in MgO

*Author to whom correspondence should be addressed.

0020-1693/84/$3.00

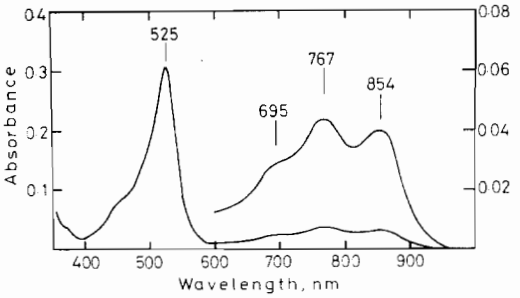


Fig. 1. Absorption spectrum of the red colored product in the reaction between CPZ and vanadate ion. Reaction mixture contained 0.25 ml of 1 m*M* CPZ, 0.25 ml of 1 m*M* $NaVO_3$ and 2.0 ml of 0.1 *M* sodium phosphate buffer, pH 7.5. Reaction time was one minute. The mixture was acidified with 0.3 ml of 4 *N* HCl, and the spectrum was recorded after 30 minutes.

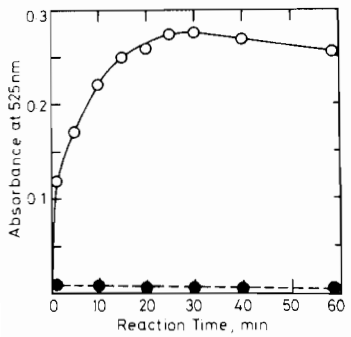


Fig. 2. Time-dependent formation of the red colored product. Reaction mixture contained 0.25 ml of 1 m*M* CPZ, 0.25 ml of 1 m*M* $NaVO_3$ and 2.0 ml of 0.1 *M* sodium phosphate buffer, pH 7.5, was acidified with 0.3 ml of 4 *N* HCl after a reaction time of one minute. The absorption at 525 nm was measured (–○–). The identical process was performed in the absence of $NaVO_3$ (--●--).

(ΔH_{34} = 86.9 G) and Fremy's salt [6], and g-values were standardized using Li–TCNQ (g = 2.0025) as a reference. All reactions were performed in 0.1 *M* sodium phosphate buffer, pH 5.5–8.0. 0.25 ml of 1 m*M* CPZ was added to the reaction mixture containing 0.25 ml of 1 m*M* $NaVO_3$ and 2.0 ml of 0.1 *M* sodium phosphate buffer, and after the reaction time an aliquot of the solution was acidified by the addition of 0.3 ml of 4 *N* HCl before application to the spectrometer.

Results

When HCl was added aerobically to the solution which contained $NaVO_3$ and CPZ in 0.1 *M* sodium phosphate buffer of pH 7.5, a red color developed having four absorption maxima at 525, 695^{sh}, 767 and 854 nm (Fig. 1). A similar spectrum was obtained by addition of H_2SO_4 or HNO_3 in place of HCl. No absorption spectrum due to the colored product was obtained at neutral pH regions. These characteristic spectra were identical to those of a free radical ion of CPZ, which is formed from CPZ during oxidation by trivalent cations such as Fe(III), Co(III) and Mn(III) [7]. The intensity of the red colored product due to CPZ free radical which was acidified by HCl and HNO_3 was higher than that of the product by H_2SO_4, indicating that the radical was stabilized in the presence of HCl and HNO_3. Although CPZ radical was formed in the absence of $NaVO_3$, the intensity of the red product was extremely low (Fig. 2).

The optimal conditions for the formation of CPZ free radical and the stoichiometry of the reaction were studied. The reaction between CPZ and vanadate ion at pH 7.5 before acidifying was very rapid (less than one minute). However, stabilization with HCl of the CPZ free radical was relatively slow, requiring 30 minutes to reach the maximum intensity at 525 nm (Fig. 2). The reaction depended

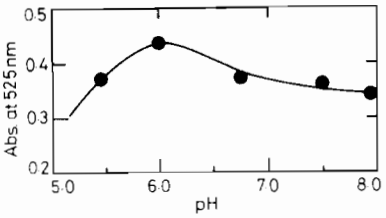


Fig. 3. pH-Dependent formation of CPZ free radical ion during CPZ-vanadate ion reaction. Reaction mixture contained 0.25 ml of 1 m*M* CPZ, 0.25 ml of 1 m*M* $NaVO_3$ and 2.0 ml of 0.1 *M* sodium phosphate buffer. Reaction time in the buffer was one minute. The mixture was acidified with 0.3 ml of 4 *N* HCl, and the absorption at 525 nm was measured after 30 minutes.

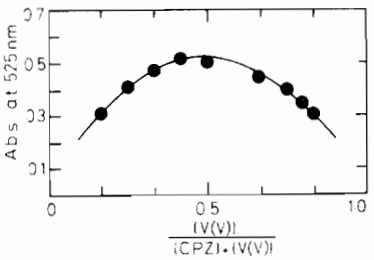
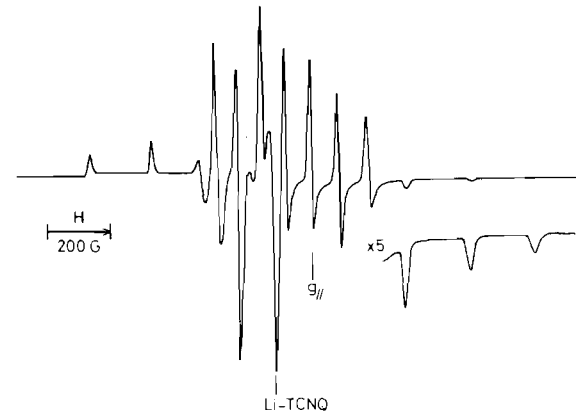
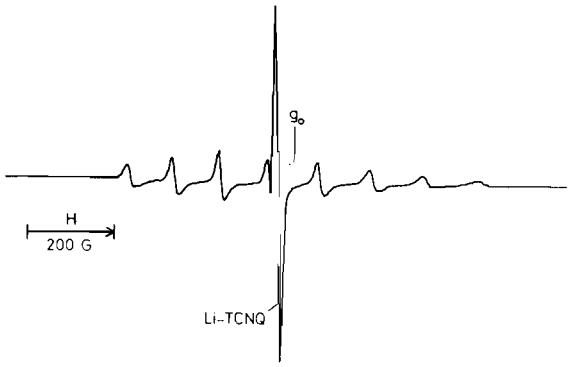


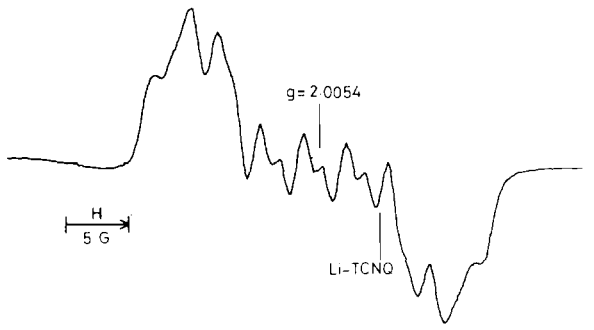
Fig. 4. Continuous variation method for determining stoichiometry in the reaction between CPZ and $NaVO_3$. Reaction mixture contained 2.4 ml of 0.1 *M* sodium phosphate buffer, pH 6.0, 2 m*M* CPZ and 2 m*M* $NaVO_3$. After reaction time of one minute, the reaction mixture was acidified with 0.3 ml of 4 *N* HCl. Total volume was 3.3 ml. Stabilizing time of CPZ free radical was 30 minutes.



(a)



(b)



(c)

Fig. 5. EPR spectra of vanadyl ion (a and b) and CPZ free radical ion (b and c). The reaction mixture contained 0.25 ml of 1 m*M* CPZ, 0.25 ml of 1 m*M* $NaVO_3$ and 2.0 ml of 0.1 *M* sodium phosphate buffer, pH 6.0. The mixture was acidified with 0.3 ml of 4 *N* HCl after a reaction time of one minute. After 30 minutes, EPR spectra were measured at 77 K (a) and 20 °C (b and c).

on the pH, the maximum value being at pH 6 (Fig. 3). The stoichiometry of the reaction was thus found to be 1:1 using the continuous variation method (Job's plot [8]) under optimal conditions (Fig. 4).

In order to study the mechanism of the reaction between CPZ and the vanadate ion, the EPR spectra for the system were measured. The EPR experiments demonstrated that a vanadyl ion was readily detected in a reaction mixture ($NaVO_3$ and CPZ in phosphate buffer of pH 7.5, reaction time of 30 minutes) treated with HCl both at room and liquid nitrogen temperature (Fig. 5, A and B). The EPR parameters (g_o = 1.984, $g_{\parallel}$ = 1.934, $g_{\perp}$ = 2.009, A_o = 115.3 Gauss, $A_{\parallel}$ = 202.5 Gauss and $A_{\perp}$ = 71.7 Gauss; $g_o = (g_{\parallel} + 2g_{\perp})/3$ and $A_o = (A_{\parallel} + 2A_{\perp})/3$), due to the vanadyl ion were found to be identical with those of an aquo vanadyl complex [9]. Furthermore, the signal due to the CPZ free radical was clearly detected in the EPR spectrum (g = 2.0054) at room temperature (Fig. 5(c)), with concomitant formation of vanadyl ion (Fig. 5(b)). The EPR spectrum of the CPZ free radical was identical with that of the reported CPZ free radical formed in the enzymatic oxidation by the peroxidase–H_2O_2 system at pH 4.8 [10].

In connection with our findings, the reduction of the vanadate ion to the vanadyl ion by CPZ has been recently reported, but the authors did not obtain the free radical derived from CPZ, and did not detect any other oxidized species by EPR spectrometry [11].

Discussion

It was demonstrated from optical and EPR spectrometries that, in the reaction between CPZ and the vanadate ion, the CPZ free radical ion and vanadyl ion were simultaneously formed. Optimal conditions were found to be: reaction time within one minute at pH 6 in 0.1 *M* phosphate buffer and stabilizing time of 30 minutes for CPZ free radical treated with HCl after the reaction at pH 6. Under these conditions, the stoichiometry for the reaction was found to be 1:1, indicating one electron redox reaction.

Direct detection of both CPZ free radical and vanadyl ion by EPR spectrometry demonstrated clearly that the reaction involved one electron transfer from CPZ to vanadate ion resulting in the formation of the oxidized CPZ free radical ion and the reduced vanadyl ion.

Based on the present results, we tentatively propose the following reaction scheme involving one electron transfer in the reaction of CPZ and vanadate ion.

VO_3^- (+5 oxidation state) + CPZ

VO^{2+} (+ 4 oxidation state) + CPZ free radical ion

The reduction of vanadate ion to vanadyl ion by CPZ may indicate a therapeutic effect of CPZ on a vanadate ion-dependent manic depressive illness [4], because the vanadate ion associates with Na^+, K^+–ATPase to inhibit enzymatic activity [2, 3] and the vanadyl ion is a non-inhibitive form [3]. However, the CPZ free radical has been reported to be a potent inhibitor of Na^+, K^+–ATPase [5]. Judging from our present findings, however, the CPZ free radical ion, formed in the reaction with the vanadate ion, is unstable at physiological pH ranges. Therefore, it may be suggested that the CPZ free radical is less inhibitive of Na^+, K^+–ATPase than the vanadate ion.

Thus, the present reaction of the vanadate ion with CPZ shown here may be useful for an explanation not only of a physiological role of the vanadate ion but also of a possible therapeutic action of CPZ. Further work in this area is in progress.

Acknowledgements

The authors thank Shionogi Pharmaceuticals KK. for the generous gift of chlorpromazine hydrochloride, Mrs K. Tajima and K. Okada for skilful assistance in measuring EPR spectra and Dr. M. Nishida for helpful discusssions during the course of this research.

References

1 J. Versieck and R. Cornelis, *Analyt. Chim. Acta, 116,* 217 (1980).
2 L. C. Cantley, Jr., L. Josephson, R. Warner, M. Yanagisawa, C. Lechene and G. Guidotti, *J. Biol. Chem., 252,* 7421 (1977).
3 I. G. Macara, *Trends in Biochem. Sci., 4,* 92 (1980).
4 G. J. Naylor and A. H. W. Smith, *Psycholog. Med., 11,* 249 (1981).
5 T. Akera and T. M. Brody, *Mol. Pharmacol., 4,* 600 (1968).
6 D. J. Cram and R. A. Reeves, *J. Am. Chem. Soc., 80,* 3094 (1958).
7 D. C. Borg and G. C. Cotzias, *Proc. Natl. Acad. Sci., 48,* 623 (1962).
8 P. Job, *Ann. Chim., 9,* 113 (1928).
9 H. Sakurai, S. Shimomura, K. Fukuzawa and K. Ishizu, *Biochem. Biophys. Res. Commun., 96,* 293 (1980).
10 L. H. Piett, G. Bulow and I. Yamazaki, *Biochem. Biophys. Acta, 88,* 120 (1964).
11 F. Vyskocil, J. Pilar, H. Zemkova and J. Teisinger, *Lancet,* 1078 (1982).