Formation of Imipramine Free Radical and Vanadyl Ion in the Reaction of Imipramine and Pentavalent Vanadium Ion

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Received November 20, 1982

Vanadium is known to be present in many tissues [1, 2] probably as a vanadyl form (+4 oxidation form) [3, 4]. Nevertheless, much interest in vanadium chemistry has currently been focused on its inhibitory effect of a vanadate ion (+5 oxidation form) to various types of enzymes [5, 6] owing to its having a stronger effect than the vanadyl form. Especially, the discovery by Josephson and Cantley [2] that vanadate is a potent inhibitor of sodium and potassium transport ($Na^+ + K^+$)–ATPase has aroused further interest in the biological function of the vanadium ion. Clinical observation of the vanadium ion has shown that the vanadate ion may be involved in the aetiology of manic-depressive illness [7], in which imipramine and phenothiazine derivatives have a therapeutic effect. Changes in the membraneous transport of sodium have been obtained by these drugs [8].

Thus, in view of the importance of these physiological functions of the vanadium ion, we have attempted to obtain information at a molecular level about the interaction of the vanadium ion with the therapeutic drugs. We found that vanadate was able to oxidize imipramine at physiological pH and detected both imipramine free radical and reduced vanadyl ion by optical and ESR spectrometries. This paper reports the redox reaction between imipramine and the vanadate ion which was characterized by the radical formation and the presence of the vanadyl ion, and proposes a possible reaction mechanism.

Vanadate (1 m*M*) which has no absorption in the visible region spectrum, exhibited an absorption maximum at 815 nm in the presence of imipramine (1 m*M*) at pH 7.5 of 0.1 *M* soidum phosphate buffer after a reaction time of 20 min under air. Thus the characteristic absorption spectrum indicated that the vanadate ion was reduced to the vanadyl ion with imipramine. Similar results were obtained in collidine-HCl, lutidine-HCl, borate and Tris-HCl buffers of pH 7.5. When HCl was added at 0.43 *M* to the solution, a blue color with an absorption maximum at 650 nm which was not due to the vanadyl ion (λ_{max} = 779 nm in HCl solution), developed and changed gradually to green. The reaction ratio of vanadate ion to imipramine was found to be 1:1. The blue product, identified as imipramine free radical by Borg [9], disappeared in the presence of reducing agents such as ascorbate and reduced glutathione. From these observations, it may be deduced that in an equimolar reaction between imipramine and vanadate, the imipramine radical and vanadyl ion are formed through a direct one-electron redox reaction. The time-dependent redox reaction in Fig. 1 showed clearly that the imipramine radical and vanadyl ion were produced at almost similar rates.

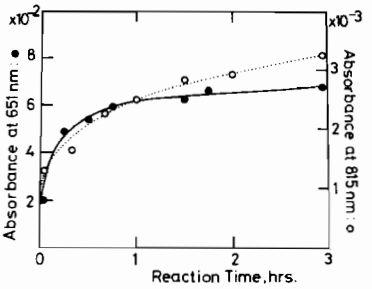

Fig. 1. Time-dependent formation of imipramine free radical and vanadyl ion in the reaction of imipramine and vanadate ion at pH 7.5. The absorption at 815 nm of a mixture containing imipramine·HCl (1 m*M*) and $NaVO_3$ (1 m*M*) at pH 7.5 of 0.1 *M* sodium phosphate buffer was measured (o-----o). To the aliquot (2.5 ml) of the reaction mixture, a 4 *N* HCl solution, 300 μl, was added and measured at 651 nm immediately (●——●).

The ESR experiments demonstrated that a vanadyl ion was detected in a reaction mixture acidified with HCl at both 77 K and 20 °C after a reaction time of 20 min (Fig. 2). The ESR parameters (g_o = 1.973, $g_\parallel$ = 1.928, $g_\perp$ = 1.996, A_o = 116 gauss, $A_\parallel$ = 203 gauss and $A_\perp$ = 73 gauss) due to the vanadyl ion were found to be identical with an aquo vanadyl ion by comparison with many other types of vanadyl complexes [2, 10–12]. The ESR spectrum of the radical (Fig. 2(C)) was a singlet with a peak-to-trough width of about 7 gauss and showed a g-value of 2.0024, which was identical with the reported value (g = 2.0026) of imipramine free radical formed with imipramine and Ce^{4+} in 0.2 *N* H_2SO_4 by Borg

*Author to whom correspondence should be addressed. Abbreviations: ESR, electron spin resonance; Tris, tris-(hydroxymethyl)aminomethane.

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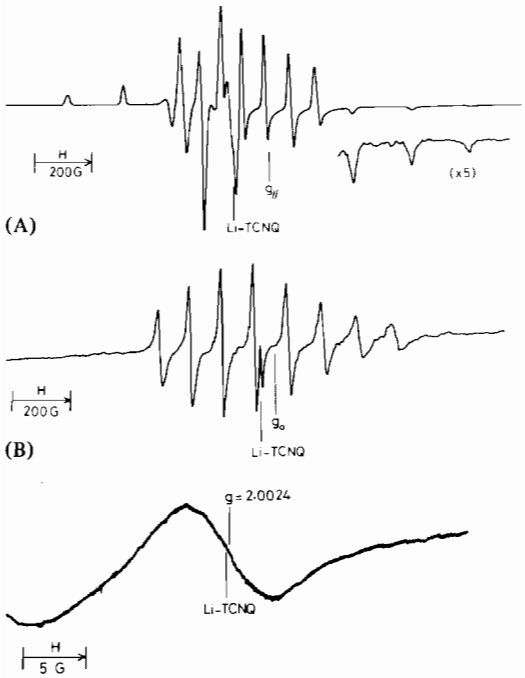
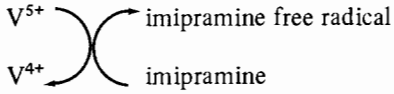


Fig. 2. ESR spectra of vanadyl ion (A and B) and imipramine free radical (B and C) in the reaction between imipramine and vanadate ion at pH 7.5. A mixture containing imipramine·HCl (1 m*M*) and $NaVO_3$ (1 m*M*) was reacted in 0.1 *M* sodium phosphate buffer at pH 7.5 and acidified with HCl (0.43 *M*) after a reaction time of 20 min. The ESR spectra were measured with 100 KHz field modulation at 77 K (A) and 20 °C (B and C). As standard, TCNQ–Li salt, MgO powder doped with Mn^{2+} salt and Fremy's salt were used.

[9]. Interestingly, the imipramine free radical was observed by ESR spectrometry in the reaction between imipramine and the vanadyl ion under the same conditions, because the vanadyl ion is readily auto-oxidized to the vanadate ion at neutral pH regions [13] and thus the vanadate ion reacts with imipramine to form a small amount of the radical. Therefore, the radical was not detected when the reaction was tested under unaerobic conditions. In connection with our findings, reduction of the vanadate ion to the vanadyl ion by imipramine was recently reported [14], however the authors did not detect an imipramine radical.

Based on these findings, we tentatively propose the following reaction scheme involving one-electron transfer from imipramine to the vanadyl ion:



The reaction of the vanadate ion and imipramine postulated here may provide an explanation not only of the physiological role of the vanadate ion but also of a possible therapeutic action of imipramine.

Acknowledgements

The authors thank Taito-Faiser KK. for the generous gift of imipramine hydrochloride and Dr. M. Nishida for helpful discussions during the course of this research.

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