SYNTHESIS, SPECTRAL CHARACTERIZATION AND ELECTROCHEMISTRY OF VANADIUM(V) COMPLEX WITH TRYPTOPHAN

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ABSTRACT

A new oxovanadium(V) complex of NH_4VO_3 and tryptophan (TrpH) has been synthesized in aqueous solution at pH 6.0 and characterized by elemental analysis, UV-Vis, FT-IR, ^1H -NMR and mass spectroscopic data. The complex ($\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$) was diamagnetic in nature as was evident from the electron spin resonance spectroscopy (ESR) and the magnetic susceptibility measurements, in conformity with the presence of vanadium(V) in the structure. The electrochemical behaviour of $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex was also studied on the hanging mercury drop electrode (HMDE) by using cyclic voltammetry (CV). The cyclic voltammograms of $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex exhibit two new reduction waves at -0.38 V and -1.01 V in Britton–Robinson buffer (pH 6.0) for the potential range from 0.0 V to -1.2 V.

Key words: Vanadium complexes, Tryptophan, Spectroscopy, Voltammetry.

INTRODUCTION

Tryptophan (TrpH, Scheme 1) is well known as one kind of essential amino acids in human and herbivores bodies, and the precursors of catecholamine synthesis such as dopamine and serotonin^{1,2}. The brain serotonin availability depends upon blood TrpH levels³, which could modulate the psychoneural control of spontaneous alternation through presynaptic inhibition of hippocampal cholinergic terminals. Therefore, TrpH is a vital constituent of proteins and indispensable in human nutrition for establishing and maintaining a positive nitrogen balance^{2,4}.

Vanadium concentrations correlated with the loss of TrpH residues in albumin⁵. Vanadium is an element which plays a variety of biochemical, pharmacological and physicochemical functions^{6,7}. The interaction of vanadium with amino acids and proteins is a subject of current interest. In living systems, vanadium is an ultratrace element and is found both anionic and cationic forms, the most common ones being vanadate H_2VO_4^- and oxovanadium(IV) (VO_2^{+})^{8,9}. It has been observed that simple inorganic vanadium compounds are more toxic than vanadium compounds with organic ligands and the efficacy of the metal based therapeutic agents changes drastically by making small changes in the organic ligands attached to the metal center¹⁰.

Vanadium complexes, particularly, vanadates and peroxovanadium compounds, have been implicated in many biological processes and therapeutic applications, as insulin-mimetics and antitumor agents¹¹. The most important physiological effect of vanadium is the stimulation of glucose uptake and glucose metabolism¹².

A variety of vanadium complexes have been introduced as structural and / or functional models for biologically active vanadium compounds¹³. Schiff base complexes with an amino acid as the amine constituent constitute a specific subfamily within this group. From the medicinal point of view, it is desirable to design compounds which are water-soluble in order to facilitate uptake¹⁴. In recent years, much interest has been focussed on oxo- and oxoperoxo complexes of vanadium with amino acid derivatives¹⁵. The complexes of vanadium are very interesting as model compounds to clarify several biochemical processes¹⁶⁻¹⁸. The interaction of vanadium with amino acids, peptides and proteins is a subject of current interest¹⁹. There are few studies on the interaction between V(V) / V(IV) and amino acids²⁰⁻²². Extensive investigations on the interaction between vanadium and biologically relevant ligands such as glycine, L-histidine and its N-carboxymethyl derivative, alkoxides, or the dipeptides glycylglycine and glycyltyrosine both in solution and in the solid state have been made¹⁵. Although the complexes of V(III) with TrpH, valin, phenylalanine and proline in the solid phase have been isolated from solution in nonaqueous solvents²³, no data are available on the complexes of vanadium(V) with TrpH in aqueous medium. In this paper, we report the synthesis, spectral characterization and electrochemistry of $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex. Electrochemical study on the interaction of vanadium(V) with TrpH is very important for the understanding of redox chemistry of vanadium and its drugs.

Scheme 1. The molecular structure of TrpH.

EXPERIMENTAL

Reagents

Tryptophan (TrpH) and NH_4VO_3 were purchased from Merck and applied without further purification. In the voltammetric experiments, Britton Robinson (B-R) buffer (pH 6) was used as supporting electrolyte. All solution were prepared daily in ultrapure triply distilled water.

Synthesis

The complex was prepared by adding a hot H_2O solution of NH_4VO_3 (0.5×10^{-3} M) to a NaOH solution which is including the TrpH (1.0×10^{-3} M) and then pH of mixture was adjusted to 6 with dilute HCl and the light yellow-orange coloured solution was kept at room temperature (25°C). After a few weeks, brown precipitated was filtered off from the solution and washed with ethanol and dried in air. Elemental analyses for C, H, N and S were performed using a LECO CHNS 932 -Rapid analyzer at TÜBİTAK Laboratories of Ankara Research Center. Anal. Calc. for $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ (592.01 g/mol) %C, 22.30; %H, 1.86; %N, 4.73. Found: %C, 22.68; %H, 1.71; %N, 4.81. According to the magnetic susceptibility measurements, the compound has diamagnetic nature. This result provides confirmatory evident for the presence of vanadium(V) in the structure.

Spectroscopy

The FT-IR spectra in the $4000\text{--}400\text{ cm}^{-1}$ regional were recorded from KBr pellets with a Jasco FT-IR 350 spectrometer at a resolution at 4 cm^{-1} based on averaging 32 sample and 16 background scans. ESR spectrum was collected using a Varian EC 109 spectrophotometre, the field being calibrated with diphenylpicrylhydrazyl (DPPH). The electronic absorption spectra in the $400\text{--}200\text{ nm}$ range were recorded on Unicam V2-100 UV/Vis spectrophotometer using 1 cm quartz cells. ^1H NMR spectra was measured in D_2O solutions using tetramethylsilane (TMS) as internal standard and recorded on a BRUKER AVANCE DPX-400 spectrometer. LCMS-ESI analysis was obtained on the AGILENT 1100 MSD spectrometer at TÜBİTAK Laboratories of Ankara Research Center.

Voltammetric measurements

The voltammetric measurements were carried out using a EG&G PAR Model 394B polarographic analyzer connected to an EG&G PARC Model 303A polarographic stand (Princeton, NJ, USA). A hanging mercury drop electrode (HMDE; as working electrode), an Ag/AgCl/KCl sat. reference electrode and a Pt wire (as counter electrode) were used. The voltammetric measurements were carried out in B-R buffer as supporting electrolyte. Prior to each experiment, a voltammogram of the solution containing only supporting electrolyte was measured. Solutions of NH_4VO_3 and TrpH and the $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex in water were separately added to the cell containing the supporting electrolyte and their voltammograms were recorded. Then, the additions of TrpH to the cell containing NH_4VO_3 were carried out and the voltammograms were recorded. Solutions were deaerated for about 8 min with pure nitrogen gas before starting the electrochemical experiments. Each measurement was performed with a fresh mercury drop at room temperature.

RESULTS AND DISCUSSION

Spectral characterization of the complex

FT – IR spectra

The infrared spectrum of the $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex has shown changes in the position and profiles of some bands as compared to those of the free TrpH (Fig. 1), suggesting participation of the groups that produce these bands in the coordination with vanadium atoms. Major changes are related to the carboxylate and amine bands. Also, new bands should also appear in the 300–497 cm^{-1} region in the spectra of vanadium complexes. These bands can be assigned to $\nu(\text{V}-\text{O})$ and $\nu(\text{V}-\text{N})$ coupled²⁴. The infrared spectrum of the $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex exhibits $\nu(\text{V}-\text{O})$ and $\nu(\text{V}-\text{N})$ stretching bands at 495 cm^{-1} and 420 cm^{-1} , respectively (Fig. 1). These bands can be attributed to binding *via* the carboxylate and amino nitrogen of Trp^- in the complex. The presence of aromatic groups in the complex is supported by the appearance of bands in the 2900–3150 cm^{-1} region. Also, the infrared spectrum of complex exhibits a sharp band at 3415 cm^{-1} , due to indole $\nu(\text{NH})$ stretching vibrations of Trp^- ion²⁵. The free TrpH shows two bands in the 1610–1660 cm^{-1} and 1395–1430 cm^{-1} regions, corresponding to the asymmetric and symmetric $\nu(\text{COO}^-)$ stretching vibrations, respectively²⁶. These bands are shifted to higher wavenumbers (1615–1678 cm^{-1}) and to lower wavenumbers (1356–1410 cm^{-1}), respectively, after complexation with vanadium atoms, thus indicating coordination through that group. The FT-IR bands at 945, 860, 835, 660 and 530 cm^{-1} are characteristics of cyclic vanadates²⁷. In the addition, the terminal $\nu(\text{V}=\text{O})$ and $\nu(\text{V}-\text{O}-\text{V})$ stretching frequencies in the complex appear as strong bands at 970 and 830 cm^{-1} and as a weak band at 650 cm^{-1} , respectively²⁸. The $\nu(\text{C}-\text{H})$ out of-plane deformations, characteristic of benzene ring of tryptophan is at 750 cm^{-1} for this complex (Fig. 1).

Fig. 1. FT-IR spectra of TrpH (A) and $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex (B) in KBr disc. The arrows show the main peaks.

Esr

A freshly prepared aqueous solution of $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex gave no ESR signal, since the present complex contains vanadium atom in the +5 oxidation state.

UV-Vis spectra

The UV-Vis spectra of NH_4VO_3 , TrpH and the mixture of NH_4VO_3 with TrpH were recorded in the 200–400 nm in water (Fig. 2). The maximum absorption bands were given in Table 1. The electronic spectra of TrpH and NH_4VO_3 gave two (219, 280 nm) and one (266 nm) the maximum absorption bands, respectively (Table 1). After the addition of TrpH to NH_4VO_3 solution, some shifts in the band positions and new bands were observed (Fig. 2 and Table 1). On the other hand, in the electronic spectra of the mixture of NH_4VO_3 with TrpH, it has been shown that the band at 219 nm of TrpH shifts to 212 nm. On raising the NH_4VO_3 concentration, a distinct increase in the intensity of 212 nm band supports the assumption of binding to TrpH. The UV-vis spectrum of $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ exhibits four absorption bands. The two bands at 212 and 261 nm correspond to LMCT transition of terminal and bridging oxygens to vanadium, respectively. The band at 247 nm can be assigned to intraligand transitions, probably superimposed with the $\text{O} \rightarrow \text{V}$ charge transfer involving the double bonded oxo group^{29,30}. The solutions of many vanadate (V) species and vanadium (V) bound to oxygen donor ligands give a yellow color which is due to intense LMCT bands tailing from the UV region³¹. A broad band at 350 nm is assigned to $\pi \rightarrow \pi^*$ transitions of the carboxylate group and interaction between the vanadium *d* orbital and π system of ligand.

Fig. 2. Electronic spectra (in 200–380 nm region) of 1×10^{-3} M TrpH solution (-----) and 1×10^{-3} M $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex solution (———).

Table 1. Characteristic absorption maxima (nm) data of NH_4VO_3 , TrpH, and of $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex in aqueous solution.

NH_4VO_3 (1×10^{-3} M)	TrpH (1×10^{-3} M)	$\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ (1×10^{-3} M)
266	219	212
	280	247
		261
		350 (br)

br: broad

Mass

The structure of the complex was further corroborated using electron spray ionisation mass spectrometry (ESI-MS). ESI-MS spectrum of the complex (Fig. 3) shows peak at m/z 592.8 which can be attributed to $\{\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})] + \text{H}\}^+$. In addition, electron spray ionisation mass spectrometry (ESI-MS) measurements (Fig. 3) show, besides the parent peak at m/z 592.8, the peaks at m/z 205 and 227, corresponding to the $[\text{M}+\text{H}]^+$ and to the sodium adduct $[\text{M}+\text{Na}]^+$ of Trp, respectively³². This complex structure was proposed based on the MS data of the Trp^- ion of m/z 203. The fragment ion of m/z 188.1, formed because of the loss of water was shown in the spectrum. The peak at m/z 390.9 can be attributed to $\{\text{Na}_4[\text{V}_3\text{O}_9] + 2\text{H}\}^+$. In addition, ESI-MS spectrum (Fig.

3) shows peaks at m/z 261.4 and 199 which may be attributed to $\text{Na}_2\text{H}_2\text{V}_2\text{O}_7$ and $[\text{V}_3\text{O}_6]^{3-}$, respectively. Finally, the results provided by ESI-MS verified the formation of $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex.

Fig. 3. Mass spectrum of $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex.

Nmr

The ^1H NMR spectrum recorded from D_2O solutions of TrpH was reported by Selvakannan et al.³³. The peak at 3.4 ppm (doublet) corresponds to the methylene protons and the peak at 4 ppm corresponds to the α -protons in the TrpH molecule^{33,34}. Moreover, the multiplet peaks around 7–8 ppm represent the aromatic protons in the TrpH molecule^{33,34}. By comparison with the ^1H NMR spectrum data of pure TrpH³³, the ^1H NMR spectrum of the complex (Fig. 4) exhibits a slight up-field chemical shift of all protons. According to this observation, it can be said that the electronic environments of the TrpH protons change with the formation of the complex. At the complex, the negatively charged $\text{V}_3\text{O}_9^{3-}$ ions affect the electronic environment of the TrpH protons, therefore shifting them slightly up-field³³. The peaks at close to 3.95 ppm correspond to protons coordinated to the α -C of the primary amine (Fig. 4). This value is shifted relative to the solution value of pure TrpH (4.1 ppm)³³ and indicates that binding of the TrpH molecule to the $\text{V}_3\text{O}_9^{3-}$ ion occurs also via the primary amine groups in the amino acid.

The other important feature of the TrpH binding to $\text{V}_3\text{O}_9^{3-}$ ion is that the resonances of the aromatic protons undergo shifts to high-field (Table 2). Consequently, the resonances observed in the ^1H NMR spectrum of the complex clearly demonstrate the existence of bound Trp⁺ ligand in the complex.

Fig. 4. The ^1H NMR spectra of $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex in D_2O . A) δ 2.7–4.2 p.p.m. region, B) δ 6.3–9.0 p.p.m. region.

The voltammetric study on the interaction of NH_4VO_3 with TrpH, and electrochemistry of the complex

CV was used to compare the electrochemical behaviors of the vanadium precursor (NH_4VO_3), TrpH and the complex. The cyclic voltammogram of

TrpH solution in the absence of NH_4VO_3 produces a cathodic reduction peak ($E_p = -1.32$ V) in the Britton-Robinson buffer (pH 6.0) (Fig. 5). As can be seen in Fig. 5, the reduction process at -1.32 V is irreversible. Because, there is no anodic counterpart is not. The peak at -1.32 V could be due to a catalytic hydrogen reduction³⁶.

Table 2. The chemical shifts ($\Delta\delta$), p.p.m., of aromatic indole ring proton resonances of L-TrpH at the free and bound forms.

Proton	L-TrpH	
	(bound)	(free) ³⁵
H2	7.20	7.33
H4	7.65	7.73
H5	7.10	7.21
H6	7.15	7.29
H7	7.45	7.55

Fig. 5. The cyclic voltammogram of 1×10^{-5} M TrpH solution (in B-R buffer pH 6.0). 1U, the catalytic hydrogen wave of TrpH. Experimental conditions: scan rate, 200 mV s^{-1} ; equilibrium time, 5 s; drop size, medium.

Under the same conditions, cyclic voltammogram of NH_4VO_3 in the absence of TrpH gives a quasi-reversible peak couple with E_{pc}/E_{pa} values at -0.16 V/ -0.08 V and irreversible a reduction peak at -0.48 V (Fig. 6). Usually two reduction steps are mentioned which are assigned from free V(V) to V(IV) and V(IV) to V(III) as follows³⁷:

Fig. 6. Cyclic voltammogram of 5.5×10^{-5} M NH_4VO_3 in the absence of TrpH (at B-R buffer pH 6.0). 1U, the reduction of free V(V) to V(IV); 2U, the reduction of V(IV) to V(III). Experimental conditions are described as Fig. 5.

A dramatic change at the voltammetric behaviour of the NH_4VO_3 in the presence of TrpH is observed. With adding of TrpH into the cell containing 1×10^{-5} M NH_4VO_3 , two new cathodic reduction peaks are observed at -0.38 V

and -1.01 V (Fig. 7), while the peak currents of free NH_4VO_3 decrease (data not shown here). The potential of this new peak (-0.38 V) is different from that of free V(V). The reversible cathodic reduction peak at -0.38 V may be due to the reduction of V(V) ions complexed with tryptophanate (Trp^-) in the aqueous media. Under the same experimental conditions, similar results have been also obtained by the aqueous solution of the solid $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex. As can be seen in Fig. 8, the cyclic voltammogram of dissolved $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex shows two new peaks at -0.39 V (reversible) and -1.06 V (irreversible). As a result, the voltammetric measurements show that there is an interaction between vanadium(V) and TrpH in aqueous solution. Moreover, the redox potentials of NH_4VO_3 and TrpH mixture are agree with those of the dissolved $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex in water.

Fig. 7. Cyclic voltammogram of 1×10^{-5} M NH_4VO_3 solution in the presence (—) and absence (-----) of 1×10^{-5} M TrpH (at B-R buffer pH 6.0). 1U, the reduction of free V(V) to V(IV); 2U, the reduction of the complex formed between NH_4VO_3 and TrpH at pH 6.0; 3U, the reduction of V(IV) to V(III); 4U, the catalytic hydrogen wave of the complex. Experimental conditions are described as Fig. 5.

Fig. 8. Cyclic voltammogram of $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex dissolved in water (at B-R buffer pH 6.0). 1U, the reduction of free V(V) to V(IV); 2U, the reduction of $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex; 3U, the reduction of V(IV) to V(III); 4U, the catalytic hydrogen wave of $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex. Experimental conditions are described as Fig. 5.

CONCLUSION

$\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex was firstly prepared by the reaction between NH_4VO_3 and TrpH in aqueous solution. Structural features of this complex were obtained from its elemental analyses, magnetic susceptibility, mass, FT-IR, UV-Vis, ^1H -NMR and ESR spectral studies. The electrochemistry of the complex has been studied using cyclic voltammetry. From the ESR and the magnetic susceptibility measurements, it is evident that the complex is diamagnetic. The characteristic frequencies of cyclic vanadates are shown in

the FT-IR spectrum of $\text{Na}_4[\text{V}_3\text{O}_9(\text{Trp})]$ complex. Also, the complex displays two new peaks at -0.39 V (reversible) and -1.06 V (irreversible) in comparison to the cyclic voltammogram of NH_4VO_3 .

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