JNBT
©2005INBDK

JNBT, Vol 2, No 2, pp. 80-84

Synthesis and characterization of insulin enhancing vanadium (Ⅳ) and zinc (Ⅱ) metal coordinated complexes

Cheol-Min Lee and Cheong-Soo Hwang*

Department of Chemistry, Institute of Nanosensor and Biotechnology, Dankook University,
8 Yongsan-ku, Hannam-dong, Seoul 140-714, Korea

ABSTRACT

In this paper, synthesis and characterization of the vanadium (Ⅳ) and zinc (Ⅱ) metal ion coordinated complexes are described. Especially, those presented complexes are expected to protect a dephosphorization reaction occurred in an insulin receptor moiety in cell by protein tyrosine phosphatase 1B (PTP-1B). As a result, theycan enhance the insulin accepting ability, by trapping the PTP-1B, in human cell. In other worlds, those vanadium and zinc complexes are suggested to be potential candidates for a synthetic drug to treat insulin related disease such as human diabetes mellitus. The presented complexes were characterized by IR, NMR (Zn complexes), ESR (V complexes), and ESI-Mass spectrometry. In addition, in order to establish structure, elemental analysis was performed.

Key words : vanadium, zinc, insulin mimic, diabetes

Introduction

For over a century vanadium salts have been shown to have insulin-like effects and improve the symptoms of diabetes type 2 in a variety of animal models (1,2). Vanadium salts and coordinated compounds have been shown to inhibit the activity of a variety of phosphatases involved in glucose metabolism and it is currently suggested that a major mode of action for vanadium's insulin-sensitizing effects is associated to PTP1B inhibition (3). More recently, great attention has been given to the development of vanadium coordination compounds with improved efficacy, such as BMOV (bis(maltolato)oxovanadium (Ⅳ)) which can enhance insulin sensitivity(4), and is an effective inhibitor of PTP1B activity (3). A common feature for coordinationcompounds is that they are composed of orally active organic ligands. Maltol for example, is the main ligand in BMOV and is a food additive approved by the US Food and Drug Administration (FDA). Since organic ligands contain many oxygen atoms,they are water soluble and can cross biological membranes (5).

Zinc is well known to play essential structural roles in many proteins and enzymes but has also been shown to have insulin enhancing activity *in vivo* (6). However, compounds containing zinc have received less attention than vanadium towards the development of potential anti-diabetic agents. The development of zinc complexes and the examination of their insulin-enhancing effects have only recently begun, and the use of similar ligands employed for vanadium compounds, such as picolinic acid and maltol, have yielded promising *in vivo* glucose lowering effects (7), although little is known on the possible modes of action, and whether zinc has similar phosphatase inhibition properties as vanadium.

Based on the recent findings on vanadium's modes of action for increasing insulin sensitivity, as well as the emergence of zinc coordination complexes as insulin-enhancers, our objective was to compare the efficacy of both classes of compounds on their ability to inhibit PTP1B in a streamlined standardscreening procedure. In the present study, we synthe-

* Corresponding author :
Cheong-Soo Hwang
Tel : +82-2-709-2736
Fax : +82-2-792-9269
E-mail : cshwang@dankook.ac.kr

sized a total of 10 vanadium and zinc coordination complexes. With the results we were able to compare the relative PTP1B inhibition potencies of vanadium and zinc compounds generated under the same set of experimental conditions.

Experimental

General Procedure

All the solvents, except water were purchased from Aldrich (reagent grade) and distilled prior to use. The starting materials including organic ligands such as maltol, $ZnSO_4$ and $VOSO_4$ were purchased from Aldrich and used as received. UV-Visspectra were recorded on a SCINCO 2001 spectrophotometer; infrared spectra were recorded as KBr pellets using FT-IR-NIR, IFS-66/S (Bruker) spectrophotometer; elemental analysis were performed using Flash EA 1112 Series analyzer operating at 500℃ (Thermo Quest). ^{1}H and ^{13}C NMR spectra were obtained by 500 MHz Unity Inova 500NB (Varian) spectrophotometer, all using DMSO-d_6 solvents (purchased from Aldrich) unless indicated otherwise. Most compounds were synthesized by following or slightly modifying procedures described previously (4,8-10).

Bismaltolatooxovanadium(Ⅳ), 〔VO(malt)$_2$〕

Vanadyl sulfate (2.50 g, 10.0 mmol) was dissolved in 100 mL H_2O and 3-hydroxy-2-methyl-4-pyrone (maltol) (2.95 g, 20 mmol) was added at once. Using a pH meter, 1N NaOH was added dropwise with stirring into the solution until the pH reached to 8.50. The mixture was refluxed overnight and the product was crystallized upon cooling to room temperature. After filtering, the product was vacuum dried and stored in a desicator. Yield: 2.21 g (70%) E.A. Found. (Calcd. for $C_{12}H_{10}O_7V$); C, 42.37 (45.45) H, 3.63 (3.18) O, 33.52 (35.31). IR data (cm^{-1}) 1610, 1550, 1485 v (C=O, C=C) ; 995 v (V=O), mp : 235 ℃ dec. EPR (3322.266 G) g = 2.09, ESI/MS; 317.1 (m/z).

Bishinokitiolatooxovandium(Ⅳ), 〔VO(hino)$_2$〕

Hinokitiol (0.20 g, 1.22 mmol) was dissolved in aqueous ethanol (1:1, 50 mL) with stirring. To this a solution of $VOSO_4.xH_2O$ (0.09 g, 0.61 mmol) in the same solvent was added, and the mixture was refluxed gently for overnight. Yield : 0.19 g (82.6%), E. A. Found. (Calcd. $C_{20}H_{22}O_5V$) ; C, 60.06 (61.07) ; H, 7.00 (5.64) ; O, 20.62 (20.34). IR data (cm-1); 1576 v (C=O) ; 1514 v (C=C) mp : 205℃ .EPR: g = 2.087, ESI / MS: 393.3 (m/z)

Bispicolinatooxovandium(Ⅳ), 〔VO(pic)$_2$〕

To 0.534 g (4.34 mmol) of picolinic acid in 20 mL of water was added 0.46 g (2.41 mmol) of $VOSO_4.3H_2O$ in 20 mL of water. The pH was raised to 4.4 with the dropwise additions of 1N NaOH. The light blue material which precipitated was isolated by filtration, washed with methanol and ether. Yield : 0.40 g (50.6%), E.A. Found. (Calcd. for $C_{12}H_{10}N_2O_6V$) ; C, 43.33 (43.79) ; H, 3.44 (3.06) ; N, 8.18 (8.51) ; O, 23.08 (29.16) IR data (cm^{-1}) ; 3500 broad v (H-O) ; 1640, 1630, 1600, 1570 v (C=N and C=C) ; 980, 970 v (V=O). EPR; g = 2.087, ESI / MS ; 329.6 (m/z)

Biskojatooxovandium(Ⅳ), 〔VO(koj)$_2$〕

$VOSO_4.5H_2O$ (2.50 g, 9.88 mmol) was dissolved in 10ml hot water and the solution was degassed with Ar for 10 min. This solution was then added to 10mL of a degassed aqueous solution of kojic acid (2.88 g, 20.3 mmol) and $NaOAc.3H_2O$ (2.97 g, 21.8 mmol). The solution was refluxed under Ar overnight. Yield : 0.59 g (17 %), E.A. Found. (Calcd. $C_{12}H_{10}O_9V$) ; C, 26.17 (41.28) ; H, 2.50 (2.89) ; O, 40.67 (41.24). IR data (cm^{-1}) ; 3500 broad v (O-H), 1610, 1550, 1500 v (C=O) ; 1470 (C=C) ; 1270, 1240, 1200, 1180, 1150, 1075, 1060, 980 v (V=O) ; 940, 800, 760, 580. EPR; g = 2.10, ESI / MS; 306 (m/z)

Bistropolonatooxovandium(Ⅳ), 〔VO(trop)$_2$〕

A solution of tropolone (0.5 g, 4.09 mmol) in water/ethanol mix (50mL/50mL) was added to a well-stirred solution of $VOSO_4.xH_2O$ (0.326 g, 2.04 mmol) in the same solvent mixture. The pH was raised to 8.5 with dropwise additions of 1N NaOH. After the solution was refluxed and stirred for another 2h and then left at room temperature. Yield : 0.44 g (69.8 %), Found. (Calcd. for $C_{14}H_{10}O_5V$) ; C, 36.77 (54.39) ; H, 2.47 (3.26) ; O, 24.36 (25.87). IR data (cm^{-1}) ; 900-1000 v (V=O),

1500-600 ν (C=O, C=C). EPR; g = 2.07, ESI / MS; 329.6 9 (m/z)

Bismaltolatozinc(Ⅱ), 〔Zn(malt)$_2$〕

Maltol (2.54 g, 20. 0 mmol) was dissolved in a mixture solution of water and ethanol (1:1) 50 mL. A solution of Zninc acetate (2.18 g, 9.92 mmol) in the same mixture solvent was added to the maltol containing solution with vigorous stirring. The resulting mixture was refluxed at 60℃ for 2 hours that produced white solid. The product was filtered and washed with ether. Yield 44.8% (1.56 g), mp : 130 -135 °C, IR (cm^{-1}); 3500 broad ν (H-O-H), 1614, 1578 ν (C=O); 1517 ν (C=C) ; 1277, 1202 ν (C-O), ^{1}H NMR [δ (ppm)]: 2.339[3H,s,CH_3]: 6.543[1H,d,H_6 *J*=5 Hz]: 8.113[1H,d,H_5 *J*=5 Hz]; ^{13}C NMR [δ (ppm)]: 15.235[C_1]: 110.296[C_5]: 150.183[C_6]: 152.386[C_3]: 154.144[C_2]: 178.169[C_4], ESI / MS ; 317.1 (m/z)

Bishinokitiolatozinc(Ⅱ), 〔Zn(hino)$_2$〕

Hinokitiol (0.40 g, 2.44 mmol) was dissolved in aqueous ethanol (1:1, 50 mL) with stirring. To this a solution of zinc(ll) acetate (0.26 g, 1.22 mmol) in the same solvent was added, and the mixture was refluxed gently for 2h. Yield : 0.19 g (82.6%). mp : 207~210 ℃, IR (cm^{-1}) 1507, 1576, ν (C=C) : 1504, ν (C=C), 1H NMR [δ (ppm), DMSO-d^6]: 7.359 (dd,1H, H_4 *J*=10.5 Hz), 7.197 (s,1H,H_7), 7.067 (d,1H,H_5 *J*=11Hz), 6.877 (d,1H,H_3 *J*=10Hz), 2.866 (m,1H,H_8), 1.212 (d,6H,CH_3 *J*=7Hz), ^{13}C NMR [δ (ppm), DMSO-d^6]: 24.362[C_9]; 38.863[C_8]; 122.770, 123.786, 123.900[$C_{4,5,7}$]; 138.335[C_3]; 160.167[C_6]; 178.434, 178.876[$C_{1,2}$], ESI / MS; 385.7 (m/z)

Bispicolinatozinc(Ⅱ), 〔Zn(pic)$_2$〕

To 0.534 g (4.34 mmol) of picolinic acid in 20 mL of water was added. 0.62 g (2.14 mmol) of $ZnSO_4.7H_2O$ in 20 mL of water. The pH was raised to 4.4 with dropwise additions of 1N NaOH. The white material which precipitated was isolated by filtration, washed with methanol and ether. Yield : 0.60 g (85.7%). mp : 100~104℃, IR (cm^{-1}) 3500 broad ν (H-O) ; 1632, 1594, 1566 ν (C=N and C=C) ; 981 ν (V=O), ^{1}H NMR [δ (ppm)]: 7.689[1H], 8.136[t,1H,*J*=7.5Hz], 8.196[d,1H,*J*=7.5Hz], 8.416[s,1H]. ^{13}C NMR [δ (ppm)]: 124.399[C_3], 127.309[C_5], 141.095[C_4], 146.911[C_2], 151.512[C_6], 165.898[C_1] , ESI / MS ; 317 (m/z)

Biskojatozinc(Ⅱ), 〔Zn(koj)$_2$〕

The methodology described for Zn (malt)$_2$.2H_2O was employed using kojic acid (1.00 g, 7.04 mmol)in water/ethanol mix (5mL/5mL)and zinc acetate (0.78 g, 3.34 mmol) in the same solvent mixture (50 mL). Yield : 0.98 g (84.5%) . . IR (cm^{-1}) 3500 broad ν (O-H), 1624, 1578 ν (C=O) ; 1517 ν (C=C) ; 1277, 1202 ν (C-O), 1H NMR [δ (ppm),DMSO-d^6]:4.360[d,2H,*J*=6Hz], 5.666[t,1H,*J*=6Hz], 6.555[s,1H]7.879[s,1H], ^{13}C NMR [δ (ppm), DMSO-d^6]: 60.588 [C_1], 106.463[C_3], 138.923[C_6], 154.426[C_5], 168.795[C_2], 180.735[C_4], ESI / MS; 360 (m/z)

Bistropolonatozinc(Ⅱ), 〔Zn(trop)$_2$〕

A solution of tropolone (0.5 g,4.09 mmol) in water/ethanol mix (50 mL/50 mL)was added to a well-stirred solution of zinc acetate (0.45 g, 2.05 mmol) in the same solvent mixture. After stirring for 2h, the solution was refluxed and stirred for another 2h and then left at room temperature. Yield : 0.41 g (65%). IR (cm^{-1}) ; 1595 ν (C=O); 1514 ν (C=C); 1224 ν (C-O), ^{1}H NMR [δ (ppm), DMSO-d^6]: 6.910 [t,1H;*J*=9.5 Hz], 7.229 [d,1H,*J*=11 Hz], 7.441 [t,1H,*J*=10.5Hz], ^{13}C NMR [δ (ppm), DMSO-d^6]: 178.468, 138.755, 124.510, 124.333, ESI / MS;306.5 (m/z)

Results and Discussion

Vanadium ions whose oxidation states from Ⅲ to Ⅴ are known to have several important biological functions (11), but the most interesting aspect was insulin mimetic effect. Both sodium vanadate and vanadyl sulfate have been used for a treatment for Type 2 diabetic patients since 1899 (12). However, some severe disadvantagesof the inorganic salts were raised from their low absorption and incorporation by human body. To overcome such problems vanadium ion containing organic complexes have been developed. For instance, bisoxalato, bissalicylaldehyde, and bismatolatooxovanadium (Ⅳ) complexes were synthesized and tested their insulin mimetic activities in terms of inhibition of free fatty acid release from isolated rat adipocytes treated with epinephrine

(13). In this study, we synthesized 10 vanadium and zinc ion containing organic complexes to compare their PTP-1B inhibition activities, which is another test method to measure the insulin mimetic ability. In **Fig 1** structures of the ligands are presented.

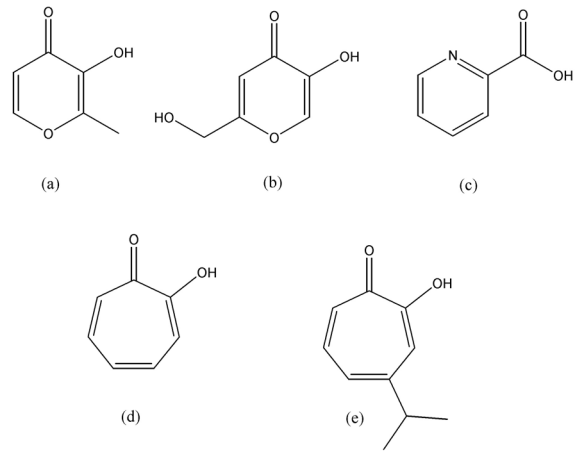

Fig 1. Used ligands: (a) maltol, (b) kojic acid (c) picoline (d) tropolone (e) hinokitiol

To characterize the corresponding metal complexes, we have employed spectroscopic methods such as IR, EPR (vanadium), and NMR (zinc). The most characteristic feature was taken from the EPR spectrum for the vanadium(Ⅳ) ion, d1 electronic configuration, complexes as shown in **Fig 2** to 6. An interesting feature of the spectrum is that $[VO(malt)_2]$, $[VO(pic)_2]$, and $[VO(koj)_2]$ showed a broad single peak with the g-factors of 2.090, 2.087, and 2.100, while $[VO(hino)_2]$ and $[VO(trop)_2]$ showed 8 line pattern with the g-factors of 2.087, 2.070 respectively. Featuring the 8 line pattern is resulted from the coupling between single d-electron and ^{51}V nucleus whose nuclear spin is 7/2.

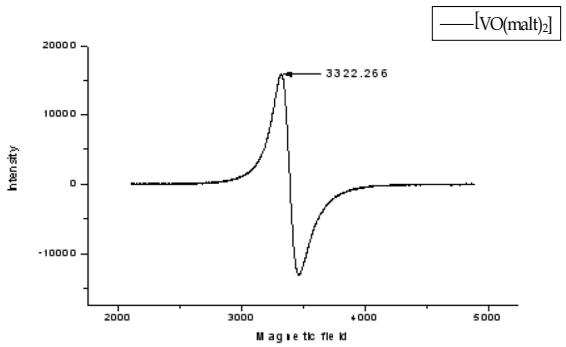

Fig 2. EPR spectrum of $[VO(malt)_2]$

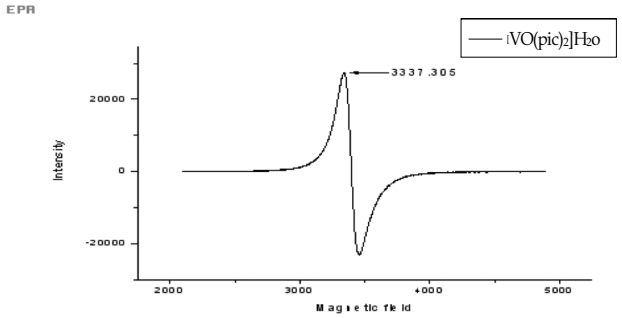

Fig 3. EPR spectrum of $[VO(pic)_2]$

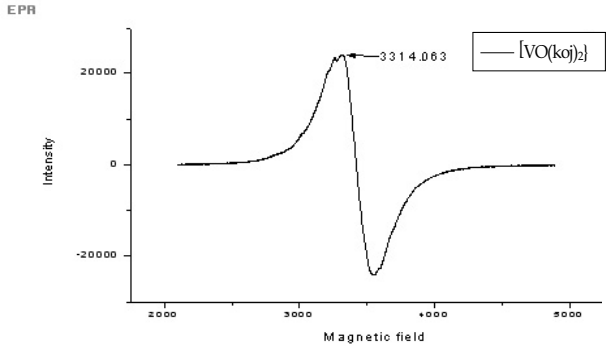

Fig 4. EPR spectrum of $[VO(hino)_2]$

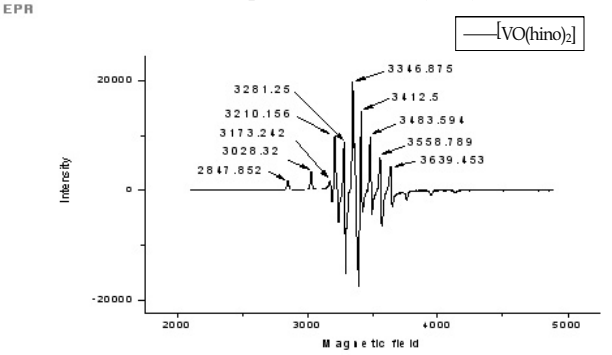

Fig 5. EPR spectrum of $[VO(koj)_2]$

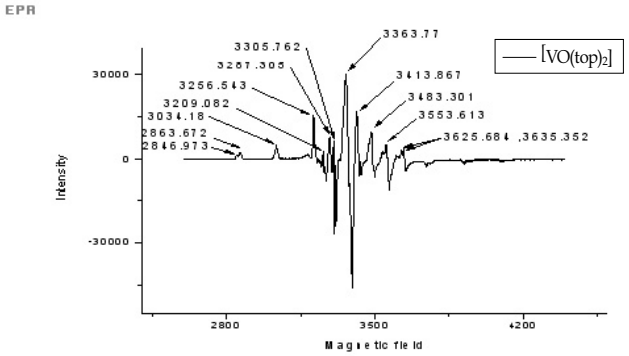

Fig 6. EPR spectrum of $[VO(trop)_2]$

The insulin mimetic activity test results for the corresponding complexes were described in our previous publication (14). In that paper, we compared the inhibitory effects of a total of 10vanadium and zinc compounds, part of which have been previously described to have anti-diabetic effects (15-17). As a result, the vanadium compounds had greater PTP1B inhibition potencies than zinc compounds, and of all compounds, $VOSO_4$ had the lowest IC_{50} value. The fact that $VOSO_4$ had greater inhibition potency than the coordination complexes is consistent with the recent finding that uncomplexed vanadate ion (VO_4) is what actually binds to the active site of PTP1B (3).

Although much more is known on the anti-diabetic effects and possible modes of action of vanadium, zinc compounds have also began to receive attention as potential organometallic anti-diabetic agents (7). Zinc coordination complexes were shown to suppress free fatty acid release from rat adipocytes, and to exhibit *in vivo* blood glucose lowering, but zinc's possible involvement in PTP1B inhibition has not been examined to date.

Conclusion

We synthesized 10 of vanadium (Ⅳ) and zinc (Ⅱ) metal ion containing complexes and fully characterized them via spectroscopic methods. Those presented compounds are expected to be potential drug candidates to treat type-2 human diabetes mellitus. The biological activities are now thoroughly examined in our laboratory.

References

(1) Rehder D (2003) Inorg Chem Comm **6**, 604
(2) Sakurai H, Fujii K, Watanabe H, Tamura H (2005) Biochem Biophys Res Commun **214**, 1095
(3) Peters KG, Davis MG, Howard BW, Pokross M, Rastogi V, Diven C, Greis KD., Eby-Wilkens E, Maier M, Evdokimov A, Soper S, Genbauffe F (2003) J Inorg Biochem. **96**, 321
(4) Yuen VG, Caravan P, Gelmini L, Glover N, McNeill JH, Setyawati IA, Zhou Y, Orvig C (1997) J Inorg Biochem **68**, 109
(5) Sakurai H, Fujii K, Watanabe H, Tamura H (1995) Biochem Biophys Res. Commun. **214**, 1095
(6) Coulston L, Dandona P (1980) Diabetes **29**, 665
(7) Sakurai H, Kojima Y, Yoshikawa Y, Kawabe K, Yasui (2002) Coord Chem Rev **226**, 187
(8) Barret MC, Mahon MF, Molloy KC, Steed JW, Wright P (2001) Inorg Chem **40**, 4384
(9) McNeill JH, Yuen VG, Hoveyda HR, Orvig C (1992) J. Med Chem **35**, 1489
(10) Melchior M, Rettig SJ, Liboiron BD, Thompson K H, Yuen VG, McNeill J H, Orvig C (2001) Inorg. Chem. **40**, 4686
(11) Stern A, Yin X., Tsang SS, Moon DJ (1993) Biochem. Cell Biol. **7**, 103
(12) Lyonnet B, Martz X, Martin E (1899) Presse. Med. **1**, 191
(13) Nakai M, Watanabe H, Fujiwara C, Kakegawa H, Satoh T, Takada J, Matsushita R, Sakurai H (1995) Biol. Pharm Bull **18**, 719
(14) Seale AP, de Jesus LA, Kim SY, Choi YH, Lim HB, Hwang CS, Kim YS (2005) Biotech. Lett. **27**, 221
(15) Kojima Y, Yoshikawa Y, Ueda E, Ueda R, Yamamoto S, Kumekawa K, Yanagihara N, Sakurai H (2003) Chem. Pharm. Bull. **51**, 1006
(16) Shechter Y, Goldwaser I, Mironchik M, Fridkin M, Gefel D (2003) Coord Chem Rev **237**, 3
(17) Thompson KH, McNeill JH, Orvig C (1999) Chem. Rev. **99**, 2561

(Received Jul 6, 2005; Accepted Aug 30, 2005)