Syntheses, Structures, and Insulin-like Activities of Oxovanadium(IV) Complexes with Tetra- and Penta-dentate Histidine Derivatives

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[VO(^{pm}H)(ClO_4)], [VO(HeY)], and [VO($^{(pm)2}$H)](ClO_4) were prepared by reactions of $VOSO_4$ and ^{pm}H (*N*-2-pyridylmethyl-(*S*)-histidine), HeY (*N,N'*-ethylene-(*S*)-histidine-(*S*)-tyrosine), and $^{(pm)2}$H (*N,N*-bis(2-pyridylmethyl)-(*S*)-histidine), respectively, and followed with $Ba(ClO_4)_2$. X-ray structure analysis of [VO(HeY)] showed that a pentadentate ligand and an oxoanion were coordinated with a vanadium(IV) ion, thereby forming a distorted octahedral geometry. In *in vitro* evaluation using isolated rat adipocytes, a tetradentate complex [VO(^{pm}H)(ClO_4)] (IC_{50} = 0.762 mM) was found to be more active at lower concentration than $VOSO_4$ (IC_{50} = 3.56 mM) in term of IC_{50} value, a 50% inhibition concentration of FFA release from the adipocytes. On the other hand, two VO complexes of pentadentate (HeY and $^{(pm)2}$H) ligands were found to be inactive for insulin-like activities.

Insulin-dependent diabetes mellitus (IDDM) is treated only by an intramuscular injection of insulin because insulin is a protein and most of it is hydrolyzed quickly in the body. Recently, the coordination chemistry of oxovanadium(IV) has gained prominence since a vanadyl cation has been found to be effective as an oral insulin-mimetic compound instead of insulin in humans.[1] In previous studies, several bis-bidentate vanadyl complexes such as [VO(picolinate)$_2$] and [VO(cysteine methyl ester)$_2$] have been found to have insulin-mimetic activity.[2] In addition, tetradentate (such as *N,N'*-ethylene-bis-amino acid (XeX: X; α-amino acid)) oxovanadium(IV) complexes have insulin-like effects.[3]

In the present investigation, the syntheses and structures of novel oxovanadium (IV) complexes with a pseudo tripod-type tetradentate alternative to linear XeX types and two pentadentate ligands including a His residue were studied, and their *in vitro* insulin-like activities were evaluated.

A novel ligand, *N*-2-pyridylmethyl-(*S*)-histidine methyl ester (^{pm}H-Me),[4] was prepared with a Schiff base that was obtained from pyridine-2-aldehyde and (*S*)-histidine methyl ester by reducting with $NaBH_3CN$. After the saponification of 0.140 g of ^{pm}H-Me with 0.085 g of $Ba(OH)_2 \cdot 8H_2O$, 0.121 g of $VOSO_4 \cdot 3.5H_2O$ was added to its aqueous solution and followed by stirring for 12 h at rt. After the filtration of $BaSO_4$ (precipitated by the addition of 0.209 g of $Ba(ClO_4)_2 \cdot 3H_2O$), a violet complex, [VO(^{pm}H)(ClO_4)] **1**,[5] was obtained with a yield of 0.094 g (42%) from the methanol solution.

A novel ligand, *N,N'*-ethylene-(*S*)-histidine-(*S*)-tyrosine methyl ethyl ester (Me-HeY-Et),[6] was obtained from (*S*)-histidine methyl and (*S*)-tyrosine ethyl esters by a one pot method similar to that used for the preparation of eHH-OMe.[7] After the saponification of 1.18g of Me-HeY-Et with 0.92 g of $Ba(OH)_2 \cdot 8H_2O$, 0.66 g of $VOSO_4 \cdot 3.5H_2O$ was added to the aqueous solution and followed by stirring for 1 h at rt. [VO(HeY)]$\cdot H_2O$ **2**[8] was obtained with a yield of 0.65 g (50%) from the mixed solution.

Another novel ligand, *N,N*-bis(2-pyridylmethyl)-(*S*)-histidine methyl ester ($^{(pm)2}$H-Me),[9] and a purple complex, [VO($^{(pm)2}$H)](ClO_4) $\cdot 4H_2O$ **3**,[10] were prepared using the methods used for those of ^{pm}H-Me and **1**, respectively. **3** was obtained with a yield of 38% by recrystallization from the methanol solution.

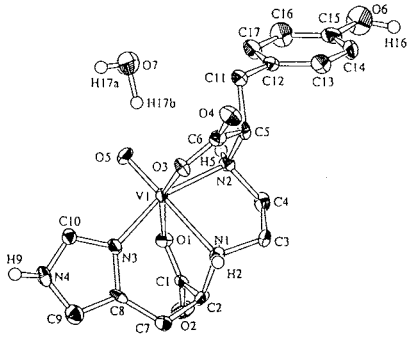

Figure 1. ORTEP drawing of [VO(HeY)]$\cdot H_2O$ (**2**) having an enantiomer, *Δ-cis-α-(RR, λδλ)*, for the skelton of HeY similar to that of MeM[3] on the basis of the absolute configuration of the ligand.

A blue crystal of **2** for X-ray structure analysis was obtained from hot water, but the single crystals of **1** and **3** were not obtained. The molecular structure of **2** was formed by only one enantiomer, *Δ-cis-α-(RR, λδλ)*, for the skelton of HeY, (which is similar to that of MeM[3]) on the basis of the absolute configuration of the ligand (Figure 1).[11] The complex has a distorted octahedral coordination structure. The V(IV) ion in **2** lies in an equatorial least-square plane (O1, N2, O3, N3) with a mean deviation of 0.001 Å. In addition, the V(IV) ion lies with a mean deviation of 0.367 Å from an equatorial least-square plane (O1, N2, O3, N3) toward an oxo ligand. The distances of the coordinated atoms around the V1 atom are 2.014(2), 1.985(2), 1.603(3), 2.324(3), 2.127(3), and 2.111(2) Å for V1-O1, V1-O3, V1-O5, V1-N1, V1-N2, and V1-N3, respectively. There are eight intermolecular hydrogen bonds in crystals primarily in O2---O7 = 2.885(4), O4---O7 = 2.805(4), N1---O2 = 3.085(4), N2---O4 = 3.001(4), N4---O1^i = 2.946(4), N4---O3^i = 2.908(4), N4---O5^i = 3.038(4), O6---O7^{ii} = 2.726(4) Å [symmetry code: (i) -x + 1/2, -y, z +1/2 (ii) x + 1/2, -y + 1/2, -z]. An O(6) atom of the phenolic OH group and an N(4) atom of the imidazolic NH group exclusively form intermolecular hydrogen bonds with an O(7) atom of the water molecule and an O(5) atom of the vanadyl oxygen, respectively.

Infrared spectroscopy has been used extensively to determine

the mode of coordination of the ClO_4^- ligand in solid state.[12] The free ClO_4^- ion belongs to the high-symmetry point group T_d and only ν_3 and ν_4 are infrared-active. In **1**, the absorption bands due to ν_3 and ν_4 appear at 1122 and 1109 and 1033 cm^{-1}, respectively. Two absorption bands due to ν_3 indicate the unidentate coordination of ClO_4^- to V(IV) ion. In **3**, ν_3 and ν_4 for a typical free ion appear at 1125-1090 and 1027 (weak) cm^{-1}, respectively. The complexes in this study with a coordination number of 6 give ν(V=O) at 974, 969, and 978 cm^{-1} for **1**, **2**, and **3**, respectively.[12] The possible geometrical isomers of **1** are shown in Figure 2. From the results of above IR spectra and the X-ray structure of [VO(*N*-(2-pyridylmethyl)iminodiacetato)(H_2O)] with a tripodal ligand,[13] **1** seems highly possible to have structures as shown in (a) and/or (b) of Figure 2.

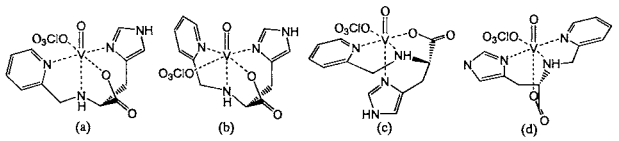

Figure 2. The possible geometrical isomers of [VO(^{pm}H)(ClO_4)] (**1**).

The insulin-like activity of these three complexes has been evaluated by *in vitro* experiments, in which the inhibition of the release of free fatty acid (FFA) from isolated rat adipocytes treated with epinephrin was estimated[14] by comparing the activity of vanadyl sulfate (VS) as a positive control. The *in vitro* experiments were performed by the method described in references. (2 and 14).

The effects of **1**, **2**, **3** and VS were found to be dose-dependent in the concentration range of 10^{-4}-10^{-3} M, as shown in Figure 3. The apparent IC_{50} value, which is the 50% inhibition concentration of the FFA release in each compound, was estimated from the results. The obtained IC_{50} = 0.762 mM for **1** was found to be more active at lower concentrations than VS (IC_{50} = 3.56 mM). On the basis of the *in vitro* results, **1** may be a potent insulin-mimetic complex in the treatment of IDDM in animals. Complexes **2** and **3**, which have two different penta-dentate ligands, were found to be inactive for insulin-like activities as shown in Figure 3.

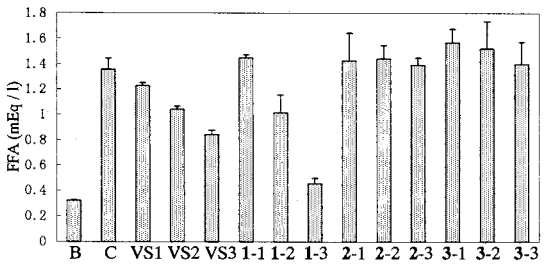

Figure 3. Inhibitory effects of vanadyl complexes on free fatty acid (FFA) release from rat adipocytes treated with epinephrine (EP).[14] B and C are blank and control, respectively. VS1-3, **1**-1-3, **2**-1-3, and **3**-1-3 are $VOSO_4$, **1**, **2**, and **3**, respectively. In each system, adipocytes (2.7×10^6 cells / ml) were treated with 10^{-4}, 5×10^{-4}, and 10^{-3} M of 1, 2, and 3, respectively, for 30 min and then incubated with 10^{-5} M EP for 3 h at 37 °C. Each column represents the mean±SD for three repeated experiments.

Oxovanadium(IV) complexes of a coordination number 5[2] such as [VO(cysteinate methyl ester)$_2$] and [VO(picolinate)$_2$] may act as potent insulin-mimics. Vanadium(IV) complexes of a coordination number 6 having a pseudo tripode-type tetradentate ligand such as ^{pm}H or a linear one such as XeX, an oxoanion and a mondentate ligand, such as a perchlorate ion or a water molecule may also act in the same manner. On the other hand, VO complexes of a coordination number 6 having a rigid penta-dentate ligand and an oxoanion are inactive in terms of insulin-like activity. Based on the above results, one coordinate site on a vanadium(IV) ion needs to be vacant or to have a monodentate ligand, such as a perchlorate ion or a water molecule, in order to give insulin-like activity.

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4 ^{pm}H-Me•3HCl: Yield: 70%. Anal. Found: C, 42.23; H, 5.23; N, 15.24%. Calcd for $C_{13}H_{16}N_4O_2$•3HCl: C, 42.24; H, 5.18; N, 15.16%. ^{1}H NMR (D_2O): δ(*J*); 3.34 (d, 2H, 6.8 Hz, H-β-CH_2), 3.76 (s, 3H, ester Me), 4.08 (t, 1H, 6.7 Hz, H-α-CH), 4.33 (d, 1H, 16.1 Hz, Py-CH_2), 4.43 (d, 1H, 16.3 Hz, Py-CH_2), 7.36 (s, 1H, Im-δ-CH), 7.86 (d, 1H, 6.3 Hz, Py-H3), 7.88 (d, 1H, 7.7 Hz, Py-H5), 8.42 (dt, 1H, 7.9, 1.4 Hz, Py-H4), 8.65 (s, 1H, Im-ε-CH), 8.55 (dd, 1H, 6.4, 1.4 Hz, Py-H6). IR (KBr): 1748 cm^{-1} for ν(C=O). mp 151-157 °C. $[\alpha]_D$ = +9.6 deg dm^{-1} cm^3 (MeOH). FAB MS m/z 261 $[M + H]^+$.

5 **1**: Anal. Found: C, 34.93; H, 3.11; N, 13.58%. Calcd for [VO($C_{12}H_{13}N_4O_2$)(ClO_4)]: C, 35.01; H, 3.18; N, 13.61%. mp 208-212 °C(dec.). $[\alpha]_D$ = -278 deg dm^{-1} cm^3 (MeOH).

6 Me-HeY-Et: Yield: 20 %. Anal. Found: C, 57.37; H, 7.02; N, 13.31%. Calcd for $C_{20}H_{28}N_4O_5$•4/5H_2O: C, 57.35; H, 7.12; N, 13.38%. ^{1}H NMR ($CDCl_3$): δ(*J*); 1.23 (t, 3H, 7.2 Hz, ethyl ester CH_3), 2.50 (d, 2H, 8.8 Hz, en), 2.75-2.84 (m, 2H, en, 2H, H-β-CH_2 & Y- β-CH_2), 2.91-2.97 (m, 2H, H-β-CH_2 & Y- β-CH_2), 3.44-3.51 (m, 2H, H-α-CH & Y-α-CH), 3.69 (s, 3H, ester CH_3), 4.15 (q, 2H, 7.2 Hz, ester CH_2), 6.71 (s, 1H, Im-δ-CH), 6.78 (d, 2H, 8.4 Hz, Ph-H3 & 5), 6.98 (d, 2H, 8.4 Hz, Ph-H2 & 6), 7.39 (s, 1H, Im-ε-CH). IR ($CHCl_3$): ν(C=O) = 1730 cm^{-1}. mp 32-36 °C. $[\alpha]_D$ = +4.8 deg dm^{-1} cm^3 (MeOH). FAB MS: m/z 405 $[M + H]^+$.

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8 **2**: Anal. Found: C, 45.86; H, 4.98; N, 12.61%. Calcd for [VO($C_{17}H_{20}N_4O_5$)]•H_2O: C, 45.85; H, 4.98; N, 12.58%. mp 205-210 °C (dec.). $[\alpha]_D$ = +691 deg dm^{-1} cm^3 (MeOH).

9 $^{(pm)2}$H-Me: Yield: 74%. Anal. Found: C, 57.95; H, 5.98; N, 17.75%. Calcd for $C_{19}H_{21}N_5O_2$•2.4H_2O: C, 57.83; H, 6.59; N, 17.75%. ^{1}H NMR ($CDCl_3$): δ(*J*); 3.06-3.18 (m, 2H, H-β-CH_2), 3.74 (dd, 1H, 11.5, 3.9 Hz, H-α-CH), 3.84 (s, 3H, ester Me), 4.01 (d, 1H, 14.2 Hz, Py-CH_2), 4.08 (d, 1H, 14.4 Hz, Py-CH_2), 6.93 (s, 1H, Im-δ-CH), 7.00 (d, 2H, 7.6 Hz, Py-H3), 7.19 (dd, 2H, 7.1, 5.1 Hz, Py-H5), 7.59 (t, 2H, 7.1 Hz, Py-H4), 8.51 (s, 1H, Im-ε-CH), 8.55 deg dm^{-1} cm^3 (d, 2H, 4.4 Hz, Py-H6). IR ($CHCl_3$): 1731 cm^{-1} for ν(C=O). $[\alpha]_D$ = -85.3 deg dm^{-1} cm^3 (MeOH). FAB MS m/z 353 $[M + H]^+$.

10 **3**: Anal. Found: C, 37.32; H, 3.90; N, 12.25%. Calcd for [VO($C_{18}H_{18}N_5O_2$)] (ClO_4) •4H_2O: C, 37.61; H, 4.56; N, 12.18%. mp 136-142 °C(dec.). $[\alpha]_D$ = +317 deg dm^{-1} cm^3 (MeOH).

11 Crystal data for **2**: $C_{17}H_{20}N_4O_6V$•H_2O, M = 445.3, orthorhombic, space group $P2_12_12_1$ (#19), *a* = 8.185(3), *b* = 36.563(5), *c* = 6.246(4) Å, *V* = 1869(1) Å^3, *F*(000) = 924.0, *Z* = 4, *Dc* = 1.582 g/cm^3, μ(Cu-Kα) = 49.00 cm^{-1}, $2\theta_{max}$ = 113.7°. Intensity data (1522 total reflections) were collected on Rigaku AFC7R diffractometer. The final cycle of full-matrix least squares refinement was based on 1474 observed reflections ($I > 3.00\ \sigma(I)$) and 351 variable parameters, and converged to *R* = 0.036 and *Rw* = 0.049. Maximum peak in final differential map is 0.35 eÅ^{-3}. Programs used to solve structure: SIR92 and DIRDIF94. All calculations including data reduction: teXsan crystallographic software package (Molecular Structure Corporation).

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