Syntheses, Structures, and Insulin-like Activities of Two Vanadyl Complexes; [VO(GeG)(H_2O)] and [VO(MeM)(H_2O)]

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We prepared [VO(GeG)(H_2O)] and [VO(MeM)(H_2O)] by a reaction of $VOSO_4$ with GeG and MeM. X-ray analyses of the complexes showed that a tetradentate ligand (GeG or MeM), an oxygen atom, and a water molecule were coordinated with the vanadium(IV) ion forming distorted octahedral geometries. In *in vitro* evaluation using isolated rat adipocytes, VO(GeG) (IC_{50} = 0.42 mM) was found to be more active at lower concentrations than VO(MeM) (IC_{50} = 17.0 mM) and $VOSO_4$ (IC_{50} = 6.0 mM) in terms of IC_{50} values, 50% inhibition concentration of the FFA release from adipocytes.

Since vanadate (+5 oxidtion) ion was found to be a potent inhibitor of Na^+,K^+-ATPase,[1] interest in the physiology, biochemistry and bioinorganic chemistry of vanadium has been focused on the structure of this element in living systems.[2,3] When vanadate ion was given to animals, vanadyl ion (+4 oxidation state or oxo-vanadium) was exclusively detected in organs and organells, indicating that it was reduced to vanadyl (+4 oxidation state, VO^{2+}) and was subsequently bound to proteins or to other higher molecule weight compounds.[4,5] The most important physiological effects are the stimulation of glucose uptake and glucose metabolism, which are the insulin-like properties of vanadyl species.[6] Thus, several types of vanadyl complexes have been shown to have insulin-mimetic activity.[7]

Recently, we have found that N_2O_2-type complexes with the metal-ligand ratio of 1:2 such as bis(picolinate)oxo-vanadium(IV) complex have insulin-like effects.[7] However, the vanadyl complex with tetradentate ligand in 1:1 molar ratio such as vanadyl-*N,N'*-ethylene bis-amino acids (XeX', X and X'; α-amino acids, mostly of tetradentate N_2O_2 type) have not been investigated. The use of α-C- and/or *N*-substituent groups of *N,N'*-ethylene bis-glycine brings about the various characteristics of VO(XeX') type complexes. The *N*-alkylation of α-amino acids increases its hydrohobicity and the replacement of bis-bidentate with mono-tetradentate will result in an increase of the complex formation constant by a chelate effect. Then we selected a leading ligand such as GeG (EDDA) = *N,N'*-ethylene bis-glycine (ethylenediamine *N,N'*-diacetic acid) as one of the XeX' = *N,N'*-ethylene bis-amino acids. The combinatorial library of XeX' will be constructed primarily with tetradentate ligands from the $_{20}C_2$ + $_{20}C_1$ = 210 species for basic α-amino acids with one absolute configuration each.

In this paper, we report the syntheses, structures and insulin-like activities of a vanadyl complex, [VO(GeG)(H_2O)], and a novel vanadyl complex, [VO(MeM)(H_2O)] (MeM = *N,N'*-ethylene bis-(*S*)-methionine).

To an aqueous solution of 0.94 g of GeG and 0.45 g of $LiOH{\cdot}H_2O$, an aqueous solution of 1.20 g of $VOSO_4{\cdot}3.5H_2O$ was added followed by stirring for 12 h at room temperature. The obtained powder was recrystallized from an aqueous solution. The blue complex, [VO(GeG)(H_2O)][8], was obtained in a 0.57 g yield (41%). The blue complex for X-ray analysis was crystallized from the aqueous solution. The [VO(GeG)(H_2O)] complex has a distorted octahedral coordination structure which is essentially the same as that reported by Kanamori et al (Figure 1).[10] The coordination distances around the V(IV) atom are 2.013(1), 1.985(1), 1.595(1), 2.047(2), 2.330(1) and 2.106(1) Å for V1-O1, V1-O3, V1-O5, V1-O6, V1-N1 and V1-N2, respectively.

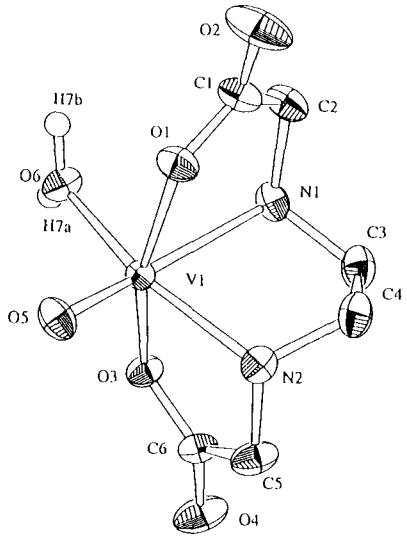

Figure 1. ORTEP drawing of Δ-cis-α-(*RR*, λδλ) of [VO-(GeG)(H_2O)].

Another ligand, MeM, was prepared according to a method previously described.[11] To an aqueous solution of 1.00 g of MeM and 0.26 g of $LiOH{\cdot}H_2O$, an aqueous solution of 0.70 g of $VOSO_4{\cdot}3.5H_2O$ were added followed by stirring for 12 h at room temperature. [VO(MeM)(H_2O)][12] was obtained in an 0.85 g yield (67%) from the mixed solution. A blue complex for X-ray analysis was crystallized from an aqueous solution. The structure of [VO(MeM)(H_2O)] shows that the V(IV) complex has an enantiomer, Δ-cis-α-(*RR*, λδλ), on the basis of the absolute configuration of the ligand (Figure 2).[13] The complex has a distorted octahedral coordination structure which is similar to that of the [VO(GeG)(H_2O)] complex. The V(IV) atom lies in an equatorial plane (O1, N2, O3, O6) with a mean deviation of 0.351 Å, and two apical atoms (N1, O5), and the

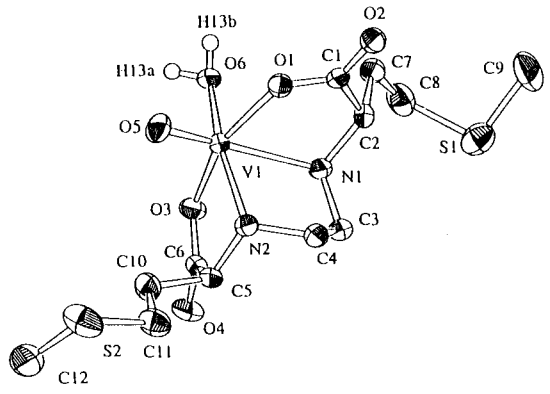

Figure 2. ORTEP drawing of Δ-cis-α-(*RR*, λδλ) of [VO-(MeM)(H_2O)].

distances around the V1 atom are 1.993(2), 1.990(2), 1.602(2), 2.033(2), 2.370(2) and 2.140(2) Å for V1-O1, V1-O3, V1-O5, V1-O6, V1-N1 and V1-N2, respectively. Coordinated aqua oxygen O6 interacts with two oxygens (O2 and O4) *via* hydrogen atoms H13a and H13b by an itermolecular hydrogen bond (O6-H13a---O2 = 2.605(4) Å and O6-H13b---O4 = 2.677(4) Å.

The insulin-like activity of both complexes have been evaluated by *in vitro* experiments, in which the inhibition of 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. Briefly, isolated rat adipocytes (2.7×10^6 cells/ml) prepared as described in ref. (14) were preincubated at 37 °C for 0.5 h with various concentrations of vanadyl complexes in 1 ml KRB buffer (120 mM NaCl, 1.27 mM $CaCl_2$, 1.2 mM $MgSO_4$, 4.75 mM KCl, 1.2 mM KH_2PO_4 and 24 mM $NaHCO_3$: pH 7.4) containing 20 mg BSA (bovin serum albumin). A 10^{-5} M epinephrine was then added to the reaction mixture and the

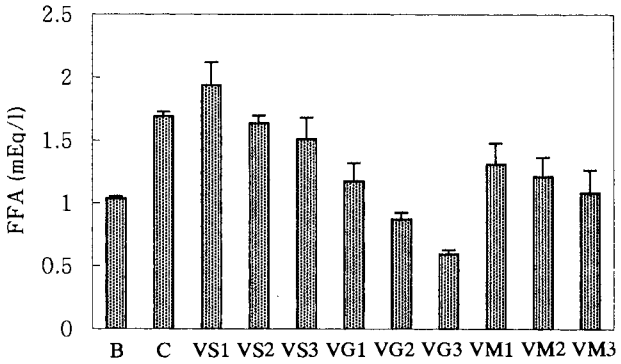

Figure 3. Inhibitory effects of vanadyl complexes on free fatty acid (FFA) release from rat adipocytes treated with epinephrine (EP). Rat adipocytes were prepared as reported.[14] B(Blank) and C(Control): Adipocytes were treated with saline for 30 m and then incubated without and with 10^{-5} M EP for 3 h at 37 °C, respectively. VS1-3, VG1-3, and VM1-3 are $VOSO_4$, VO(GeG), and VO(MeM), respectively. In each system, adipocytes (2.7×10^6 cells /ml) were treated with 10^{-4}, 5×10^{-4}, 10^{-3} M for 1, 2, and 3, respectively, for 30 m and then incubated with 10^{-5} M EP for 3 h at 37 °C. Each column represents the mean ± SD for three repeated experiments.

resulting solutions were incubated at 37 °C for 3.0 h. The reactions were stopped by soaking in ice water and the mixtures were centrifuged at 1,200 rpm for 10 min. For outer solution of the cells, FFA levels were determined with an FFA kit Wako.

The effects of VO(GeG), VO(MeM) and VS were found to be dose-dependent in the concentration range of 10^{-4}-10^{-3} M as shown in Figure 3. From the results, the apparent IC_{50} value, 50% inhibition concentration of the FFA release in each compound was estimated. The obtained IC_{50} = 0.42 mM for VO(GeG) was found to be more active at lower concentrations than VO(MeM) (IC_{50} = 17.0 mM) and VS (IC_{50} = 6.0 mM). On the basis of the *in vitro* results, the VO(GeG) has been proposed to be a potent insulin-mimetic complex to treat insulin-dependent diabetes mellitus of experimental animals. Further investigations are under way.

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References and Notes

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8 Although the preparation of this complex has been reported,[9] its method was different from our method. [VO(GeG)(H_2O)]; Anal. Found: C, 27.79; H, 4.67; N, 10.82%. Calcd for $C_6H_{12}N_2O_6V$: C, 27.81; H, 4.67; N, 10.81%. mp 245-250 °C(dec.). IR (Nujol): 953 cm^{-1} for $\nu_{V=O}$.
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10 Although X-ray data for [VO(GeG)(H_2O)][9] has been reported, we used our own data because of its improved quality. [VO(GeG)(H_2O)]: $C_6H_{12}O_6N_2V$, M = 259.11, monoclinic, space group *Pn*(#7), *a* = 8.307(3), *b* = 6.745(2), *c* = 9.526(2) Å, β = 111.40(2)°, *V* = 496.9(2) Å^3, *F*(000) = 266.0, *Z* = 2, *Dc* = 1.732 g/cm^3, μ(Mo-Kα) = 10.10 cm^{-1}, $2\theta_{max}$ = 59.9°. Intensity data (1662 total (1568 independent) reflections) were collected on Rigaku AFC 7R diffractometer. The final cycle of full-matrix least squares refinement was based on 1432 observed reflections ($I > 2.00\ \sigma(I)$) and 185 variable parameters, and converged to *R* = 0.019 and *Rw* = 0.030. Maximum peak in final differential map is 0.23 eÅ^{-3}. Programs used to solve structure: SHELX86 and DIRDIF. All calculations including data reduction: TEXSAN crystallographic software package (Molecular Structure Corporation).
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12 [VO(MeM)(H_2O)]: Anal. Found: C, 35.18; H, 6.04; N, 6.89%. Calcd for $C_{12}H_{24}N_2O_6S_2V$: C, 35.38; H, 5.94; N, 6.88%. mp 215-223 °C(dec.). The visible (ε; dm^3 mol^{-1} cm^{-1}) and CD (Δε; dm^3 mol^{-1} cm^{-1}) absorption maxima in DMSO; 12.8 (27) and 16.7 (17), and 11.5 (+0.36), 13.7 (-0.50) and 16.4 (-0.40) kcm^{-1}. IR (Nujol): 974 cm^{-1} for $\nu_{V=O}$.
13 Crystal data for [VO(MeM)(H_2O)]: $C_{12}H_{24}O_6N_2S_2V$, M = 407.39, triclinic, space group *P*1(#1), *a* = 7.614(2), *b* = 9.056(2), *c* = 6.782(1) Å, α = 99.19(2)°, β = 102.23(2)°, γ = 78.96(2)°, *V* = 445.1(2) Å^3, *F*(000) = 213.0, *Z* = 1, *Dc* = 1.520 g/cm^3, μ(Mo-Kα) = 8.20 cm^{-1}, $2\theta_{max}$ = 60.0°. Intensity data (2786 total (2603 independent) reflections) were collected on Rigaku AFC 7S diffractometer. The final cycle of full-matrix least squares refinement was based on 2378 observed reflections ($I > 2.00\ \sigma(I)$) and 273 variable parameters, and converged to *R* = 0.028 and *Rw* = 0.029. Maximum peak in final differential map is 0.25 eÅ^{-3}. Programs used to solve structure and all calculations including data reduction are the same as that of [VO(GeG)(H_2O)].[10]
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