# A New Type of Orally Active Insulin-Mimetic Vanadyl Complex: Bis(1-oxy-2-pyridinethiolato)oxovanadium(IV) with $VO(S_2O_2)$ Coordination Mode

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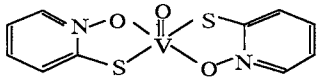
A new purple vanadyl complex, bis(1-oxy-2-pyridinethiolato) oxovanadium(IV), VO(OPT), with $VO(S_2O_2)$ coordination mode, was prepared by mixing 2-mercaptopyridine-N-oxide or 1-hydroxy-2-pyridinethione and $VOSO_4$, and characterized by UV, IR and EPR spectra, magnetic susceptibility and partition coefficient. Based on the higher *in vitro* insulin-mimetic activity of VO(OPT) ($IC_{50}$=0.19 mM) than that of $VOSO_4$ ($IC_{50}$=0.9 mM), the complex was found to be a potent agent for treating insulin-dependent diabetes mellitus in rats when given by daily intravenous injection or oral administration.

The *in vitro* findings in 1980,[1,2] that both vanadyl (+4 oxidation state of vanadium) and vanadate (+5 oxidation) have an insulin-mimetic activity, stimulated *in vivo* research on insulin-mimetic vanadium compounds. In 1985[3] and 1987,[4] the blood glucose level of rats with streptozotocin (STZ)-induced diabetes (STZ-rats) was reported to be normalized by addition of vanadate to their drinking water. These observations prompted us to develop orally active insulin-mimetic complexes in STZ-rats. In our research, we used vanadyl, which is less toxic than vanadate[5] and present exclusively in rats.[6] Since we reported first that vanadyl-cysteine methyl ester, -malonate and -tartarate complexes[7] are effective on oral administration in STZ-rats, several types of vanadyl complexes with different coordination modes such as $VO(O_4)$,[7,8] $VO(S_4)$,[9,10] $VO(S_2N_2)$,[7] $VO(N_2O_2)$[11-14] and $VO(N_3O_2)$[15] have been proposed.[16,17]

In general, vanadyl is a hard acid and thus this metal ion binds preferentially with hard bases such as oxygen and nitrogen ligands.[18] However, vanadyl complexes such as vanadyl-cysteine methyl ester[7,19] and –dithiocarbamate[9,10] complexes with $VO(S_2O_2)$ and $VO(S_4)$ coordination mode, respectively, have been found to form stable complexes and exhibit high insulin-mimetic activity when administered orally. We examined another types of vanadyl complexes and found that a purple bis(1-oxy-2-pyridinethiolato)oxovanadium(IV) complex ($C_{10}H_8N_2O_3S_2V$, VO(OPT)) with $VO(S_2O_2)$ coordination mode has a strong insulin-mimetic activity. This paper reports the results on the synthesis, *in vitro* and *in vivo* insulin-mimetic activities, and pharmacokinetic analysis of the complex.



VO(OPT)

VO(OPT) was prepared by mixing, 2-mercaptopyridine-N-oxide (Aldrich, Japan) or 1-hydroxy-2-pyridinethione (Tokyo Kasei, Japan) and vanadyl sulfate ($VOSO_4$) at molar ratio 2:1 of ligand:metal ion in aqueous solution of pH 5–6. The yield of the complex on the basis of ligand was 80% each. The structure of VO(OPT) was characterized by visible absorption, infrared (IR) and electron paramagnetic resonance (EPR) spectrometries, magnetic susceptibility and partition coefficient.[20]

In evaluating *in vitro* insulin-mimetic action of VO(OPT), we examined its inhibitory effect on free fatty acid (FFA) release in isolated rat adipocytes treated with epinephrine.[16,17,21] When VO(OPT) was added to the adipocytes, it inhibited the FFA release dose-dependently, the $IC_{50}$ value, 50% inhibitory concentration of the complex on the FFA release in the adipocytes, being 0.19 mM, indicating that VO(OPT) is one of the most effective complexes among others examined previously,[12,15-17] whereas the value of $VOSO_4$ was 0.9 mM under the same experimental conditions.[22]

Since VO(OPT) was found to be a potent insulin-mimetic complex, we examined the change of serum glucose levels in male Wistar STZ-rats on daily intraperitonial injection (*ip*) and oral administration (*po*) of VO(OPT). When STZ-rats received daily *ip* injection of the VO(OPT) at the dose of 5.0 mg (98 $\mu$ mol) or 2.5 mg (49$\mu$ mol) of vanadium(V)/kg of body weight for the first 14 days, the serum glucose level decreased to the normal range within 7 days and they were maintained in the

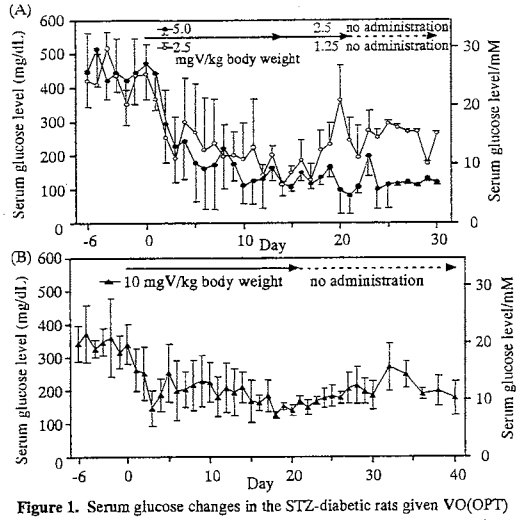



**Figure 1.** Serum glucose changes in the STZ-diabetic rats given VO(OPT) complex by daily *ip* (A) and *po* (B) administrations. Data are expressed as the means ± SDs for 6 rats.

normal range by the following administration of the same complex at the dose of 2.5 mg (49$\mu$ mol) or 1.25 mg (2.45$\mu$ mol) V/kg for further 7 days, respectively (Figure 1(A)). The glucose level of STZ-rats given higher doses of the complex (5.0 and then 2.5 mgV/kg) remained normal for more than 10 days after the end of treatment, however, that given lower doses of the complex (2.5 then and 1.25 mgV/kg) gradually increased. Based on the observations, the VO(OPT) complex was given to STZ-rats by oral administration. When STZ-rats received daily oral administration of the complex at the dose of 10 mg (196$\mu$ mol)V/kg for 21 days, the serum glucose level was normalized within 4 or 5 days and it was maintained in the normal range for more than 20 days after the end of treatment (Figure 1(B)). These results indicated that VO(OPT) is effective on both *ip* and *po* administrations. The normalization of the serum glucose level of STZ-rats by oral administration of VO(OPT) was supported in part by the improvement of serum FFA, and blood urea nitrogen (BUN) levels (Table 1). However, the serum insulin level was not recovered as expected, indicating that the effect of VO(OPT) is not peripheral.

Then, we evaluated the vanadyl level in the blood of rats to

**Table 1.** Serum parameters in STZ-rats treated with VO(OPT) complex

| Treatment | Dose of VO(OPT) (mgV/kg body weight) | Day after treatment | Glucose (mg/dL) | Insulin ($\mu$U/mL) | FFA (mEq/L) | BUN (mg/dL) |
|---|---|---|---|---|---|---|
| Normal rats | — | — | 165±25 | 25.9±1.6 | 0.38±0.06 | 14.0 ± 0.7 |
| STZ-rats | — | — | 406±64 | 5.1±3.8 | 1.25±0.14 | 20.0 ± 3.1 |
| STZ-rats + VO(OPT) *(ip)* | — | 0 | 366±76 | 9.1±4.4 | 0.77±0.18 | 20.2 ± 5.2 |
| 2.5mgV/kg body weight | 2.50 | 14 | 177±49 | 4.9±5.2 | 0.74±0.15 | 29.7 ± 11.8 |
| 1.25mgV/kg body weight | 1.25 | 21 | 215±93 | 6.2±4.8 | 0.39±0.11 | 39.9 ± 21.3 |
| no administration | 0 | 30[a] | 263 | 5.3 | 1.21 | 28.0 |
| STZ-rats + VO(OPT) *(ip)* | — | 0 | 442±188 | 5.3±2.6 | 0.83±0.27 | 22.4 ± 4.8 |
| 5.0mgV/kg body weight | 5.00 | 14 | 108± 8 | 1.9±1.7 | 0.36±0.10 | 106.0 ± 36.1 |
| 2.5mgV/kg body weight | 2.50 | 21 | 82±71 | 6.8±5.8 | 0.26±0.20 | 58.4 ± 38.6 |
| no administration | 0 | 30[a] | 190 | 6.8 | 0.87 | 22.2 |
| STZ-rats + VO(OPT) *(po)* | — | 0 | 339±59 | 9.4± 9.9 | 0.99±0.17 | 18.2 ± 3.1 |
| 10mgV/kg body weight | 10.00 | 14 | 210±45 | 2.5± 2.0 | 0.99±0.20 | 13.6 ± 1.6 |
| no administration | 0 | 30 | 184±42 | 11.9±12.0 | 0.99±0.10 | 19.4 ± 4.0 |

Data are expressed as the means ± standard deviations for 6 rats. [a] Data are the means of 2 or 3 rats.

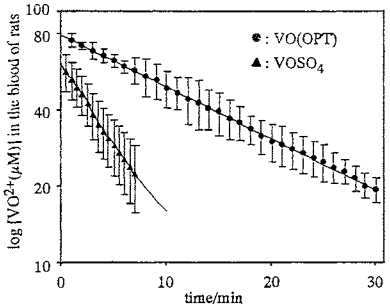



**Figure 2.** Time courses for vanadyl concentrations in the blood of rats as monitored by BCM-EPR method. Data represent the means ± SDs for 4 rats and the theoretical curves were fitted to the mean values.

clarify the disposition of VO(OPT) or $VOSO_4$ at the dose of 0.5 mg (9.8 $\mu$mol) V/kg body weight, in which blood circulation monitoring-EPR (BCM-EPR)[23] was used to measure the paramagnetic vanadyl species in the blood of rats received intravenous injection of the complex. As shown in Figure 2, the concentrations of vanadyl species in the blood of rats given VO(OPT) or $VOSO_4$ were decreased almost linearly in the semilogarithmic plot. The pharmacokinetic parameters[24] for VO(OPT) indicated that vanadyl concentrations in the blood of rats given VO(OPT) remain higher than those given $VOSO_4$.[25] From these results, VO(OPT) complex was concluded to have a long-acting character, as supported by the partition coefficient (P=12) of VO(OPT) and that (P=0.16) of $VOSO_4$.[20]

On the basis of these results, we propose here a new VO(OPT) complex with $VO(S_2O_2)$ coordination mode as an orally active and long-acting characters for treating insulin-dependent diabetes mellitus.

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**References and Notes**


1 Y. Shechter and S. J. D. Karlish, *Nature*, **286**, 556 (1980).
2 G. R. Dubyyak and A. Kleinzeller, *J. Biol. Chem.*, **255**, 5306 (1980).
3 C. E. Heyliger, A, G. Tahiliani, and J. H. McNeil, *Science*, **227**, 1474 (1985).
4 J. Meyerovitch, Z. Farfel, J. Sach, and Y. Shechter, *J. Biol. Chem.*, **262**, 6658 (1987).
5 F. T. G. Hudson, " Toxicology and Biological Significance," Elsevier Publication Co., New York (1964).
6 H. Sakurai, S. Shimomura, K. Fukuzawa, and K. Ishizu, *Biochem. Biophys. Res. Commun.*, **96**, 293 (1980).
7 H. Sakurai, K. Tsuchiya, and M. Komatsu, *J. Clin. Biochem. Nutr.*, **8**, 193 (1990).
8 J. H. McNeil, V. G. Yuen, and H. R. Hoveya, *J. Med. Chem.*, **35**, 1489 (1992).
9 H. Watanabe, M. Nakai, K. Komazawa, and H. Sakurai, *J. Med. Chem.*, **37**, 876 (1994).
10 H. Sakurai, H. Watanabe, H. Tamura, H. Yasui, R. Matsushita, and J. Takada, *Inorg. Chim. Acta*, **283**, 175 (1998).
11 H. Sakurai, K. Fujii, H. Watanabe, and H. Tamura, *Biochem. Biophys. Res. Commun.*, **214**, 1095 (1995).
12 S. Fujimoto, K. Fujii, H. Yasui, R. Matsushita, J. Tanaka, and H. Sakurai, *J. Clin. Biochem. Nutr.*, **23**, 113 (1997).
13 K. Kawabe, M. Tadokoro, Y. Kojima, Y. Fujisawa, and H. Sakurai, *Chem. Lett.*, **1998**, 9.
14 R. Tawa, K. Uchida, J. Taniyama, Y. Kojima. Y. Fujisawa, S. Fujimoto, T. Nagaoka, K. Kanamori, and H. Sakurai, *J. Pharm. Pharmacol.*, **51**, 119 (1999).
15 K. Kawabe, T. Suekuni, T. Inada, K. Yamato, M. Tadokoro, Y. Kojima, Y, Fujisawa, and H. Sakurai, *Chem. Lett.*, **1998**, 1155.
16 H. Sakurai and A. Tsuji, in "Vanadium in the Environment, Part 2: Health Effects," ed by J. O. Nriagu, John Wiley & Sons, New York (1998), p. 297.
17 H. Sakurai, K. Fujii, S. Fujimoto, Y. Fujisawa, K. Takechi, and H. Yasui, in "Vanadiumu Compounds: Chemistry, Biochemistry, and Therapeutic Applications," ACS Symp. Ser., 711, ed by A. S. Tracy and D. C. Chans, *Am. Chem. Soc.*, (1998) p. 344.
18 R. G. Pearson, *J. Am. Chem. Soc.*, **85**, 3533 (1963).
19 H. Sakurai, Z. Taira, and N. Sakai, *Inorg. Chim. Acta*, **151**, 85 (1988).
20 Physical characteristics of VO(OPT): Anal. Calcd for $C_{10}H_8N_2O_3S_2V$: C, 37.60; H, 2.51; N, 8.78%. Found: C, 37.41; H, 2.53; N, 8.78%. $\lambda$ max, 512 nm ($\varepsilon$ =42.3 $M^{-1}cm^{-1}$) and 600 nm ($\varepsilon$ =13.0 $M^{-1}cm^{-1}$) in millipore water; $\nu_{v=0}$ 960 $cm^{-1}$ (KBr disk); EPR spectra in DMSO at 77K exhibited the formations of two vanadyl complexes, the dominant and minor species formed in trace amount. EPR parameters (dominant form), $g_0$=1.990, $A_0$=85.1×$10^{-4}cm^{-1}$, $g_{//}$=1.954, $A_{//}$=162.0×$10^{-4}cm^{-1}$, $g_\perp$=2.008, $A_\perp$=46.7×$10^{-4}cm^{-1}$. Magnetic susceptibility, 3.55×$10^{-6}$ cgs units and $\mu_{eff}$ =1.74. Partition coefficient, 12 for VO(OPT) and 0.16 for vanadyl sulfate ($VOSO_4$) in chloroform: saline=1:1 for mixing of 6 h at 37 °C. VO(OPT) complex was slightly soluble in most organic solvents such as DMSO, DMF, $CHCl_3$, methanol and ethanol, but insoluble in *n*-butanol. VO(OPT) in 5% acacia suspension is stable
21 M. Nakai, H. Watanabe, C. Fujiwara, H. Kakagawa, T. Satoh, J. Takada, K. Matsushita, and H. Sakurai, *Biol. Pharm. Bull.*, 18, 719 (1995).
22 The Insulin-mimetic activity of the complex has been evaluated by *in vitro* experiments,[21] in which the inhibition of release of FFA from isolated rat adipocytes treated with epinephrine was estimated. Briefly, isolated rat adipocytes (2.7×$10^6$ cells/mL) prepared as described[21] were preincubated at 37 °C for 0.5 h with three different concentrations of vanadyl compound in 1mL 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 bovine serum albumin (BSA, Sigma, USA). A $10^{-5}$ M epinephrine was then added to the reaction mixture and the resulting solutions were incubated at 37 °C for 3 h. The reactions were stopped by soaking in ice water and the mixtures were centrifuged at 12000 rpm for 1 min. For outer solution of the cells, FFA levels were determined with an FFA kit (Wako Pure Chemicals, Japan).
23 K. Takechi, H. Tamura, K. Yamaoka, and H. Sakurai, *Free Rad. Res.*, 26, 483 (1997).
24 K. Yamaoka, Y. Tanigawa, T. Nakagawa, *J. Pharmacobio-Dyn.*, 4, 879 (1981).
25 Pharmacokinetic parameters for VO(OPT) and $VOSO_4$: were obtained as follows. One-compartment model [$C_b=D/Vd\cdot exp(-k_e\cdot t)$] was fitted to each individual profile of the concentrations in the blood of rats given VO(OPT) or $VOSO_4$ using nonlinear least squares regression program, MULTI,[24] where $C_b$ is the blood concentration, $D$ is the dose of a comppound, $Vd$ is the distribution volume, $k_e$ is the elimination rate constant, and $t$ is time. The area under the concentration curve ($AUC$), mean residence time ($MRT$), total clearance ($CL_{tot}$), and half life ($t_{1/2}$) were calculated from the following equations: $AUC=D/Vd/k_e$, $MRT=I/k_e$, $CL_{tot}=Vd\cdot k_e$, and $t_{1/2}=0.693/k_e$. VO(OPT): $AUC$=1700±170 (nmol·min/mL), $MRT$=21.2±2.3 (min), $CL_{tot}$=5.81±0.63 (mL/min/kg), $Vd$=122±5 (mL/kg), $k_e$=0.0476±0.0054 ($min^{-1}$), and $t_{1/2}$=14.6±1.6 (min). $VOSO_4$: $AUC$=461±100 (nmol · min/mL), $MRT$=7.2±1.6 (min), $CL_{tot}$=22.2±5.1 (mL/min/kg), $Vd$=154±26 (mL/kg), $k_e$=0.136±0.028 ($min^{-1}$), and $t_{1/2}$=5.2±1.2 (min).