# A New Type of Orally Active Insulin-mimetic Vanadyl-porphyrin Complex: *meso*-Tetrakis({4-sulfonatophenyl}porphyrinato)oxovanadium(IV)

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Vanadyl-*meso*-tetrakis(4-sulfonatophenyl)porphyrin, VOTPPS, was synthesized and evaluated for its in vitro and in vivo insulin-mimetic activities in streptozotocin (STZ)-induced diabetic mice. Based on the high in vitro insulin-mimetic activity in terms of inhibition of free fatty acid release and enhancement of glucose-uptake in adipocytes, and matallokinetic parameters, VOTPPS was revealed to have a significant hypoglycemic activity in STZ-mice following a single oral gavage of the complex. VOTPPS is thus found to be the first example for orally active vanadyl-porphyrin complex to treat type 1 diabetic animals.

In recent years, the number of subject suffering from diabetes mellitus (DM) is increasing worldwide. DM is classified mainly into two types; type 1 insulin-dependent DM is associated with absolute insulin deficiency and type 2 non-insulin dependent DM is associated with lowering of insulin sensitivity in all organs, which ultimately follows absolute insulin deficiency.[1] Type 1 DM is treated only by daily insulin injections to normalize the high blood glucose levels; type 2 DM is treated by several types of synthetic therapeutics together with a controlled diet and physical exercise. Even with these measures, the daily necessity for several insulin injections is painful both physically and mentally, whereas the synthetic therapeutics used over the long term often exhibit severe side effects. For these reasons, the creation and development of a new class of pharmaceuticals for the treatment of DM would be extremely desirable in the 21st century. In 1990, we first reported that vanadyl-cysteine methyl ester complex with the $VO(S_2N_2)$ coordination mode is very useful in the treatment of streptozotocin (STZ)-induced type 1 diabetic rats by daily oral administrations.[2] Since then, many types of insulin-mimetic vanadyl complexes with different coordination modes around vanadyl, such as $VO(O_4)$, $VO(S_4)$, $VO(S_2O_2)$, $VO(S_2N_2)$, $VO(N_2O_2)$, and $VO(N_3O)$ have been proposed,[3–7] however, few complexes with $VO(N_4)$ coordination mode have been examined. Recently, we found that *meso*-tetrakis({1-methylpyridinium-4-yl}porphyrinato)oxovanadium(IV), VOTMPyP with $VO(N_4)$ coordination mode, is a potential insulin-mimetic vanadyl complex for the treatment of type 1 diabetic model STZ-rats, where the complex was given simultaneously with sodium ascorbate.[8] This important finding promoted us to develop more active vanadyl-porphyrin complexes. In this study, we have synthesized and characterized *meso*-tetrakis({4-sulfonatophenyl}porphyrinato)oxovanadium(IV) complex and estimated its in vitro and in vivo insulin-mimetic activities, comparing with those of VOTMPyP as a positive control.

VOTPPS was prepared according to the method of Adler et al.[9] with slight modification. *meso*-Tetrakis(4-sulfonatophenyl)-porphyrin ($H_2$TPPS; 0.20 g, 0.20 mmol (Funakoshi, Tokyo, Japan) and $VOSO_4 \cdot 3H_2O$ (VS; 0.86 g, 3.99 mmol) were heated to reflux in *N*,*N*-dimethylformamide (DMF; 50 mL) on a stirring hot plate at 150 °C for 24 h. The refluxing solution was then evaporated to about 5 mL and cooled in an ice bath. Acetone (20 mL) was added to the remaining solution. The resulting precipitate was redissolved in methanol and reprecipitated with acetone six times. The crude material was purified by gel chromatography (Sephadex LH-20, Amersham Pharmacia Biotech, Tokyo, Japan; eluent: $H_2O$). Finally, the aqueous solution was concentrated and dried under high vacuum. The composition was determined by elemental analyses, mass spectrometry, and IR and ESR spectra, and then compared with the data for the VOTMPyP complex.[8,10]

The stability of vanadyl porphyrin complexes were studied in 4% BSA (Sigma, St. Louis, MO., USA) as well as in rat blood serum (RBS). RBS was collected from supernatant after centrifugation of normal Wistar rat blood at 3000 rpm for 10 min. The vanadyl state of porphyrin complexes was monitored with ESR. The spectra of VOTMPyP exhibited eight lines, however, VOTPPS showed an anisotropic spectrum at room and liquid nitrogen temperatures, which indicates a strong interaction of the VOTPPS with the positive charge on BSA[11] and RBS. Similar results were observed by Kadish et al.,[12] in which the addition of either 5% neutral surfactant Triton X-100 (TX-100) or 0.05 M cationic surfactant cetyltrimethylammonium bromide (CTAB) to the solutions of VOTPPS induced an anisotropy in the spectrum at room temperature. The ESR signal intensities due to vanadyl species of VOTPPS was very stable in 4% BSA as well as in RBS in the absence of ascorbate for 48 h.

In vitro insulin-mimetic activity of the complexes was examined by simultaneous inhibition of free fatty acids (FFA) release and enhancement of glucose-uptake in isolated rat adipocytes treated with epinephrine (Sigma).[13,14] The apparent $IC_{50}$ value, which is a 50% inhibitory concentration of the complex for FFA-release from the adipocytes, was estimated to be $488.3 \pm 49.1\,\mu M$ for VOTMPyP and $18.6 \pm 13.0\,\mu M$ for VOTPPS, respectively. Moreover, the apparent $EC_{50}$ value, which is a 50% enhancing concentration of the compound with respect to the maximal glucose-uptake concentration in epinephrine-treated adipocytes, was not detected for VOTMPyP, but found for VOTPPS as $46.3 \pm 6.4\,\mu M$. These results strongly indicated that VOTPPS has a higher potent insulin-mimetic activity than that of VOTMPyP under the same experimental conditions.

In evaluating in vivo insulin-mimetic activity of the vandayl-porphyrin complexes, both complexes were assessed in streptozotocin (STZ)-induced diabetic mice, which is a rodent model of type 1 DM. The changes in the blood glucose level of the STZ-induced diabetic mice after a single oral gavage of vanadyl porphyrin complexes without ascorbate at a dose of 15 mg V/kg of body weight were monitored (Figure 1).

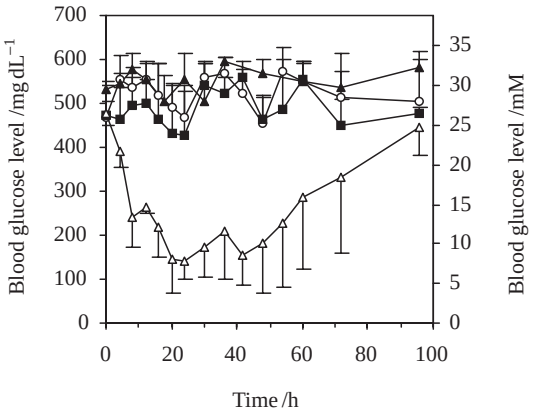


**Figure 1.** Change of blood gluocse levels in STZ-mice after a single oral administration of saline (○, $n = 4$), vanadyl-porphyrin complexes without ascorbate, VOTMPyP (■, $n = 5$) and VOTPPS (△, $n = 5$), at a dose of 15 mg V/kg of body weight and ligand, $H_2$TPPS, (▲, $n = 5$) at a dose as equimolar concentration (297 mg $H_2$TPPS/kg of body weight) of complex. Complexes and $H_2$TPPS were dissolved in saline.

VOTPPS showed evidence of significant hypoglycemic activity within at least 8 h after the administration, the effect being sustained for at least 48 h. The results suggest that the vanadyl species of VOTPPS is remained in the blood for a long time without ascorbate, probably due to the strong interaction between RBS and VOTPPS as observed in ESR data.

In contrast, VOTMPyP did not show hypoglycemic effects at the same dose of VOTPPS (Figure 1). In addition, no hypoglycemic effect was noted for the ligand ($H_2$TPPS) at equimolar concentration of VOTPPS. Based on these results, VOTPPS was found to have much higher hypoglycemic activity than VOTMPyP.

To understand hypoglycemic activity of the compounds, metallokinetic analysis in rats was examined in terms of vanadyl concentration by using blood circulation monitoring-ESR (BCM-ESR).[15,16] Vanadyl species remained longer in the blood of rats receiving VOTPPS by intravenous (iv) injection without ascorbate than in that of rats receiving iv injection of VOTMPyP (Figure 2),[17] suggesting its stronger and longer hypoglycemic activity.

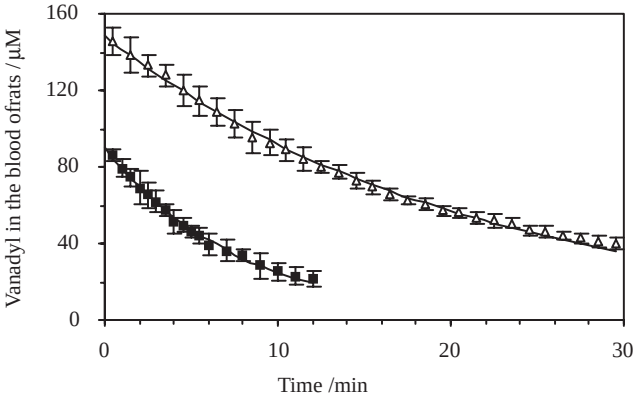


**Figure 2.** Vanadyl clearance curves as monitored by BCM-ESR from the blood-circulation of rats, which received iv bolus injection of vanadyl-porphyrin complexes, VOTPPS (△, $n = 3$) and VOTMPyP (■, $n = 3$), without ascorbate at a dose of 0.5 mg V/kg of body weight under anesthesia. Complexes were dissolved in saline.

In conclusion, VOTPPS was found to be the first example for orally active vanadyl-porphyrin complex, proposing an insulin-mimetic complex for treating type 1 diabetic mellitus animals.

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**References and Notes**

1 S. Wild, G. Roglic, R. Sicree, A. Green, and H. King, "Global Burden of Disease," WHO, Geneva (2003).
2 H. Sakurai, K. Tsuchiya, M. Nukatsuka, J. Kawada, S. Ishikawa, H. Yoshida, and M. Komatsu, *J. Clin. Biochem. Nutr.*, **8**, 193 (1990).
3 H. Sakurai, H. Yasui, and Y. Adachi, *Expert Opin. Invest. Drugs*, **12**, 1189 (2003).
4 H. Sakurai, Y. Kojima, Y. Yoshikawa, K. Kawabe, and H. Yasui, *Coord. Chem. Rev.*, **226**, 187 (2002).
5 H. Sakurai, *Chem. Rec.*, **2**, 237 (2002).
6 K. H. Thompson, J. H. McNeill, and C. Orvig, *Chem. Rev.*, **99**, 2561 (1999).
7 L. C. Woo, V. G. Yuen, K. H. Thompson, J. H. McNeill, and C. Orvig, *J. Inorg. Biochem.*, **76**, 251 (1999).
8 H. Sakurai, T. Inohara, Y. Adachi, K. Kawabe, H. Yasui, and J. Takada, *Bioorg. Med. Chem. Lett.*, **14**, 1093 (2004).
9 A. D. Adler, F. R. Longo, F. Kampas, and J. Kim, *J. Inorg. Nucl. Chem.*, **32**, 2443 (1970).
10 VOTPPS:[9] $\nu_{V=O}$: 1005 cm$^{-1}$ (KBr disk); $\lambda_{max}$ in nm ($\varepsilon/10^3$ M$^{-1}$ cm$^{-1}$): 436 (219.5), 564 (18.8), 604 (7.3) in $H_2O$. Anal. Calcd for $C_{44}H_{28}O_{12}S_4N_4VO\cdot 8.5H_2O\cdot 2.1C_3H_7NO$: C, 46.24; H, 4.61; N, 6.54%; Found: C, 46.21; H, 4.48; N, 6.55%; FAB$^-$ MASS $m/z$: $[M-H]^- = 998$. ESR parameters: $g_0 = 1.967$, $A_0 = 106 \times 10^{-4}$ cm$^{-1}$; $g_{//} = 1.938$, $A_{//} = 183 \times 10^{-4}$ cm$^{-1}$; $g_\perp = 1.982$, $A_\perp = 67 \times 10^{-4}$ cm$^{-1}$.
11 D. Matulis and R. Lorrian, *Biophys. J.*, **74**, 422 (1998).
12 K. M. Kadish, G. B. Maiya, C. Araullo, and R. Guilard, *Inorg. Chem.*, **28**, 2725 (1989).
13 M. Nakai, H. Watanabe, C. Fujiwara, H. Kakegawa, T. Satoh, J. Takada, R. Matsushita, and H. Sakurai, *Biol. Pharm. Bull.*, **18**, 719 (1995).
14 Y. Adachi and H. Sakurai, *Chem. Pharm. Bull.*, **52**, 428 (2004).
15 K. Takechi, H. Tamura, K. Yamaoka, and H. Sakurai, *Free Radical Res.*, **26**, 483 (1997).
16 H. Yasui, A. Tamura, T. Takino, and H. Sakurai, *J. Inorg. Biochem.*, **91**, 327 (2002).
17 Metallokinetic parameters of VOTPPS and VOTMPyP were obtained as follows. One-component model [$C_b = D/V_d \cdot \exp(-k_e \cdot t)$] was used to fit each individual profile of the concentrations in the blood of rats given VOTPPS or VOTMPyP using nonlinear least squares regression program, MULTI,[17] where $C_b$ is the blood concentration, $D$ is the dose of a compound, $V_d$ is the distribution volume, $k_e$ is the elimination rate constant, and $t$ is time. The area under the concentration curve ($AUC$), mean resistance time ($MRT$), total clearance ($CL_{tot}$), and half life ($t_{1/2}$) were calculated from the following equations: $AUC = D/V_d/k_e$, $MRT = 1/k_e$, $CL_{tot} = V_d \cdot k_e$ and $t_{1/2} = 0.693/k_e$. VOTPPS: $AUC = 3.09 \pm 0.18$ (μmol·min/mL), $MRT = 20.9 \pm 0.6$ (min), $CL_{tot} = 3.2 \pm 0.2$ (mL/min/kg), $V_d = 66 \pm 4$ (mL/kg), $k_e = 0.048 \pm 0.001$ (min$^{-1}$), and $t_{1/2} = 14.5 \pm 0.4$ (min). VOTMPyP: $AUC = 0.70 \pm 0.10$ (μmol·min/mL), $MRT = 7.8 \pm 0.8$ (min), $CL_{tot} = 14.1 \pm 2.1$ (mL/min/kg), $V_d = 109 \pm 5$ (mL/kg), $k_e = 0.129 \pm 0.013$ (min$^{-1}$), and $t_{1/2} = 5.4 \pm 0.6$ (min). All parameters of VOTPPS were significantly different from those of VOTMPyP at the 1% level of ANOVA (Analysis of variance; using SPSS 12.0, SPSS Inc.).
18 K. Yamaoka, Y. Tanigawa, and T. Nakagawa, *J. Pharmacobio-Dyn.*, **4**, 879 (1981).

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