Stereospecific and Structure-Dependent Insulin-Mimetic Oxovanadium(IV) Complexes with *N,N'*-Ethylenebis(amino acids)

Kenji Kawabe,[†] Makoto Tadokoro,[†] Akio Ichimura,[†]
Yoshitane Kojima,*,[†] Toshikazu Takino,[‡] and
Hiromu Sakurai*,[‡]

*Department of Chemistry, Graduate School of
Science, Osaka City University, Sugimoto
Sumiyoshi-ku, Osaka 558-8585, Japan
Department of Analytical and Bioinorganic Chemistry
Kyoto Pharmaceutical University, Nakauchi-cho 5
Misasagi, Yamashina-ku, Kyoto 607-8414, Japan*

Much interest has been focused on the oxovanadium(IV) ion and its complexes, since a variety of oxovanadium(IV) complexes, when administered orally, have been shown to normalize the hyperglycemia of streptozocin-induced rats (STZ-rats) with insulin-dependent diabetes mellitus (IDDM),[1−4] and orally administered vanadyl sulfate in place of insulin has been reported to improve human noninsulin-dependent diabetes mellitus (NIDDM).[5−9]

During our investigations on the development of insulin-mimetic oxovanadium(IV) complexes,[10,11] we found that the complexes with a $VO(N_2O_2)$ coordination mode, such as oxovanadium(IV)−picolinate complexes, exhibit excellent insulin-mimetic activities in terms of both the *in vitro* inhibition of free fatty acids (FFA) release and glucose uptake in isolated rat adipocytes. There is also an *in vivo* normoglycemic effect on not only the STZ-rats with IDDM[10,11] but also KKA[y] mice with NIDDM[12] by both intraperitoneal (ip) injection and oral administration of the complexes. On the basis of these results, we examined further the structure−activity relationship of the oxovanadium(IV) complexes with different stereochemistries of VO-(N_2O_2) coordination mode and found that tetradentate oxovanadium(IV) complexes of $[VO(XeX)(H_2O)]$, where XeX is *N,N'*-ethylenebis(amino acid), are good models for the purpose.[13] We report here stereospecific and structure-dependent insulin-mimetic
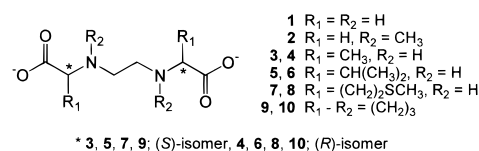

[†] Osaka City University.
[‡] Kyoto Pharmaceutical University.
(1) (a) Sakurai, H.; Tsuji, A. In *Vanadium in the Environment*; Nriagu, J. O., Ed.; Wiley: New York, 1998; p 297. (b) Sakurai, H.; Fujii, K.; Fujimoto, S.; Fujisawa, Y.; Takechi, K.; Yasui, H. In *Vanadium Compounds: Chemistry, Biochemistry, and Therapeutic Applications*; ACS Symposium Series 711; Tracey, A. S., Crans, D. C., Eds.; American Chemical Society: Washington, DC, 1998; p 344.
(2) Caravan, P.; Gelmini, L.; Glover, N.; Herring, F. G.; Li, H.; McNeill, J. H.; Rettig, S. J.; Setyawati, I. A.; Shuter, E.; Sun, Y.; Tracey, A. S.; Yuen, V. G.; Orvig, C. *J. Am. Chem. Soc.* **1995**, *117*, 12759.
(3) Sakurai, H.; Watanabe, H.; Tamura, H.; Yasui, H. *Inorg. Chim. Acta* **1998**, *283*, 175.
(4) Tawa, R.; Uchida, K.; Taniyama, J.; Fujisawa, Y.; Fujimoto, S.; Nagaoka, T.; Kanamori, K.; Sakurai, H. *J. Pharm. Pharmacol.* **1999**, *50*, 1.
(5) Cohen, N.; Halberstam, M.; Shlimovich, P.; Chang, C. J.; Shamoon, H.; Rossetti, L. *J. Clin. Invest.* **1995**, *95*, 2501.
(6) Goldfine, A. B.; Simonson, D. C.; Folli, F.; Patti, M. E.; Kahn, C. R. *J. Clin. Endocrinol. Metab.* **1995**, *80*, 3311.
(7) Goldfine, A. B.; Simonson, D. C.; Folli, F.; Patti, M. E.; Kahn, C. R. *Mol. Cell Biochem.* **1995**, *153*, 217.
(8) Halberstam, M.; Cohen, N.; Shlimovich, P.; Rossetti, L.; Shamoon, H. *Diabetes* **1996**, *45*, 659.
(9) Boden, G.; Chen, X.; Ruiz, J.; van Rossum, G. D. V.; Turco, S. *Metabolism* **1996**, *45*, 1130.
(10) Sakurai, H.; Fujii, K.; Watanabe, H.; Tamura, H. *Biochem. Biophys. Res. Commun.* **1995**, *214*, 1095.
(11) Fujimoto, S.; Fujii, K.; Yasui, H.; Matsushita, R.; Takada, J.; Sakurai, H. *J. Clin. Biochem. Nutr.* **1997**, *23*, 113.
(12) Sakurai, H.; Fujisawa, Y.; Yasui, H.; Fujimoto, S. *33th Int. Conf. Coord. Chem.* **1998**, Abstract 614.
(13) Kawabe, K.; Tadokoro, M.; Kojima, Y.; Fujisawa, Y.; Sakurai, H. *Chem. Lett.* **1998**, 9.

Figure 1. Ligand structures.

1 $R_1 = R_2 = H$
2 $R_1 = H$, $R_2 = CH_3$
3, 4 $R_1 = CH_3$, $R_2 = H$
5, 6 $R_1 = CH(CH_3)_2$, $R_2 = H$
7, 8 $R_1 = (CH_2)_2SCH_3$, $R_2 = H$
9, 10 $R_1 - R_2 = (CH_2)_3$

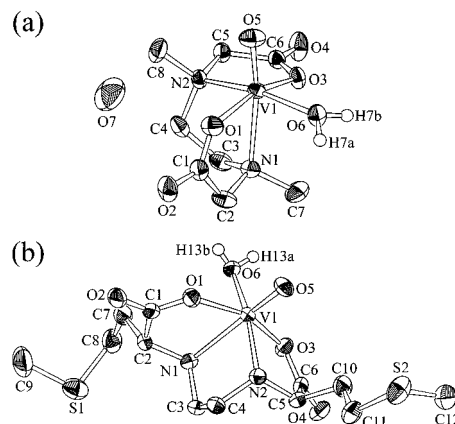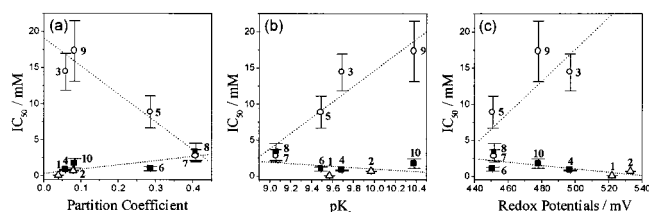* 3, 5, 7, 9; (*S*)-isomer, 4, 6, 8, 10; (*R*)-isomer

Figure 2. (a) X-ray structure of **2** (Δ-type) as the $0.5H_2O$ adduct (ORTEP representation; ellipsoids at 50% probability level). The optical isomer (Λ-type) was omitted. The O(7) atom of water molecule is disordered between two positions and calculated by an occupancy factor at 0.5. (b) X-ray structure of **8** (ORTEP representation; ellipsoids at 50% probability level).

oxovanadium(IV) complexes. Before our work, Shechter et al. reported the correlation of insulin-mimetic activity to the chirality and lipophilicity of oxovanadium(IV) complexes with $VO(O_4)$ coordination mode, in which the D-isomer has been found to be less active than the L-isomer.[14]

The structures of the ligands used in the present study are shown in Figure 1. The complexes $[VO(XeX)(H_2O)]$, where X = G (Gly) (**1**), mG or Gm (*N*-methylglycine) (**2**), L- and D-A (Ala) (**3** and **4**), L- and D-V (Val) (**5** and **6**), L- and D-M (Met) (**7** and **8**), and L- and D-P (Pro) (**9** and **10**) were prepared according to the previously reported method.[13,15] The physical properties of complexes **1**−**10** were analyzed by elemental analyses and electronic, IR, CD, and ESR spectra. Partition coefficients of the complexes were evaluated in an *n*-octanol/saline system.[16] X-ray structures were analyzed as reported for complexes **1** and **7**.[13]

The molecular structure of the new complex **2** has been determined by X-ray analysis (Figure 2a), which contains two optical isomers of Δ-*cis*-α-(*RR*, λδλ) (Δ-type) and Λ-*cis*-α-(*SS*, δλδ) (Λ-type) similar to that of complex **1**.[17] Those of the new complexes **3**, **5**, and **9** have only Δ-type configuration, as is the case for complex **7**.[18] An ORTEP view of the molecular structure

---

(14) Shechter, Y.; Shisheva, A.; Lazar, R.; Libman, J.; Shanzer, A. *Biochemistry* **1992**, *31*, 2063.
(15) Watanabe, M.; Kojima, Y.; Kawabe, K.; Hatamoto, E.; Tsuru, E.; Miyake, H.; Yamashita, T. *Synthesis* **1996**, 452.
(16) The partition coefficients of the complexes were determined by a conventional method in an *n*-octanol/saline system using a UV−vis spectrometer (Hitachi U-3500L). The concentrations of the complexes in each phase were monitored at their characteristic absorption bands due to the vanadyl state. The partition coefficient of the complexes was calculated by the equation $P = C_{oct}/C_w$, where C_{oct} and C_w are the equilibrium concentrations of the complex in *n*-octanol and saline, respectively, after shaking for 2 h at 37 °C.
(17) Crystal data for **2**·$0.5H_2O$: monoclinic, $P2_1/n$ (No. 14), $a = 8.962(3)$ Å, $b = 11.569(2)$ Å, $c = 12.316(2)$ Å, $β = 105.48(1)°$, $V = 1230.6(4)$ Å³, $Z = 4$, GOF = 1.52. Data collected over the range $2θ_{max} = 60.0°$ at 23 °C on a Rigaku AFC7S diffractometer with Mo Kα radiation. Convergence to the final R values of $R = 0.031$ and $R_w = 0.051$ ($I > 3.00σ(I)$) for **2**·$0.5H_2O$ achieved by using 3761 reflections and 164 variable parameters. Details of X-ray crystallographic analyses are in Supporting Information.

Figure 3. Correlation between the IC_{50} and (a) partition coefficient, (b) pK_a of the constituent amino acids as the ligands, and (c) redox potentials of complexes **1**−**10**: (○) chiral complexes having Δ-*cis*-α-(*RR*, $\lambda\delta\lambda$) configuration (Δ-type); (■) chiral complexes having Λ-*cis*-α-(*SS*, $\delta\lambda\delta$) configuration (Λ-type); (△) achiral complexes made up of both Δ- and Λ-types. Data are expressed as the means plus or minus the standard deviations of three experiments.

of the blue complex **8** has been revealed to have an optical isomer of Λ-type (Figure 2b).[19] In addition, the optical isomers of complexes **4**, **6**, and **10** were recognized to have Λ-type configuration by measuring their CD spectra.[18]

We have found that the serum glucose level correlates well with serum FFA level in both normal rats and STZ-rats.[20] The results indicate that the *in vitro* evaluation method of FFA release using adipocytes is not only useful to test and find insulin-mimetic compounds but also is simple, accurate, and sensitive when compared with the glucose incorporation method using radio-isotopes.[1b,14,21−24] In fact, with this evaluation method in terms of FFA release from adipocytes, many oxovanadium(IV) complexes with different coordination modes, which show blood glucose normalizing effects in *in vivo* evaluation, have been found.[1,3,10−12] Therefore, the insulin-mimetic activity of the complexes was examined in the *in vitro* system using adipocytes, where the inhibition of the release of FFA from isolated rat adipocytes treated with epinephrine was estimated in terms of IC_{50} value, the 50% inhibition concentration of the FFA release from adipocytes in the presence of the complex.[25] The relationship between the insulin-mimetic activity and the partition coefficient of the complexes **1**−**10** was examined and summarized, as shown in Figure 3a. The insulin-mimetic activities of the complexes **4**, **6**, **8**, and **10** with Λ-type configurations, which contain D-amino acids, were higher than those of the corresponding L-amino acids except for the activity of **8**, which is almost equivalent to that of complex **7**. In contrast, the insulin-mimetic activities of the complexes **3**, **5**, **7**,and **9** with Δ-type configurations containing L-amino acids increased remarkably with increasing of the partition coefficients, indicating that the activity depends on the lipophilicity of the complex.[26] However, the insulin-mimetic activities of the complexes **1** and **2** with both Δ- and Λ-type configurations, which contain achiral amino acids, were found to be greater than those of others as well as that of VS.[10,11] In

particular, complex **1** (IC_{50} = 0.070 mM) without side chains in the GeG ligand exhibited the strongest activity among the complexes examined. These results indicate that the complex with Λ-type configuration or high lipophilicity reaches the target organs and sites such as insulin-receptor, glucose-transporter or related enzyme systems and exhibits insulin-mimetic activity. However, to verify our assumption we need more experiments with other cell systems involving muscle cells[27] and then *in vivo* evaluation with animals with IDDM[10,11] and NIDDM.[12] In the same way, the relationship between the insulin-mimetic activity of the complexes and pK_a values[28] of the amino acid ligands and redox potentials[29] of complexes **1**−**10** were examined, as shown in Figure 3b and 3c, respectively. In Δ-type complexes containing L-amino acids, the activities were markedly lowered with the increase of the pK_a values and the redox potentials. However, the Λ-type complexes have little dependence on them.

On the basis of these observations, the complexes with Λ-type configuration which contain achiral amino acids or D-amino acids were concluded to have high *in vitro* insulin-mimetic activity, being less dependent on the physical properties such as pK_a value of the ligand, partition coefficient, and redox potential of the complexes. However, the insulin-mimetic activities of the complexes with Δ-type configuration (L-isomer) were very sensitive to those physical parameters but were less active than the complexes with Λ-type configuration (D-isomer). These results suggest that the complexes with Λ-type configuration have high affinity to the insulin receptor or glucose transporter in terms of chiral recognition sites. In contrast, in oxovanadium(IV) complexes with leucine-based ligands, the D-isomer has been found to be less active than the L-isomer.[14] Such a difference might depend on the absolute configuration (Δ- and Λ-isomers) of chelation or the difference in the coordination modes, $VO(O_4)$ and $VO(N_2O_2)$, of the complexes, which in turn alters inhibition mechanisms at the enzyme levels. Thus, the stereospecific and structure-dependent insulin-mimetic oxovanadium(IV) complexes will be useful for further studying their binding features with the receptor or the transporter.

Furthermore, the present results are useful for designing and developing new and active insulin-mimetic oxovanadium(IV) complexes. In addition, the *in vivo* activity of the complexes as well as the action mechanism for the activity are quite interesting, and thus the investigations are now under way in this regard.

(18) The molecular structures of complexes **3**, **5**, and **9** and the CD spectra of the complexes **3**−**10** in water are available as Supporting Information. The detailed results and discussions of these studies will be reported elsewhere.

(19) Crystal data for **8**: triclinic, $P\overline{1}$ (No. 1), a = 7.6043(5) Å, b = 9.049-(1) Å, c = 6.7747(9) Å, α = 99.20(1)°, β = 102.186(8)°, γ = 78.951(9)°, V = 443.77(9) Å³, Z = 1, GOF = 1.36. Data collected over the range $2\theta_{max}$ = 136.02° at 23 °C on a Rigaku AFC7R diffractometer with Cu Kα radiation. Convergence to the final R values of R = 0.028 and R_w = 0.038 (I > 3.00$\sigma(I)$) for **8** achieved by using 1602 reflections and 293 variable parameters. Details of X-ray crystallographic analyses are in Supporting Information.

(20) Nakai, M.; Watanabe, H.; Fujiwara, C.; Kakegawa, H.; Satoh, T.; Takada, J.; Matsushita, R.; Sakurai, H. *Biol. Pharm. Bull.* **1995**, *18*, 719.

(21) Dubyak, G. R.; Kleinzeller, A. *J. Biol. Chem.* **1980**, *255*, 5306.

(22) Sakurai, H.; Tsuchiya, K.; Nukatsuka, M.; Sofue, M.; Kawada, J. *J. Endocrinol.* **1990**, *126*, 451.

(23) Duckworth, W. C.; Solomon, S. S.; Liepnieks, J.; Hamel, F. G.; Hand, S.; Peavy, D. E. *J. Endocrinol.* **1988**, *122*, 2285.

(24) Fantus, I. G.; Ahmad, F.; Deragon, D. *J. Endocrinol.* **1990**, *127*, 2716.

(25) Isolated rat adipocytes (2.7 × 10⁶ cells/mL) prepared as for refs 3, 11, and 20 were preincubated at 37 °C for 0.5 h with various concentrations of oxovanadium(IV) complexes in 1 mL of KRB buffer (120 mM NaCl, 1.27 mM CaCl₂, 1.2 mM MgSO₄, 4.75 mM KCl, 1.2 mM KH₂PO₄, and 24 mM NaHCO₃; pH 7.4) containing 20 mg of BSA (bovin serum albumin). A 10⁻⁵ M epinephrine solution was then added to the reaction mixture, and the

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 1200 rpm for 10 min. For the outer solution of the cells, FFA levels were determined with an FFA kit (Wako Pure Chemicals, Osaka, Japan). The IC_{50} values are expressed on the basis of the concentration of oxovanadium(IV) complex to inhibit 50% of the FFA released from the adipocytes.

(26) The correlation coefficient of the linear regression was greater than 0.966 for a total of four points (complexes **3**, **5**, **7**, and **9**) in the triplicated measurements. However, this coefficient was greater than 0.687 for a total four points (complexes **4**, **6**, **8**, and **10**).

(27) Bevan, A. P.; Burgess, J. W.; Yale, J.-F.; Drake, P. A.; Lachance, D.; Baquiran, G.; Shaver, A.; Posner, B. I. *Am. J. Physiol.* **1995**, *268*, E60.

(28) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum Press: New York, 1974; p 1.

(29) Redox potentials with respect to Ag/AgCl were electrochemically measured at a glassy carbon electrode in 0.1 M phosphate buffer (pH 7.0).