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Accounts

Chemistry and Biochemistry of Insulin-Mimetic Vanadium and Zinc Complexes. Trial for Treatment of Diabetes Mellitus

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The number of patients suffering from diabetes mellitus (DM), which is a chronic metabolic disorder and mainly classified as either insulin-dependent type 1 or non-insulin-dependent type 2, is increasing throughout the world. The sequence of DM progression makes this disease a major health risk in regard to microvascular disease that leads to kidney failure, blindness, and nerve damage as well as macrovascular disease that leads to amputations, cardiovascular disease, and stroke. To treat DM, several types of insulin preparations and synthetic drugs for type 1 and type 2 DM, respectively, are in clinical use. However, there are several problems concerning the insulin preparations and synthetic drugs, such as physical and mental pain due to daily insulin injections and defects involving several side effects, respectively. Thus, the disease demands extraordinary effects to define pathobiochemical pathways and strategies for prevention and to find new therapeutic approaches. For this purpose, oxovanadium(IV) (vanadyl, VO^{2+}) and zinc(II) containing complexes have been explored as treatments for both types of DM. This article reviews the current state of research on insulin-mimetic and antidiabetic metal complexes, with special focus on paramagnetic vanadyl and diamagnetic zinc(II) complexes with different coordination modes, together with possible reaction mechanisms. New drug delivery systems involving enteric-coated capsulation and a biopolymer are also reviewed.

One of the most important findings in the 20th century was that metal ions and their biomolecular complexes are essential for life on the earth and play roles in not only metalloproteins and metalloenzymes but also gene expression.[1,2] This fundamental knowledge showed that our health, aging, physiological disorders and diseases are related to the state of the metal ions and their biomolecular complexes in our body. Some clinically useful metal-containing compounds and metal complexes known as metallopharmaceuticals that can regulate human health have been developed to treat or cure many types of diseases. The most distinctive examples are the platinum (Pt) anti-tumor agents, cisplatin and related newly developed Pt complexes, the gold (Au) orally active anti-rheumatoidal agent auronofin, the aluminum (Al) and zinc (Zn) anti-ulcer agents scralfate and polaprezinc, respectively, the selenium (Se) anti-inflammatory agent ebselen, and the lithium (Li) anti-manic-depressive agent lithium carbonate.[1–3]

Since the 20th century, lifestyle in all nations of the world have changed, which in turn alters development of diseases. One of the most widespread lifestyle-related diseases in the 21st century is thought to be diabetes mellitus (DM). The number of patients that suffer from DM in the world has been forecasted to increase to approximately 333 million in 2025 from 194 million in 2003. DM is generally classified as either type 1 insulin-dependent that is caused by destruction of pancreatic B cells or type 2 non-insulin dependent that is caused by aging, lifestyle-related obesity, spiritual stress or other environmental factors.[4] Untreated DM sometimes causes many severe secondary complications, such as atherosclerosis (a disease resulting in loss of elasticity from destruction of the elastic fibers in the blood vessels), microangiopathy (disorders of the blood capillary), renal (kidney) dysfunction and failure, cardiac (heart) abnormalities, diabetic retinopathy (functional defect of the retina, which relates to the whole body disorder), and ocular disorders (eye disorders that often induce blindness). Thus, type 1 DM is controlled only by several insulin injections per day, and type 2 DM is treated by several types of synthetic therapeutics. However, both treatment methods have undesirable defects, such as painful injections after the measurement of blood glucose levels, and severe side effects, respectively. To eliminate such undesirable defects, creation and development of new therapeutic compounds to replace insulin injections and synthetic therapeutics are being explored to improve the lives of diabetic patients.

Vanadium(V) has been a part of DM research since the discovery of DM. In 1899, 23 years before Banting and Best re-

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ported the discovery of insulin and used it clinically to treat patients with DM,[5] French researchers reported the improvement of DM in human diabetes by administration of sodium metavanadate ($NaVO_3$).[6] Later in 1987, the in vivo insulin-mimetic effect of vanadium ions was confirmed.[7] Since these findings, the relationship between DM and vanadium has been intensively studied with a focus on the development of new antidiabetic vanadium complexes. In 1990, the first orally active antidiabetic vanadyl(+IV oxidation state of vanadium, VO^{2+}) complexes were proposed.[8] On the other hand, orally active antidiabetic zinc (Zn^{2+}) complexes were discoverd in 2002,[9] following the observations of in vitro insulin-mimetic activity of zinc(II) in 1980[10] and in vivo antidiabetic effect at high doses of zinc(II) ions after 1990.[11,12] This article reviews recent progress in the development of insulin-mimetic and antidiabetic vanadyl complexes, which follows recently dicovered zinc complexes.

Solution Chemistry of Vanadium and Zinc Complexes

Vanadium has an electronic configuration of $[Ar]3d^34s^2$, and the common oxidation states of vanadium include +II, +III, +IV, and +V. The +I oxidation state is rarely seen. Under physiological conditions, both the +IV and +V oxidation states of vanadium are accessible kinetically and thermodynamically. On the other hand, zinc has an electronic configuration of $[Ar]3d^{10}4s^2$, and therefore, the most common oxidation state of zinc is +II. In this section, the speciation, characterization and reactivity of vanadium and zinc complexes in solution are discussed.

Zinc forms three different complexes with monoprotonated didentate ligands (L): ZnL^+, ZnL_2, and ZnL_3^-.[13] Using the over-all stability constant of the complex ($\log \beta$) and proton dissociation constant of the ligand (pK_a), the speciation for the $[Zn^{II}]/[L]$ system can be calculated. Considering that neutral zinc(II) complexes probably permeate membranes by simple diffusion, this species distribution is useful to determine the optimum conditions for a given zinc concentration and pH. The determination of $\log \beta$ for zinc(II) complexes made it possible to discuss the relationship between the stability constant and the insulin-mimetic activity, e.g., zinc(II) complexes with lower $\log \beta$ than 10.5 exhibited higher insulin-mimetic activities than those of $ZnSO_4$ which was used as a control.[14,15] The speciation diagram, in which the fraction of each complex formed in solution is plotted against various pH values, is very useful for understanding which chemical species are predominant at given pH values. The detailed speciations of vanadyl-oligopeptides,[16] vanadyl-acetohydroxamic acid,[17] oxovanadium(IV,V)-D-saccharic, and mucic acids,[18] vanadyl-1,2-dimethyl-3-hydroxy-4(1*H*)-pyridinone in the presence of oxalate, citrate, and phosphate,[19] and vanadyl-acetylactamides[20] have also been intensively investigated.

IR spectroscopy is a very useful tool for analyzing the existence of functional groups in a molecule. The absorption band due to V=O stretching vibration of vanadyl complexes is usually observed at a higher wavenumber compared to those of vanadate complexes. The V=O stretching vibration, however, is susceptible to a number of influences including electron donation from basal plane ligand atoms, solid-state effects, and coordination of additional molecules. Therefore, there have been much work done to assign the V=O stretching vibration toward oxovanadium(IV) and (V) complexes.[21–23]

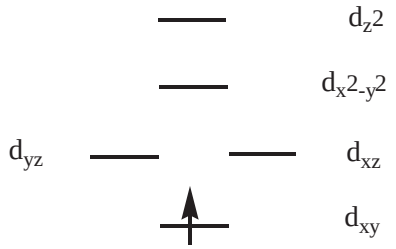
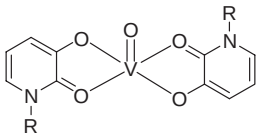


Fig. 1. Orbital energy levels for the d electrons of oxovanadium(IV) ion (VO^{2+}).

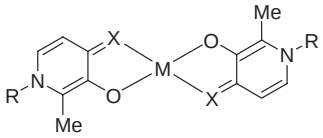
Electronic absorption spectra of vanadyl complexes are normally interpreted in terms of the energy level scheme derived from a molecular orbital study for a square-pyramidal structure with C_{4v} symmetry at the metal center,[24,25] in which the *z*-axis is taken as the vanadium–oxygen double bond, and the *x*- and *y*-axes are taken along the equatorial bonds (Fig. 1). In this scheme, b_2 (d_{xy}) < e (d_{xz}, d_{yz}) < b_1 ($d_{x^2-y^2}$) < a_1 (d_{z^2}), three electronic transitions are predicted, and indeed three absorption bands due to the d–d transitions are usually observed for vanadyl complexes.[21,22,26] However, in case of distorted vanadyl complex, four absorption bands are observed owing to the splitting of d_{xz} and d_{yz}.[27] The absorption bands that have extinction coefficients (ε) in the thousands and are assigned as ligand-to-metal charge transfer (LMCT) are sometimes observed.

Vanadyl ion, which has an unpaired electron, is paramagnetic. Electron paramagnetic resonance (EPR) spectroscopy can be used to investigate the electronic structure of vanadyl complexes.[28] A typical EPR spectra of bis(1,2-dihydro-4,6-dimethyl-2-oxo-1-pyrimidinolato)oxovanadium(IV) [VO(hopy-Me,H,Me)$_2$] (Fig. 2) measured at room and liquid nitrogen (77 K) temperatures are shown in Fig. 3. When a vanadium(IV) is in the vanadyl (VO^{2+}) state, the nuclear spin quantum number I of vanadium(IV) is 7/2. The number (n) of hyperfine signal of vanadyl state exhibits eight according to the equation; $n = 2I + 1$. Thus, in the EPR spectrum, eight resonance signals characteristic to the vanadyl state are observed in DMSO at room and liquid nitrogen temperatures, indicating that [VO(hopy-Me,H,Me)$_2$] exists as a single isomer. EPR parameters, such as universal constants (g-values) and hyperfine coupling constants (A-values) are calculated from the spectra. Based on a comparison of the parameters with those of different vanadium coordination modes, e.g., $VO(O_4)$, $VO(N_2O_2)$, $VO(O_2O_2^-)$, $VO(S_2N_2)$, and $VO(S_2O_2)$ reported previously,[29] [VO(hopy-Me,H,Me)$_2$] contains a V^{IV} ion with four equatorial O atoms.[16] The sensitively of the EPR parameters reflects the coordination environment around vanadyl ion. Therefore, the interaction of vanadyl complexes with blood serum components, such as oxalic acid,[19] lactic acid,[19] citric acid,[19] phosphoric acid,[30] saccharic acid,[18] mucic acid,[18] catecholamines,[31] apo-transferin,[32] and albumin,[32] has been extensively investigated by means of EPR spectroscopy. EPR spectra of various freshly isolated organs, such as liver and kidney, were also measured to estimate the coordination environment around the detected vanadyl species in the organs.[29,33] Detailed characterization of vanadyl complexes in various solvents[34] and the assignment of species in aqueous solutions of [VO(acac)$_2$][35]



X = O, M = VO: [VO(3,2-hopo-R)$_2$]

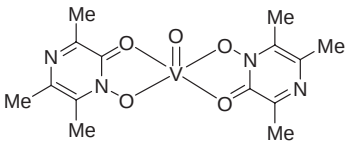
X = S, M = Zn: [Zn(3,2-hops-R)$_2$]



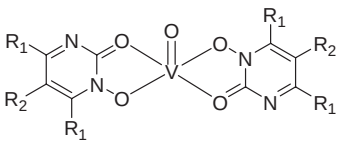
X = O, M = VO: [VO(3,4-hopo-R)$_2$]

X = S, M = VO: [VO(3,4-hops-R)$_2$]

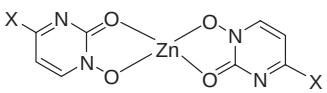
X = S, M = Zn: [Zn(3,4-hops-R)$_2$]



[VO(hopr)$_2$]



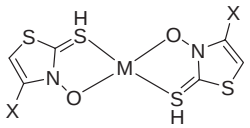
[VO(hopy-R$_1$,R$_2$,R$_1$)$_2$]



X = NH-R: [Zn(hopy-NH-R)$_2$]

X =(*S*)-NH-CH(Me)-CO$_2$Me: [Zn(hopy-L-ala)$_2$]

X = (*R*)-NH-CH(Me)-CO$_2$Me: [Zn(hopy-D-ala)$_2$]



X = *p*-RC$_6$H$_4$, M = VO: [VO(thia-Phe-R)$_2$]

X = *p*-RC$_6$H$_4$, M = Zn: [Zn(thia-Phe-R)$_2$]

X = Me, M = Zn: [Zn(thia-Me)$_2$]

Fig. 2. List of the vanadyl(IV) and zinc(II) complexes.

have also been performed using EPR spectroscopy.

The magnetic moment is used to determine the number of unpaired electrons in vanadium complexes. For example, the room temperature magnetic moment (μ_{eff}) of [VO(hopy-Me,H,Me)$_2$] is 1.80 μ_B, which corresponds to a spin-only value of a d^1 system. The value of the magnetic moment, therefore, is widely used for determining the oxidation states of vanadium complexes.[36–39]

The metallokinetic analysis of vanadyl species in the blood

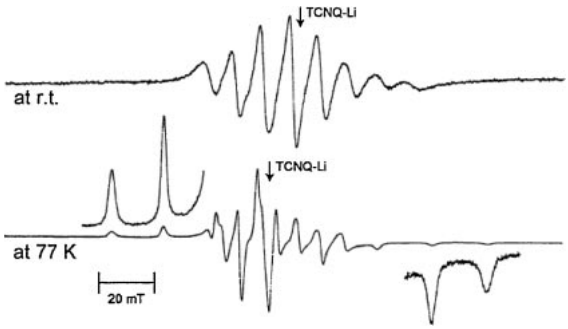


Fig. 3. EPR spectra of [VO(hopy-Me,H,Me)$_2$] in DMSO at room and liquid nitrogen temperatures.

of live rats that were given $VOSO_4$ and vanadyl complexes intravenously has been carried out using blood-circulation monitoring-electron paramagnetic resonance (BCM-EPR) method.[40–43] BCM-EPR is a useful method not only to determine the concentration of paramagnetic vanadyl species in the blood in real time but also to elucidate the global disposition of the vanadyl species in living animals.

Electron nuclear double resonance (ENDOR) is another tool for studying the fine structure of complexes containing paramagnetic ions, such as the vanadyl ion, because inhomogeneous line bordering in conventional EPR spectra often hides hyperfine splittings that arise from ligand nuclei.[28] ENDOR can sometimes recapture this lost resolution. For example, the positions of inner- and outer-sphere-coordinated solvent molecules were assigned by using ENDOR and selectively deuterated analogues of methanol.[44]

Electron spin-echo envelope modulation (ESEEM) is a specialized, pulsed EPR technique, that is used to detect weak nuclear hyperfine couplings. ESEEM spectroscopy is one of best techniques for characterization of vanadyl coordination environments. It has been demonstrated that ESEEM results not only show the presence or absence of nitrogen nuclei coordinated to vanadyl (and possibly the number of the coordinating nitrogen atoms) but also makes it possible to distinguish different types of nitrogen donors based on the empirical correlation between the type of the nitrogen and the ^{14}N-hyperfine coupling parameters.[28] The application of the ESEEM technique, for example, to vanadyl complexes with ligands, such as hydroxamic acids,[45] *N*-(2-hydroxyethyl)iminodiacetic acid,[46] 4-[2-(salicylidenamino)ethyl]imidazole,[47] *N*-{2-[(2-pyridylmethylene)amino]phenyl}pyridine-2-carboxamide (Hccapca),[48] and 1,2-bis(2-hydroxybenzamido)benzene (H_4hybeb)[49] is well documented.

NMR spectroscopy is not as useful to study the vanadyl moiety as it is to study oxovanadium(V) (vanadate) because vanadyl is paramagnetic. In other words ^{51}V NMR, ^{1}H NMR, and ^{13}C NMR are powerful tools for determining the solution conformations of vanadate complexes. For example, in the ^{51}V NMR spectrum of bis(1,4-dihydro-1,2-dimethyl-4-oxo-3-pyridinolato)methoxooxovanadium(V) [VO(3,4-hopo-Me)$_2$-(OMe)] that was measured in D_2O at pD 8 with $VOCl_3$ as an external standard, three vanadate species were shown to exist in D_2O, because three separated signals were observed at −480, −503, and −535 ppm (Fig. 4a). When an excess of the ligand 3,4-hopo-Me was added to the solution, the signals

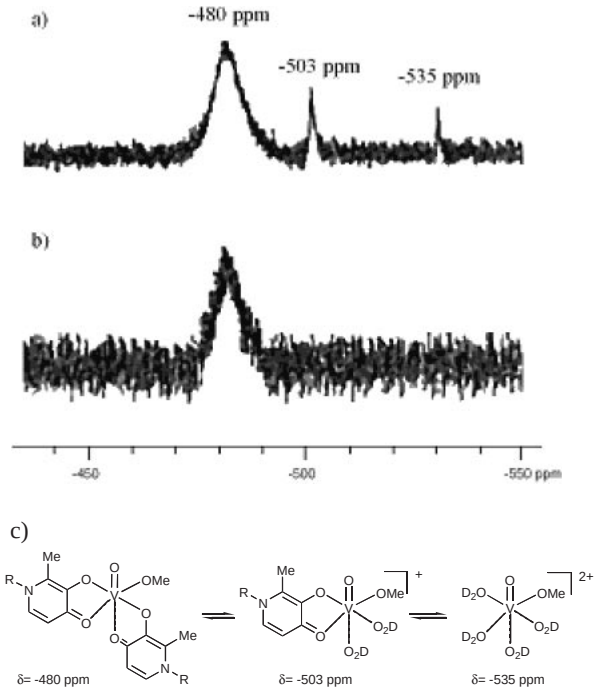


Fig. 4. ^{51}V NMR spectra of [VO(3,4-hopo-Me)$_2$(OMe)] in D_2O at pD 8. a) Oxovanadium(V) complex; b) after addition of the ligand 3,4-hopo-Me; c) the assignment of observed three signals.

at −503 and −535 ppm completely disappeared (Fig. 4b).[50] Based on this and other data,[51,52] the two minor signals were tentatively assigned to be the ligand dissociation products [VO(3,4-hopo-Me)$(D_2O)_2$(OMe)]$^+$ and [VO$(D_2O)_4$(OMe)]$^{2+}$ as shown in Fig. 4c. The oxidation kinetics of bis(maltolato)-oxovanadium(IV) [VO$(ma)_2$] in MeOH,[52] the interaction of *N*-hydroxyacetoamide with oxovanadium(V) ion in aqueous solution,[17] the solution structure of ammonium (dipicolinato)-oxovanadate(V),[53] the chelation properties of vanadate to mono-ionized diols and carbohydrates,[54] and the complex-forming tendency of vanadate with α-hydroxycarboxylic acids[55] have been examined in detail by means of ^{51}V NMR spectroscopy.

Zinc(II) is also diamagnetic, and thus the measurement of ^{1}H NMR spectrum of zinc(II) complexes is possible. When a complex is administered by oral administration, its stability under acidic conditions, like in the stomach (pH ≈ 2), is crucial. The stability of bis(1,2-dihydro-4-*N*-butylamino-2-oxo-1-pyrimidinolato)zinc(II) [Zn(hopy-NH-Bu)$_2$] (Fig. 2) was determined by measuring its ^{1}H NMR spectra in D_2O under various pD (2–7) conditions (Fig. 5). DCl (deuterium chloride) was used to adjust the solution's pD. In this experiment, the chemical shifts of the olefinic 5-H and 6-H protons shifted upon complexation with zinc(II). The spectrum of [Zn(hopy-NH-Bu)$_2$] at pD 2.60 was identical with that of the ligand hopy-NH-Bu at almost same pD, indicating that [Zn(hopy-NH-Bu)$_2$] is stable at a pD 4 or higher, but it decomposes below pD 2 to give the corresponding ligand hopy-NH-Bu and zinc(II).[56]

Electrochemistry have been used to examine the electronic effects of ligands as well as the electronic effects of substituents on ligands in metal complexes. The measurement of cyclic voltammogram provides the following useful information: 1) the reversibility of the redox process, 2) the standard redox

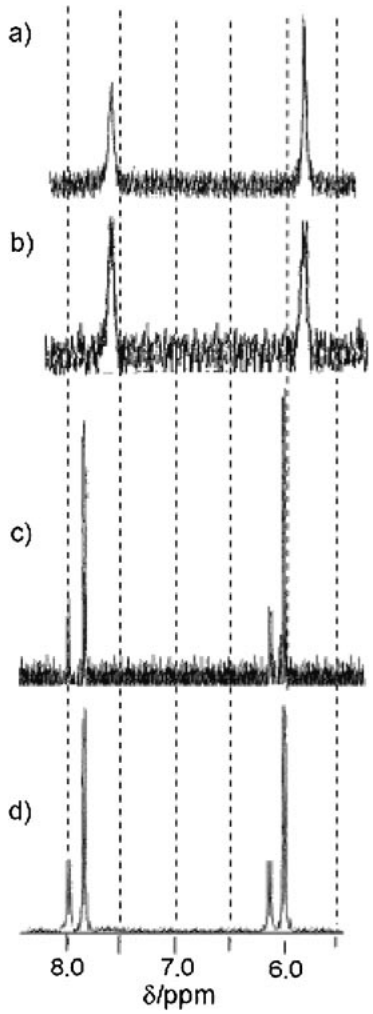


Fig. 5. ^{1}H NMR spectra of [Zn(hopy-NH-Bu)$_2$] and its ligand (hopy-NH-Bu) in various pD conditions. a) Complex at pD 7.0; b) complex at pD 4.4; c) complex at pD 2.6; d) hopy-NH-Bu at pD 2.4.

potential, 3) the stability of each oxidation state (of the resulting redox species), and 4) the adsorption behavior to the electrode. A typical cyclic voltammogram of bis(1,4-dihydro-1,2-dimethyl-4-oxo-3-pyridinolato)oxovanadium(IV) [VO(3,4-hopo-Me)$_2$] (Fig. 2) is shown in Fig. 6.[50] The electron-transfer process was nearly reversible because the i_{pa}/i_{pc} ratio (0.91) was close to unity, although the value of the peak-to-peak separation (ΔE_p = 110 mV) was greater than 60 mV. The $E_{1/2}$ vs Ag/AgCl value (315 mV) is approximately 130 mV lower than that of [VO$(ma)_2$],[51] indicating that the complex is less stable toward the oxidation. Because the ΔE_p value was larger than 60 mV, cyclic voltammgrams were measured at various scan rates (v). A plot of i_{pc} and $v^{1/2}$ gave a straight line, indicating that the electron transfer is diffusion controlled. The electrochemistry of vanadyl complexes with ethylenebis[(*o*-hydroxyphenyl)glycine],[57] Schiff-base ligands derived from optically active 1,2-diamines and salicylaldehydes,[58] bis(catecholamide),[59] mixed ligands of tridentate hydrazones and 2,2′-bipyridine,[60] maltol,[51] *N*-(2-hydroxybenzyl)-*N*′-(2-hydroxyethyl)-*N*,*N*′-bis(2-pyridylmethyl)ethane-1,2-diamine (H_2bbpeten),[27] *N*,*N*′-ethylenebis(amino acids),[61] and 3-hydroxypyri-

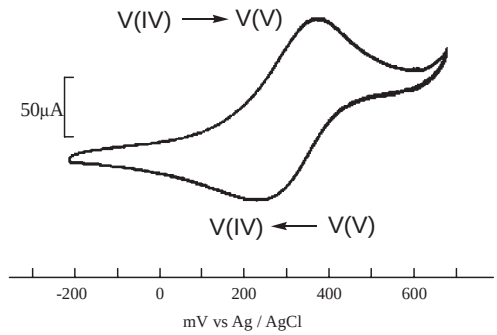


Fig. 6. A cyclic voltammogram of $[VO(3,4\text{-hopo-Me})_2]$ in 0.1 M aqueous NaCl solution. Scan rate: 100 mV s^{-1}.

dine-2-carboxylic acid[38] have been reported.

In general, circular dichroism (CD) spectroscopy provides valuable information about the changes in conformation and configuration, the absolute configuration, and the secondary structure of peptides and proteins in solution. In the case of vanadyl complexes with optically active ligands, CD spectrometry has mainly been used to clarify the coordination modes of ligands and the metal ion-promoted deprotonation/coordination of terminal functional groups.[61–67] Further, CD spectroscopy was used to analyze the specific binding of the vanadyl ion with the thiolate of the cysteine-34 residue in bovine serum albumin (BSA).[68]

Radiolabeled vanadium (vanadium-48; $t_{1/2} = 15.97$ days) has been used to examine the behavior of vanadyl and vanadate ions in canine blood[69] and to study the exchange behavior of vanadium between transferrin and ferritin.[70] Also, zinc (zinc-65; $t_{1/2} = 244.26$ days) has been used to investigate the uptake of zinc by erythrocytes in the presence of maltol and its analogues.[13]

Biochemical and Physiological Functions of Vanadium and Zinc

Vanadium. Vanadium, which was found in 1831 by Sefström, was proposed to be an essential nutrient in some animals by different research groups in 1971,[71,72] and then in 1973.[73] However, the extent to which it is essential in not only animals but also human being is still been argued in 21st century.[74]

A dramatic and surprising change in vanadium biochemistry occurred in 1977 when Cantley et al. identified that endogenous vanadium (vanadate) strongly inhibited sodium and potassium ATPase (Na,K-ATPase), because vanadium was present in an ATP preparation derived from equine muscle.[75] This finding, which was confirmed by other researchers the next year,[76–78] started a new era of vanadium biochemistry and stimulated the studies on vanadium in many enzyme systems involving adenylate cyclase, tyrosine kinase, phosphotyrosyl phosphatase and ribonuclease.[79]

In addition to the vanadate in equine muscle, a vanadium-containing protein, bromoperoxidase, was found by Vilter in marine algae in 1984,[80] research on the vanadium-containing haloperoxidases that were isolated from a terrestrial lichen and fungi increased. Haloperoxidases catalyze the oxidation of a halide (chloride, bromide, or iodide) by hydrogen peroxide to the corresponding hypohalous acid, which in turn results in halogenation of organic compounds. Many of the organohalogens generated by these enzymes are biocidal and provide defense. Thus, three types of haloperoxidases identified to date include vanadium-containing haloperoxidases, heme-iron haloperoxidases and nonmetallo haloperoxidases. Interestingly, the oxidation state of vanadium in the enzymes is +V (vanadate), and the redox state of the metal does not change during the catalytic cycle, in which an activated peroxo-intermediate was identified during the catalytic reactions.[81]

Another type of vanadium-containing enzyme was initially discoverd by Bortels in 1930.[82] Vanadium-containing nitrogenase was purified from *Azotobacter vinelandii* which was grown in the presence of vanadium. To date, vanadium-nitrogenase have been purified from *A. vinelandii* and *Azotobacter chroococcum*. Genes coding for the structural components of vanadium-nitrogenase have been detected by DNA hybridization in a variety of strains of the family *Azotobacteriaceae* and in the cyanobacterium *Anabaena variabilis*.[83]

A unique natural vanadium(IV)-containing compound, (*S*,*S*)-2,2′-(hydroxyimino)dipropionic acid $[V(hida)_2]$, was found in some *Amanita* fungi, such as *Amanita muscaria*, and named as amavadine by Bayer and Kneifel in 1972.[84] This compound was initially proposed to be a vanadyl complex; however, later an unusual octa-coordinate structure of the bare vanadyl complex was suggested in 1987. The octa-coordinate structure was confirmed by X-ray crystal structure analysis in 1988.[85] Although amavadine was thought to be involved in some biological electron-transfer processes, recent electrochemical studies show that it acts as a mediator in the oxidation of thiol compounds with carboxylic or ester groups that have biological significance, such as cysteine and glutathione.[86]

Since vanadium was proposed to be an essential nutrient in 1971,[71,72] a wealth of new results on the physiological roles of vanadium in cells and organisms has been produced (Fig. 7).[79] In 1899, French physicians found that sodium metavanadate ($NaVO_3$) improved state of human patients with diabetes mellitus[6] before the discovery of insulin in 1922 by Banting and Best.[5] In 1912, vanadium was suggested to be a panacea for human disorders,[87] and it has been proposed numerous times to be pharmacologically and nutritionally important.

Originally vanadium was studied as possible pharmaceuticals for treating syphilis, reducing serum cholesterol and pre-

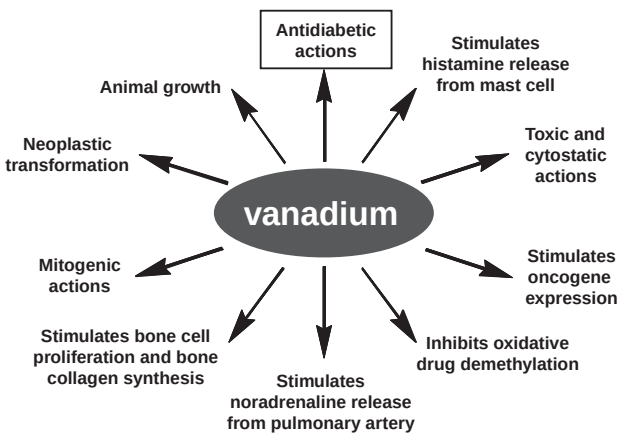


Fig. 7. Physiological roles of vanadium (Ref. 79).

venting caries, but current focus is to create pharmaceuticals that will take advantage of its insulin-mimetic and antidiabetic properties.[74]

Zinc. Zinc, which was found by Marggrab in 1746, was determined to be an essential trace element in animals and human beings in 1977.[88] However, its potential for growth and development and the transmission of the genetic message was not determined until 1993,[89] because of its chemical properties like being colorless and diamagnetic.

Unlike iron, of which 80% of the 3 g in humans occurs in heme iron proteins, the 2.3 g of zinc in humans, is spread among thousands of proteins. The broad distribution of zinc has made it difficult to establish the biochemical and physiological roles of zinc. Nevertheless, there are now approximately 200 three-dimensional structures for zinc proteins representing all six classes of enzymes and covering a wide range of phyla and species. Three primary types of zinc proteins and enzymes are known: structural, catalytic, and cocatalytic. The most common amino acids involved in these three types are His, Gln, Asp, and Lys.[90]

Unique zinc proteins were first discovered in 1980's. The first transcription factor found in 1983 was identified as a zinc enzyme,[91] which led to introduce the term DNA-binding finger protein in 1985.[92] Within 15 years after the finding of the zinc-finger protein, hundreds of proteins have been identified.[93] In 1995, a zinc transporter, which participates in a homeostatic system in cell, was discovered.[94] Metallothionein (MT), which was found in 1957, was revealed to link zinc distribution to the redox state of cell in 1998.[95] In 2000, a zinc-containing regulatory protein was found to have a role in neurotransmission.[96]

The physiological roles of zinc in cells and organs are summarized in Fig. 8. Among them, the antidiabetic activity is currently thought to be an important role of zinc. In fact, zinc and diabetes intersect at several points during metabolism in a cell.[97,98]

In 1980, zinc was found to stimulate rat adipocyte lipogenesis similar to the action of insulin,[10] which was followed by the observations on in vivo antidiabetic effects of oral $ZnCl_2$ in STZ-rats and ob/ob mice in 1992[11] and 1998,[12] respectively. Because the bioavailability of $ZnCl_2$ is relatively low, the coordination chemistry of zinc(II) ion was explored, and the first orally active insulin-mimetic and antidiabetic zinc(II) complexes were discovered in 2002.[9] Since then, a wide variety of zinc(II) complexes with different coordination structures have been synthesized.[97,99]

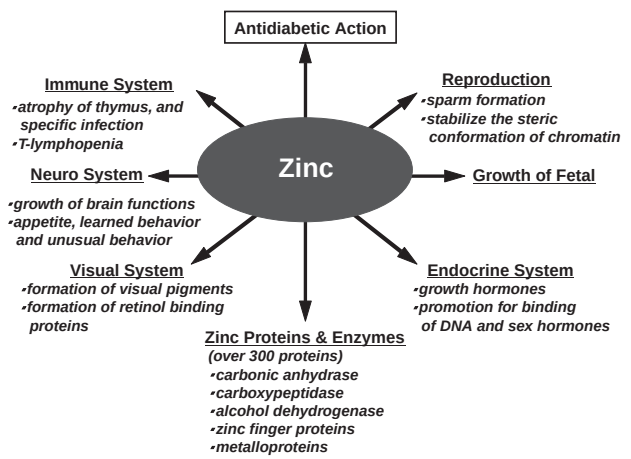


Fig. 8. Biochemical and physiological roles of zinc.

Treatment of Diabetes by Vanadium and Zinc Complexes

Because of new types of metallodrugs, progress in the research for new treatments for diabetes mellitus began in 1990, when the first orally active insulin-mimetic vanadyl-cysteine methyl ester complex was introduced to treat type 1 diabetic model animals.[8,97] Metal complexes that are to be used as metallodrugs are required to possess high bioavailability, which involves many important factors, such as low molecular weight, neutral charge, coordination environment around the metal center, moderate stability constant, moderate partition coefficient, high stability in the presence of many proteins and other biomolecules, and zero-toxicity.

On the other hand, the establishment of a reliable in vitro appraisal system is required to evaluate metal ions and metal complexes for their potential antidiabetic activity. For this purpose, an appraisal system was developed with respect to the interaction of metal ions with isolated rat adipocytes (adipose cells prepared from the epididymal fat tissue) treated with adrenaline (epinephrine).[100]

Although the mechanism by which metal ion works as insulin-mimetics has not yet been fully elucidated, there is evidence that insulin receptors are activated by inhibiting protein tyrosine phosphatase (PTP1B), which is related to the activation of cytosolic nonreceptor tyrosine kinase, direct phosphorylation of insulin receptor substrate 1 (IRS1), and activation of phosphatidylinositol 3 kinase (PI3K), leading to glucose transporter 4 (GLUT4) translocation, as well as the activation of phosphodiesterase (PDE).[97,101–104] As expected, $VOSO_4$ showed insulin-mimetic activity with regard to both incorporation of the glucose in the rat adipocytes as well as inhibition of the free fatty acids (FFA) release from the adipocytes.[29,100,105] It is well known that insulin has only one action site, insulin receptor. However, both vanadyl and zinc(II) ions have been found to have multiple action sites in the adipocytes. Therefore, the mechanism of action was called an "ensemble mechanism" (Fig. 9). Both glucose incorporation and FFA release can be examined with simple determination kits,[100,105] which are convenient to use in a normal laboratory whether or not metal ions and metal complexes have insulin-mimetic activity.

With the goal of developing active insulin-mimetic and antidiabetic metal complexes, a wide variety of metal complexes with different coordination environment around the metal centers has been prepared (Fig. 10).[97,101,102,106–110] In the next section, insulin-mimetic and antidiabetic vanadyl and zinc complexes and in vivo evaluations are discussed.

Vanadyl and Zinc Complexes with (N_2O_2) Coordination Set. The insulin-mimetic vanadyl complex with a $VO(N_2O_2)$ coordination environment, bis(picolinato)oxovanadium(IV) $[VO(pa)_2]$ complex, was tested in 1995 following evaluations of in vitro insulin-mimetic activity in isolated rat adipocytes and in vivo hypoglycemic ability in streptozotocin (STZ)-induced type 1 diabetic rats (STZ-rats), that received daily intraperitoneal (*ip*) injections and oral administrations.[111] From $[VO(pa)_2]$, which has a partition coefficient ($\log P$) of -0.48 in an *n*-octanol/buffer (pH 7.4) system, many analogues were

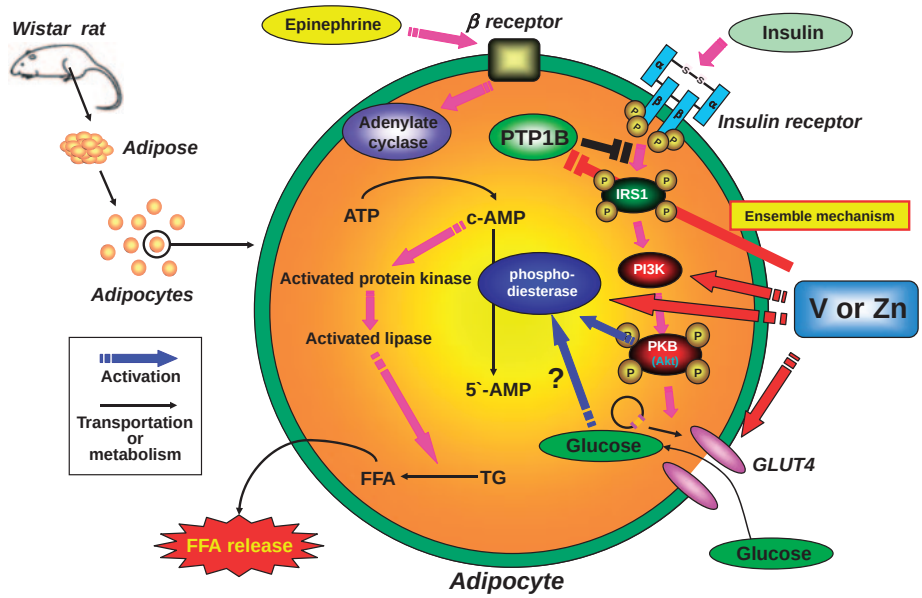


Fig. 9. A proposed "ensemble mechanism" of glucose incorporation and free fatty acids (FFA) release catalyzed by vanadium and zinc in isolated rat adipocytes.

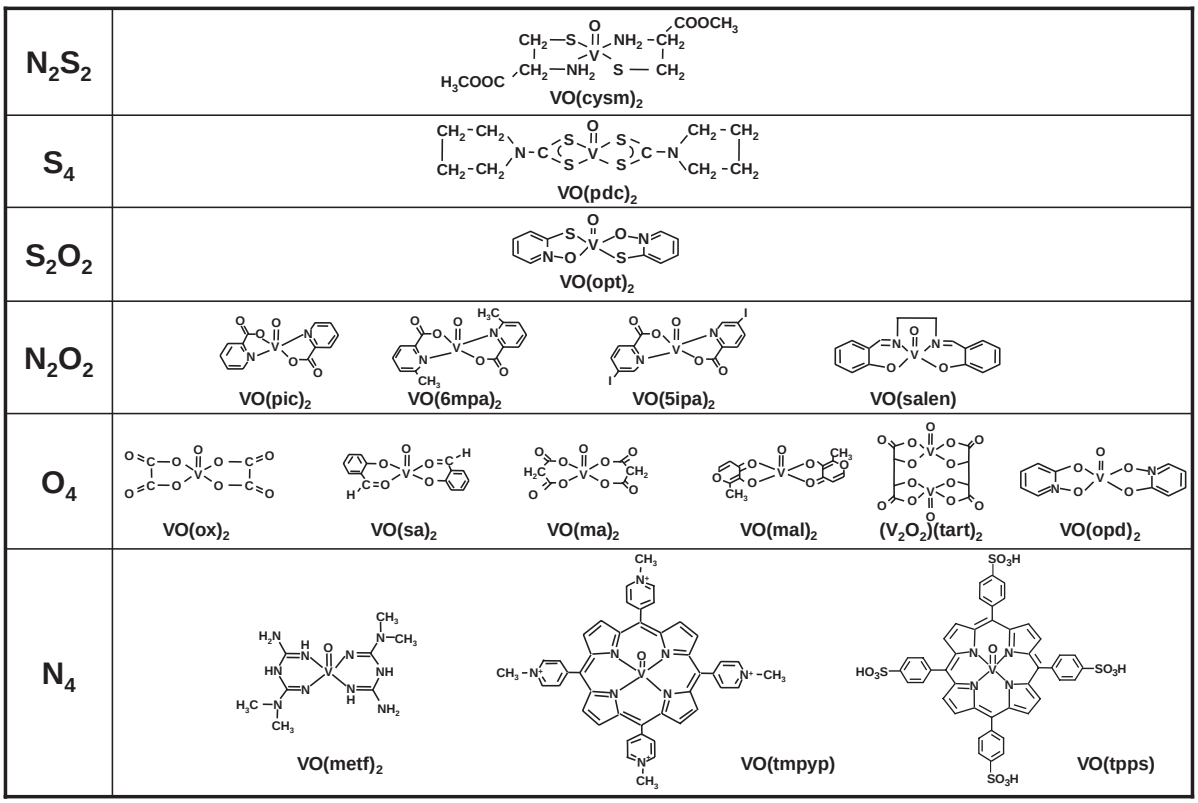


Fig. 10. Insulin-mimetic and antidiabetic vanadyl complexes with different coordination modes. The complex abbreviations appear in the text.

prepared in order to examine relationship between the structure, and insulin-mimetic and antidiabetic activities by introducing electron-donating or electron-withdrawing groups at different positions of the pyridine ring in picolinate ligand. In fact, both bis(6-methylpicolinato)oxovanadium(IV) [VO(6mpa)$_2$] ($\log P = -0.23$), prepared in 1997,[112] and bis(5-iodopicolinato)oxovanadium(IV) [VO(5ipa)$_2$] prepared in 2001,[113] exhibited better in vitro insulin-mimetic activity and in vivo hypoglycemic effects in STZ-rats than the leading [VO(pa)$_2$] complex. The former, in particular, was found to continue working for at least 80 days after stopping of daily oral administrations which lasted 20 days. It was suggested that the long lasting effects were due to both the accumulation of vanadium in the bone tissue, which was determined by neu-

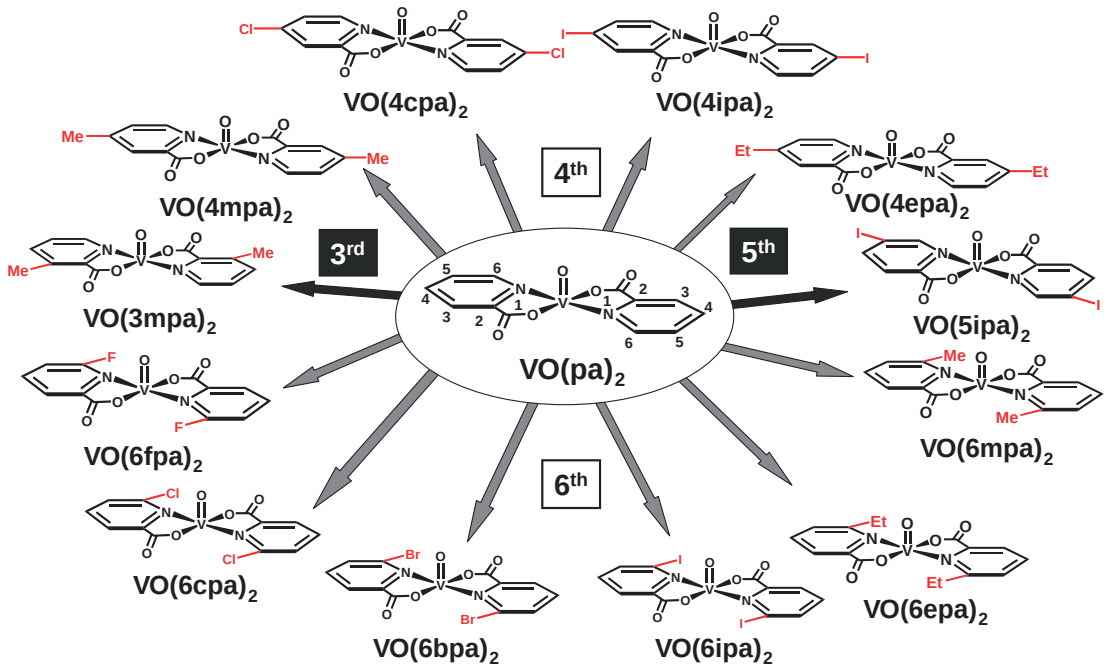


Fig. 11. Thirteen vanadyl-picolinate related complexes used for the study of the structure–activity relationship. The positions of the added substituent in picolinate ligand are indicated as 3rd, 4th, 5th, and 6th.

tron activation analysis (NAA), and the formation of a ternary complex composed of picolinate–vanadyl–protein or amino acids complexes in the liver and kidney, which was observed by ESEEM study.[114] In addition, $[VO(6mpa)_2]$ was found to cause a hypoglycemic effect in a hereditary type 2 diabetic animal, the KK-A^y mouse, as a result of daily *ip* injections and oral administrations.[115] In 2005, among 13 vanadyl analogue complexes of $[VO(pa)_2]$ (Fig. 11), both bis(4-methylpicolinato)oxovanadium(IV) $[VO(4mpa)_2]$ and bis(4-iodopicolinato)oxovanadium(IV) $[VO(4ipa)_2]$ were found to be better at inhibiting FFA release and causing in vivo hypoglycemic effect in STZ-mice that received a single *ip* injection than the previously studied $[VO(6mpa)_2]$ and $[VO(5ipa)_2]$,[116] suggesting the importance of the substituent position on the ligand.

Detailed structural conformation from X-ray crystallographic analysis of the complexes is essential for discussing their insulin-mimetic and antidiabetic activities in a molecular level. However, because good crystals of $[VO(pa)_2]$ and related complexes suitable for X-ray structure analysis have been difficult to obtain, the structures of the complexes were determined and characterized by electronic absorption, IR, EPR, EXAFS, and MS spectra. $[VO(pa)_2]$, $[VO(3mpa)_2]$, and $[VO(5ipa)_2]$ have a six-coordinate structure with an additional V–OH_2 bond. In contrast, $[VO(6mpa)_2]$ and $VOSO_4$ do not have a coordinated H_2O molecule and, therefore, have a five-coordinate structure.[117] However, the structure of $[VO(6epa)_2]$ could be analyzed by X-ray,[118] where two distinct molecules were observed in an asymmetric unit cell. Each vanadium ion in $[VO(6epa)_2]$ is coordinated by two carboxylate oxygens, two pyridine nitrogens, one vanadyl oxygen and one water oxygen, forming a distorted octahedral geometry. The two carboxylate oxygens and the pyridine nitrogens occupy an equatorial plane, and the two ligands coordinated to the vanadium center in a *trans* arrangement.

Similarly to the vanadyl complexes, zinc(II)–picolinate complexes, such as $[Zn(pa)_2]$,[119] $[Zn(6mpa)]$,[119] and bis(6-methylpicoline methylamido)zinc(II) $[Zn(6mpa\text{-}ma)_2]$,[118] were prepared. In a colorless single crystal of $[Zn(6mpa)_2(H_2O)]\cdot H_2O$ complex, the coordination geometry around the zinc(II) ion is a distorted trigonal bipyramidal structure with the equatorial plane containig O1, O1′, and O3 and with two apical N atoms (N1 and N1′).[119]

Zinc complexes with $\log\beta$ lower than 10.5 exhibit higher in vitro insulin-mimetic activities than those of $ZnSO_4$ and $VOSO_4$. And $[Zn(pa)_2]$ with a $\log\beta = 9.52$ showed higher activity than those of $ZnSO_4$ and $VOSO_4$. However, zinc complexes with $\log\beta$ values higher than 11.0 show essentially no insulin-mimetic activity.[14,119]

Based on the in vitro evaluation, both $[Zn(pa)_2]$ and $[Zn(6mpa)_2]$ exhibited high hypoglycemic effects in KK-A^y mice that were subjected to a single *ip* injection and daily *ip* injections at a dose of 3.0 mg Zn kg^{-1} body weight for two weeks.[9] The improvement in diabetic state of the KK-A^y mice was related to the reduction in the hemoglobin A_{1C} (HbA_{1C}) level, which is an index for glycemic control, from 8.3 to 6.5% by the complex administration.

Vanadium and Zinc Complexes with (O_4) Coordination Set. Orally active insulin-mimetic vanadyl complexes with a $VO(O_4)$ moiety were first prepared in 1990,[8] and the order of hypoglycemic effect in STZ-rats is $[VO(malonato)_2] > [VO_2(tartarato)_2] > [VO(salicylaldehydato)_2] > [VO(oxalato)_2]$. Bis(maltolato)oxovanadium(IV) $[VO(ma)_2]$ was then prepared McNeill et al. in 1992.[121] The X-ray crystal structure of $[VO(ma)_2]$ was determined in 1995,[51] and it has a structure similar to bis(3-hydroxypyronato)oxovanadium(IV) $[VO(3hp)_2]$. Since both of these complexes are bioactive, the structure–activity relationship with $[VO(3hp)_2]$ as a starting point was examined in detail (Fig. 12).[122]

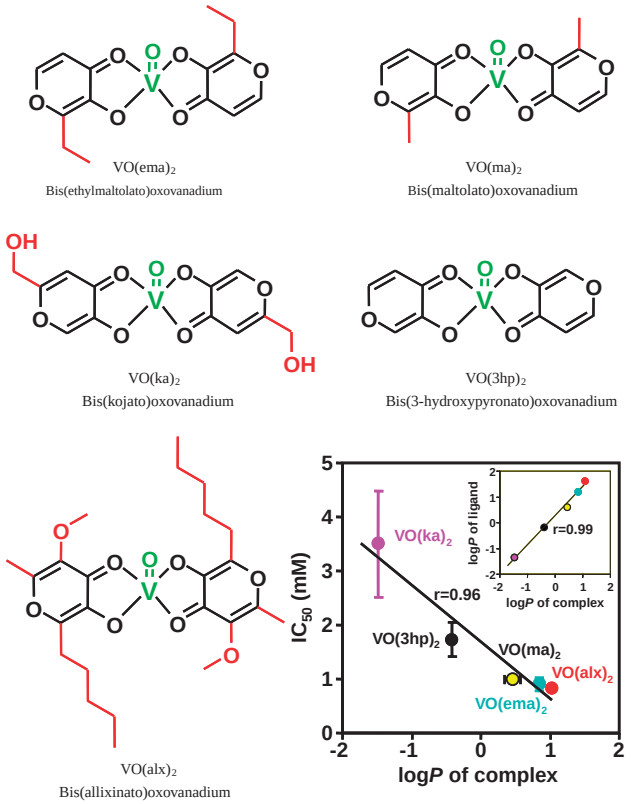


Fig. 12. Structures of vanadyl-3-hydropyronate related complexes, and the correlation between IC_{50} value and partition coefficient (log *P*) of the complexes. In vitro insulin-mimetic activity is expressed in terms of IC_{50}, which is defined as 50% inhibitory concentration of the complex on the FFA release from isolated rat adipocytes treated with adrenaline (epinephrine).

A vanadyl complex with allixin, which was derived from dried garlic after standing for approximately two years, bis(allixinato)oxovanadium(IV) $[VO(alx)_2]$, afforded the best in vitro insulin-mimetic complex in terms of both suppression of FFA release and glucose incorporation in isolated adipocytes.[122] This complex has an advantage in treating not only type 1 diabetes (STZ-rats) but also type 2 diabetes (KK-A^y mice) on oral administration (Fig. 13). In addition, $[VO(alx)_2]$ has several characteristics, that fit the requirements for clinical use in the future. These include maintaining body weight through an antiobesity effect, which also maintains food intake and suppression of plasma leptin levels, improvements in lipid metabolism in terms of reducing the total cholesterol levels, and a blood pressure lowering effect by daily oral administrations for one month.

Vanadyl complexes with the ligands maltol, allomaltol, and isomoltol (ima) were prepared, and their in vivo hypoglycemic effects in STZ-rats were examined.[109] Among the complexes, $VO(ima)_2$ was found to show a significant hypoglycemic effect. However, none were better than $VO(ma)_2$.

Prepared in 2003, bis(1-oxy-2-pyridonato)oxovanadium(IV) $[VO(opd)_2]$[42] with $VO(O_4)$ coordination environment is an oxygen analogue of bis(1-oxy-2-pyridinethiolato)oxovanadium(IV) $[VO(opt)_2]$ with $VO(S_2O_2)$ coordination environment that was reported in 1999 (Fig. 10).[40,123,124] $[VO(opd)_2]$

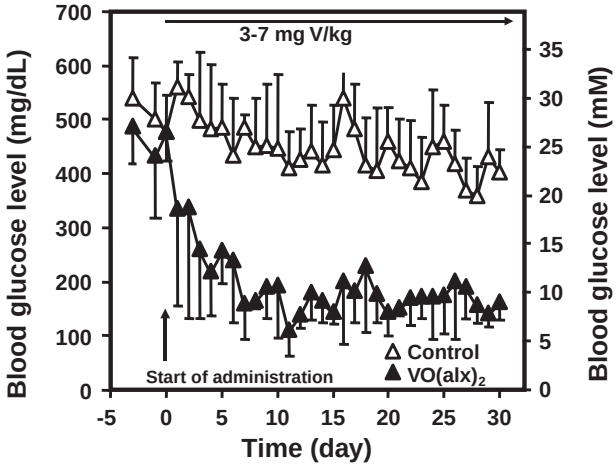


Fig. 13. Hypoglycemic (blood glucose lowering) effect in KK-A^y mice treated with $[VO(alx)_2]$ complex via daily oral administrations for one month.

has an advantage over $[VO(opt)_2]$ for treating type 1 diabetes because $[VO(opd)_2]$ is more effective at lower doses than $[VO(opt)_2]$ when they are orally administered.

Similar to the vanadyl complexes, zinc(II) complexes with 3-hydroxypyronate and related ligands were prepared, and the X-ray crystal structure of bis(maltolato)zinc(II) $[Zn(ma)_2]$ was determined.[125] Two different geometries around the zinc center in the colorless complex *trans*-$[Zn(ma)_2(H_2O)][Zn(ma)_2(H_2O)]\cdot 2(H_2O)$ were found in the unit cell. One zinc ion has two maltols and two water molecules coordinated in a *trans* octahedral conformation, and the other has two maltols and an apical water molecule found in a square pyramidal conformation.

All of the zinc(II) complexes have been found to be effective in treating type 2 diabetes through oral administration. Bis(allixinato)zinc(II), $[Zn(alx)_2]$, not only lowered high blood glucose levels but also improved HbA_{1C} levels in type 2 diabetic KK-A^y mice after daily oral administrations at 15 mg Zn kg^{-1} body weight for 2 weeks (Fig. 14).[126]

Bis(*S*-allixinato-*N*-methyl)zinc(II) $[Zn(sanm)_2]$ was determined by in vitro evaluations to be an excellent complex for suppressing FFA release in adipocytes. It has 20 fold higher activity than that of $[Zn(ma)_2]$.[127] The in vivo evaluations on this complex will be reported.

A new zinc(II) complex involving hinokitiol[128] (β-thujaplicin) is also effective when administered by *ip* injection.

Dinuclear Vanadyl Complexes. Among many vanadyl complexes, a dinuclear vanadyl(IV)–tartrate complex, dioxobis(L-tartrato)divanadium(IV) $[(V_2O_2)(L\text{-tart})_2]$ with $VO(O_4)$ coordination structure was also shown to have hypoglycemic effect in STZ-rats on oral administration in 1990 (Fig. 10).[8] However, although the importance of dinuclear structure and chirality of the vanadyl complex for exhibiting the antidiabetic effects has been demonstrated, these aspects have received little attention since 1990. Thus, several dinuclear vanadyl complexes of L-, D-, and racemic tartaric acids were prepared using different cations, such as NH_4^+, Na^+, and K^+. After structural analysis of the complexes, of which the structure of $[Na_4(V_2O_2)(D\text{-tart})(L\text{-tart})]$ was being determined in 1968,[129] the in vitro insulin-mimetic activity was examined, and both $[Na_4$-

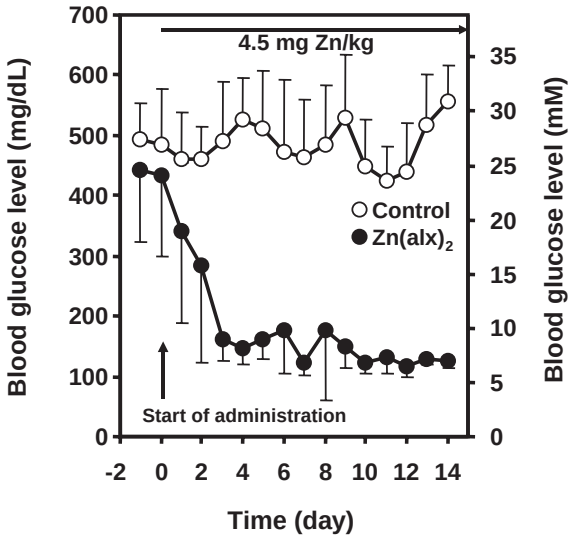


Fig. 14. Hypoglycemic (blood glucose lowering) effect in KK-A^y mice given [$Zn(alx)_2$] complex on daily oral administrations for two weeks.

(V_2O_2)(L-tart)$_2$] with the naturally occurring ligand L-tartaric acid and racemic [$Na_4(V_2O_2)$(D-tart)(L-tart)] exhibited the highest activity.[130] Following single oral administration of both complexes in STZ-mice at a dose of 20 mg V kg^{-1} body weight, both exhibited a normoglycemic effect. Since the blood glucose levels of STZ-mice given [$Na_4(V_2O_2)$(L-tart)$_2$] were less dispersed than those of the racemic complex, the former was subjected to daily oral administrations for 10 days, in which both high-response and low-response groups to the complex were observed. In the high-response group, a complete normoglycemic effect of the complex was seen as long as the daily complex administrations were continued. The occurrence of tolerance of the complex for the STZ-mice suggests a need for biochemical study involving some enzymatic systems in cells and tissues that are relevant to lipid and glucide metabolisms.

Other insulin-mimetic macrocyclic binuclear vanadyl complexes, for example, 6,6′-piperazine-1,4-diylmethylenebis(4-methyl-2-formylphenolato)dioxovanadium(IV), have been reported to be effective in STZ-rats.[131]

Vanadium and Zinc Complexes with 1-Hydroxy-2(1*H*)-pyrimidinones and 3-Hydroxythiazole-2(3*H*)-thiones. A heterocyclic compound is defined as "a cyclic compound in which one or more of the atoms of the ring are heteroatoms such as nitrogen, oxygen, sulfur, and so on."[132] A typical example for a heterocyclic compound is pyridine and of homocyclic compound is benzene. Heterocyclic compounds are very attractive compounds because they are found in natural products and in bioactive molecules such as nucleic acid bases in the living body. In addition, heterocyclic compounds are found in dyes, pigments, agrochemicals, pharmaceuticals, and electronic materials. Therefore, the use of heterocyclic compounds as ligands for vanadyl and zinc ions is quite interesting for the development of new insulin-mimetic complexes.

In this section, heterocyclic compounds, such as didentate ligands for zinc and vanadyl complexes, especially, hydroxymonoazine, hydroxydiazines,[26] and hydroxythiazole-2(3*H*)-thiones (Fig. 15) are discussed. These heterocyclic compounds

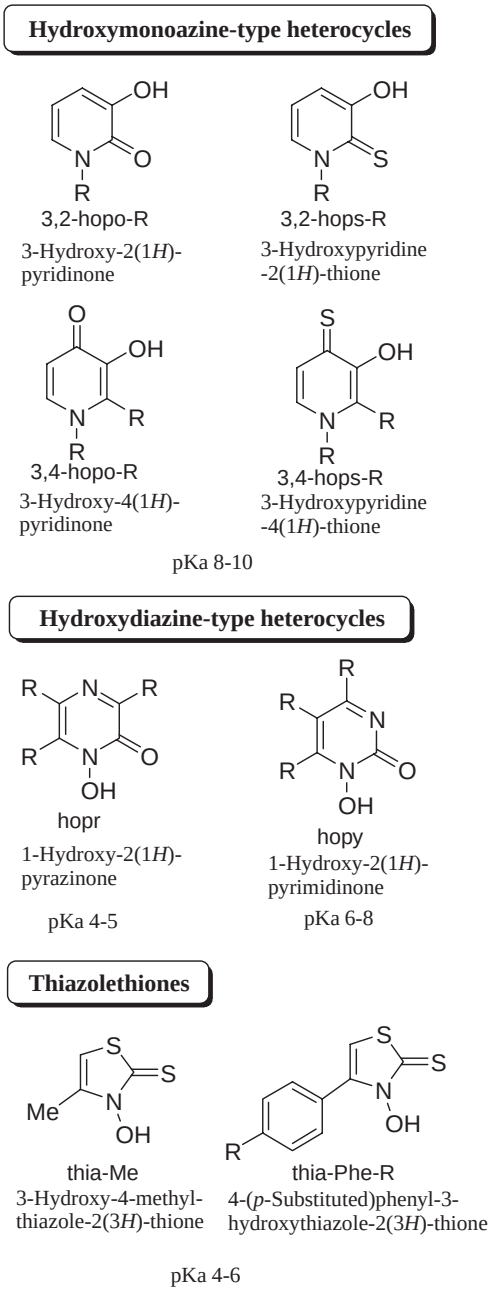


Fig. 15. The structures of hydroxymonoazine-type and hydroxydiazine-type heterocycles, and hydroxythiazolethiones.

have a variety of characteristics as follows: 1) low-molecular-weight compound, 2) easiness of synthesis, 3) possession of unshared electron pair and hydroxy group contributes to increase the solubility in water, 4) a wide range of pK_a values which make it possible to prepare metal complexes with a wide range of the stability constants, 5) easiness of the conversion of the C=O into a C=S group, which make it possible to change the coordination environment, 6) easiness of the introduction of various substituents, which make it possible to change the hydrophilic/hydrophobic balance, and 7) easiness of the introduction of *p*-substituted phenyl group which makes it possible to change the electronic structure.[132]

Four types of zinc(II) complexes with O_4 and S_2O_2 donor

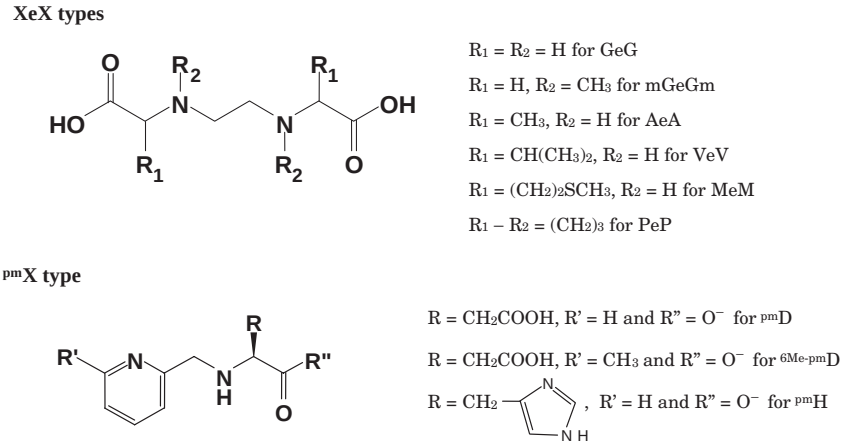


Fig. 16. Ligand structures of XeX and ^{pm}X, and their abbreviations.

sets were prepared by reacting heterocyclic didentate ligands with $ZnSO_4$ in the presence of LiOH or with $Zn(OAc)_2$. Also, five types of vanadyl complexes were prepared by reacting heterocyclic didentate ligands with $VOSO_4$ at pH (pK_a of the ligand $= +1$) (Fig. 2).

From the results of the in vitro insulin-mimetic activities, the following points became clear. 1) Seven kinds of zinc(II) complexes, [Zn(3,2-hops-R)$_2$], showed extremely high insulin-mimetic activity regardless of the substituent R.[133] 2) Bis-(1,2-dihydro-4-(R)-1′-methoxycarbonylethylamino-2-oxopyrimidinolato)zinc(II) [Zn(hopy-D-ala)$_2$] with a D-alanine residue showed higher insulin-mimetic activity than its L-isomer. It may be due to the difference in the sensitivity toward enzymes.[61] In fact, when [Zn(hopy-D-ala)$_2$] was treated with rabit liver esterase, no hydrolysis was observed. On the other hand, [Zn(hopy-D-ala)$_2$] smoothly underwent enzymatic hydrolysis. 3) [Zn(thia-Phe-X)$_2$] with 3-hydroxythiazole-2(3*H*)-thiones showed almost the same insulin-mimetic activity regardless of the electronic effect of the substituent R at the *p*-position.[134] 4) Among five kinds of vanadyl complexes with 3-hydroxythiazole-2(3*H*)-thiones, [VO(thia-Phe-NO_2)$_2$], which has the strongest electron-withdrawing nitro group, showed the highest insulin-mimetic activity,[134,135] demonstrating that the insulin-mimetic activity apparently correlates to the Hammett's substituent constant (σ_p) of the ligands.

[VO(hopy-Me,H,Me)$_2$] with the highest insulin-mimetic activity in vitro improved the diabetic state of STZ-rats regarding loss of body weight, oral glucose tolerance test (OGTT), HbA_{1c} level and other serum parameters.[56] On the other hand, [Zn(thia-Phe-Cl)$_2$] lowered the high blood glucose levels in KK-A^y mice. Interestingly, this complex improved both insulin and leptin resistance.[134] In addition, bis(2,3-dihydro-2-thioxo-4-methyl-3-thiazololato)zinc(II) [Zn(thia-Me)$_2$] exhibited hypoglycemic effect on oral administration.[136] Thus, vanadyl and zinc complexes with heterocyclic compounds, especially 1-hydroxy-2(1*H*)-pyrimidinones and 3-hydroxythiazole-2(3*H*)-thiones, may be potent agents for the treatment of type 1 and type 2 DM.

Vanadium Complexes with Porphyrin Compounds (N_4) Coordination Set. Porphyrins are good ligands to metal ions because they form stable complexes. To develop insulin-mimetic vanadyl complexes with a $VO(N_4)$ coordination environment, two types of water soluble prophyrin ligands were used, and their vanadyl complexes, such as *meso*-[tetrakis(1-methylpyridinium-4-yl)porphyrinato]oxovanadium(IV) [VO-(tmpyp)][137] and vanadyl-*meso*-[tetrakis(4-sulfonatophenyl)-porphyrinato]oxovanadium(IV) [VO(tpps)][43] were prepared (Fig. 10). Both complexes were stable in 4% bovine serum albumin (BSA) for 6 h; however, the urinary clearance of vanadyl species in STZ-rats that received [VO(tmpyp)] was quick and 40% of [VO(tmpyp)] was oxidized to the vanadate form. When [VO(tmpyp)] and sodium ascorbate were administered simultaneously, a hypoglycemic effect was observed, suggesting a synergistic effect, i.e. the individual compound did not cause a change in the blood glucose levels. In contrast, [VO(tpps)] showed evidence of significant hypoglycemic activity within at least 8 h after the single oral administration without ascorbate, giving much higher hypoglycemic activity than [VO(tmpyp)].

To analyze the difference in the hypoglycemic activity of both complexes, metallokinetic analysis in rats was performed in terms of vanadyl concentration in the blood of rats by using BCM-EPR method. Vanadyl species remained longer in the blood of rats receiving VOTPPS by *iv* injection without ascorbate than that of rats receiving *iv* injection of [VO(tmpyp)]. These results supported the stronger and longer hypoglycemic activity of [VO(tpps)] than that of [VO(tmpyp)]. [VO(tpps)] was thus found to be the first example for orally active insulin-mimetic vanadyl-porphyrin complex.[43]

Vanadium and Zinc Complexes with Amino Acids and Peptides. In 1993, bis(glycinato)oxovanadium(IV) [VO-(gly)$_2$] was reported to exhibit insulin-mimetic activity, or a blood glucose lowering effect, in diabetic rats, the effects were weak without a correlation with the physicochemical data.[138] This was, however, really the first example of an insulin-mimetic complex using an amino acid ligand.

Since 1998, insulin-mimetic vanadyl and zinc complexes with amino acid and peptide ligands have been extensively examined, and vanadyl complexes with *N*,*N*′-ethylenebis(amino acids) derivatives (XeX type ligand), such as [VO(GeG)], [VO(AeA)], [VO(VeV)], [VO(MeM)], [VO(PeP)], and [VO-(mGeGm)],[15,139] as well as tetradentate (^{pm}X type ligand) and pentadentate (^{pm2}X type ligand) amino acid derivatives (Fig. 16), and peptide derivatives, such as [Zn(glythr)$_2$], [Zn-

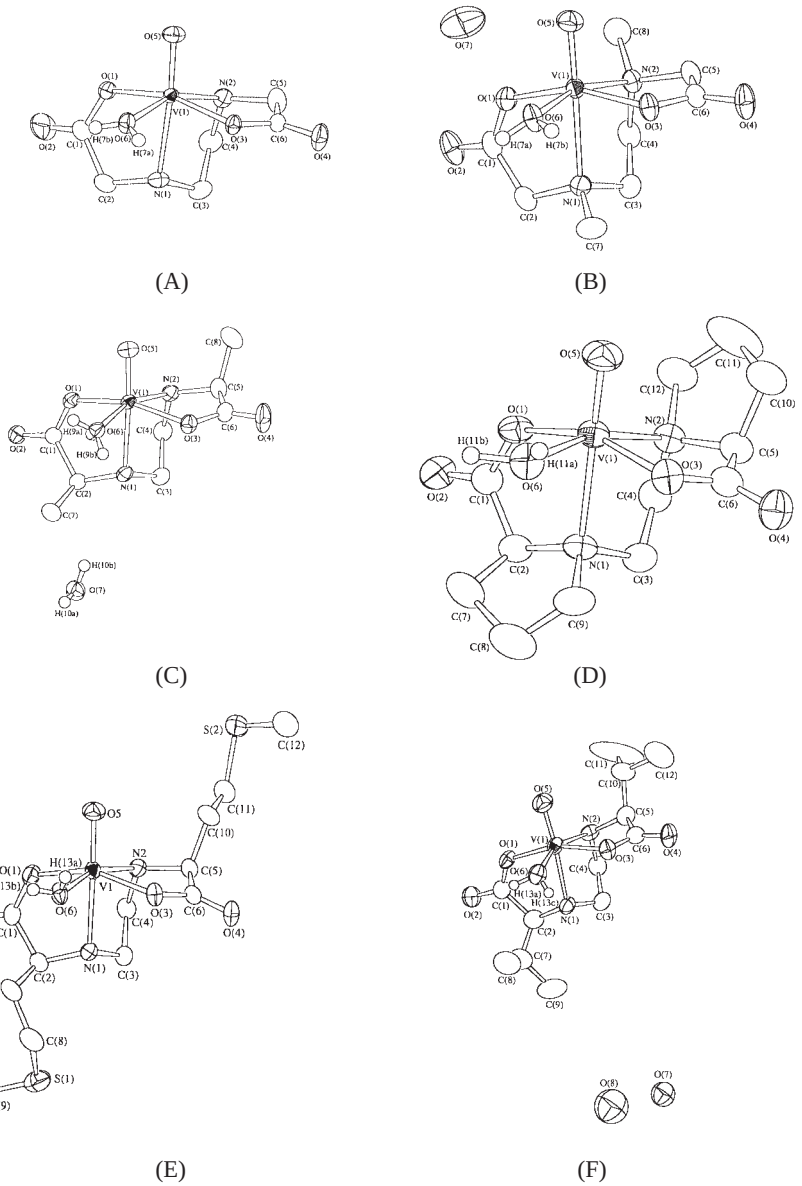
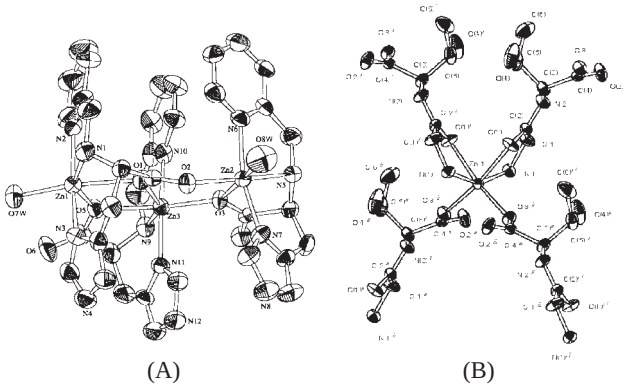


Fig. 17. ORTEC diagrams of vanadyl(IV) complexes (ellipsoids at 50% probability level). (A) [VO(GeG)] (Δ-type), (B) [VO(mGeGm)] (Δ-type) as the $0.5H_2O$ adduct, (C) [VO(AeA)] as the H_2O adduct, (D) [VO(PeP)], (E) [VO(MeM)], and (F) [VO(VeV)] as the $2H_2O$ adduct (Refs. 61, 65, 139, and 181).

$(glyval)_2]$, and $[Zn(glypro)_2]$[14,15,140] were prepared and analyzed by X-ray crystallography. In vitro insulin-mimetic activity and in vivo antidiabetic activity were also evaluated. The vanadium centers in [VO(GeG)], [VO(mGeGm)], [VO(AeA)], [VO(VeV)], [VO(MeM)], and [VO(PeP)] with *N*,*N*′-ethylenebis(amino acids) ligands are coordinated by two carboxylate oxygen atoms, two amine nitrogen atoms, one water oxygen atom, and one vanadyl oxygen atom, and they have distorted octahedral environments. The water oxygen occupies an equatorial coordination position, and one of the two secondary or tertiary nitrogen atoms is bound *trans* to the vanadyl oxo moiety. Furthermore, the two carboxylate ligands coordinate *trans* to each other (Fig. 17).

The structures of the zinc(II) complexes with insulin-mimetic activity that were determined by X-ray structure analysis are as follows: (A) Five-coordinate trigonal bipyramidal geometry in $[Zn(pro)_2]_n$;[141] (B) Five-coordinate square pyramidal geometry in $[Zn(gly)_2]_n$;[141] (C) Six-coordinate octahedral geometry in $[Zn(thr)_2]$,[143] $[Zn(asn)_2]$,[144] $[Zn(glygly)_2]_n$,[145] $[Zn_3(^{pm}H)_3(H_2O)_2](ClO_4)_3$ ((*N*,*N*-bis(2-pyridylmethyl)-(*S*)-histidinato)zinc(II)),[15] and $[Zn(glythr)_2]_n$.[140] The strudures of the (C) type zinc(II) complexes have been examined in detail as follows. The X-ray structures of two zinc(II) complexes are shown in Fig. 18. $[Zn_3(^{pm}H)_3(H_2O)_2](ClO_4)_3$, a trinuclear complex with six-coordinate distorted octahedral geometry, in which three ^{pm}H ligands and two H_2O molecules were coordinated to three zinc(II) ions (Fig. 18A).[15] The mean deviations of Zn1, Zn2, and Zn3 from a least-square plane are within ca. 0.06 Å. On the other hand, $[Zn(glythr)_2]$ has a polymeric structure with a distorted octahedral six-coordinate geometry. The ligand $GlyThr^-$ in the $Zn(glythr)_2$ complex acts as a tridentate ligand, via a monodentate oxygen atom of the terminal carbox-



(A) (B)

Fig. 18. ORTEC diagrams of zinc(II) complexes (ellipsoids at 50% probability level). (A) [Zn(^{pm}H)] and (B) [Zn-(glythr)$_2$] (Refs. 15 and 140).

yl group of threonine residue and a bidentate ligand containing a nitrogen atom of the amino group and the oxygen atom of the peptide group of glycine (Fig. 18B). The zinc ion in the structure binds with two terminal carboxyl groups and two bidentate ligands with a *cis*-configuration. The zinc is located on a crystallographic inversion center, and it is distorted by 0.103 Å from the least-squares plane with Zn(1), O(1), C(2), C(1), and N(1).[140]

Vanadium complexes that contain D-amino acids were found to have higher insulin-mimetic activities than the corresponding L-isomers. In particular, for alanine-based complexes, [VO(AeA)] with the D-isomer was 20 times more effective than [VO(AeA)] with the L-isomer. The relationships between the insulin-mimetic activity and the partition coefficient (log *P*) of the complexes, pK_a value of the ligands, or redox potential of the complexes were determined (Fig. 19).[61]

The complexes with Λ-type configuration that contain achiral amino acids or D-amino acids were determined to have high in vitro insulin-mimetic activity because they are less dependent on the physical properties such as the pK_a value of the ligand, log *P*, 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 they were less active than the complexes with Λ-type configuration (D-isomer).[61] No correlation was found

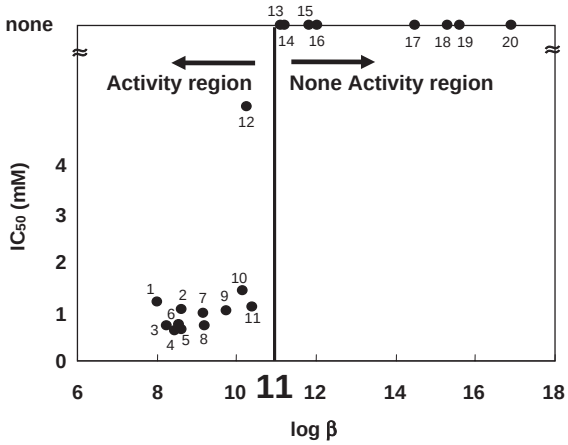


Fig. 20. Correlation between the IC_{50} values and the overall stability constants (log β) of Zn^{II} complexes (Ref. 15). 1. [Zn($^{6Me\text{-}pm}$D)] 2. [Zn(ala)$_2$] 3. [Zn(val)$_2$] 4. [Zn(thr)$_2$] 5. [Zn(VtV)] 6. [Zn(asn)$_2$] 7. [Zn(gln)$_2$] 8. [Zn(gly)$_2$] 9. [Zn(pro)$_2$] 10. [Zn(asp)$_2$] 11. [Zn(^{pm2}V)] 12. [Zn(GtG)] 13. [Zn(^{pm2}A)] 14. [Zn(GeG)] 15. [Zn(mGeGm)] 16. [Zn-(his)$_2$] 17. [Zn(^{pm2}L)] 18. [Zn(^{pm}H)] 19. [Zn(^{pm2}S)] 20. [Zn(^{pm}D)].

between the insulin-mimetic activity and the geometric structure among the vanadium complexes with amino acid derivatives.

It is interesting to point out that a correlation was observed between the insulin-mimetic activity and the log β of some zinc(II) complexes (Fig. 20), and that insulin-mimetic activity was not shown in the zinc(II) complex with a log β value more than 11.[15] It is well known that the absorption of zinc is promoted by albumin. Many researchers have reported log β values of zinc(II) complexes with albumin, being 7.6–9.6.[146,147] If the log β of zinc(II) complexes is higher than that of zinc(II)–albumin complex, they will not be replaced by albumin in absorption processes. In fact, zinc(II) complexes with a log β above 11 did not distribute in the cells, and therefore, no insulin-mimetic activity was observed.

Insulin-mimetic activities were observed for the three (A), (B), and (C) types of complexes, i.e. (A), (B), and (C) that were metioned before; however, no particular correlation

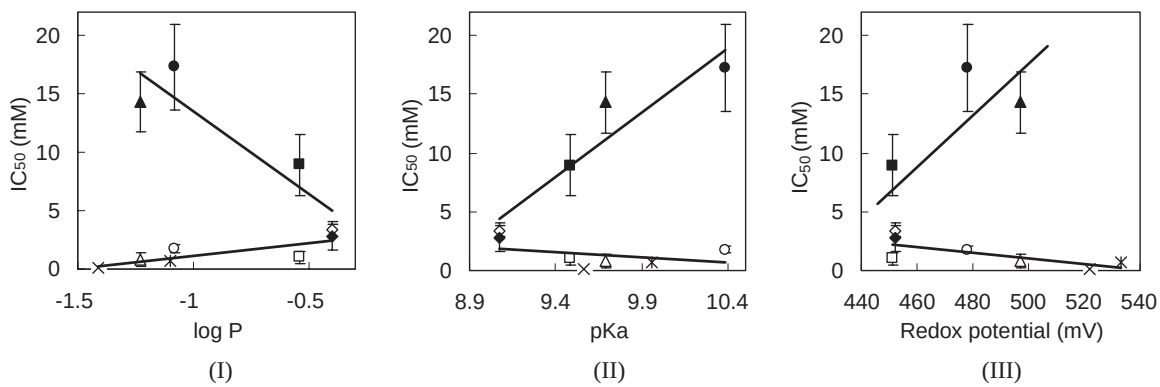


Fig. 19. Correlation between the IC_{50} values and (I) partition coefficients (log *P*), (II) pK_a of the ligands, and (III) redox potentials of vanadyl(IV) complexes. Each symbol is expressed as the mean ± S.D. for three repeated experiments. [VO(GeG)] (×), [VO-(mGeGm)] (∗), [VO(AeA)] (L-isomer ▲; D-isomer △), [VO(VeV)] (L-isomer ■; D-isomer □), [VO(MeM)] (L-isomer ◆; D-isomer ◇), and [VO(PeP)] (L-isomer ●; D-isomer ○) (Ref. 61).

was found between the insulin-mimetic activity and the geometric structure.

Among the wide variety of zinc(II)–amino acids or –peptides complexes prepared, $[Zn(thr)_2]$ complex[14] showed the functions, such as blood glucose lowering effect and HbA_{1c} normalizing effect, suggesting that it is a clinically useful agent to treat type 2 diabetes.

Vanadium Complexes with Sulfur-Containing Ligands. The purple *trans*-bis(methylcysteinato)oxovanadium(IV) $[VO(cysm)_2]$ with a V–S bond, which was analyzed by X-ray crystallography, was proposed to be a good reagent for treating type 1 diabetes via oral administration in 1990 (Fig. 10).[8] In general, the coordination bond between vanadyl ion, which is as a hard Lewis acid, and the thiolate, which is a soft Lewis base, is not stronger than those of the combinations of hard acid/hard base or soft acid/soft base, according to Pearson's HSAB (hard and soft acids and bases) principle.[148] Nevertheless, the purple $[VO(cysm)_2]$ complex with $VO(S_2N_2)$ coordination environment was found to form a strong V–S bond.

This first example stimulated the investigation on insulin-mimetic vanadyl complexes with V–S bonds. The second example was bis(pyrrolidine-*N*-carbodithioato)oxovanadium(IV) $[VO(pcd)_2]$ complex prepared in 1994,[149] which was the most effective among six prepared complexes with $VO(S_4)$ coordination environment. The effect was dose-dependent in the in vitro systems, as well as being effective in treating type 1 diabetic STZ-rats by both daily *ip* injections and oral administrations.[150]

Bis(1-oxy-2-pyridinethiolato)oxovanadium(IV) $[VO(opt)_2]$ with a $VO(S_2O_2)$ coordination sphere, reported in 1999,[40] was the third complex with a V–S bond and demonstrated a strong in vitro insulin-mimetic activity in isolated rat adipocytes (Fig. 10). $[VO(opt)_2]$ was actually the second example which could be used to treat both type 1 diabetic STZ-rats and type 2 obese diabetic ob/ob mice,[124] when administered via daily *ip* injections and orally, after $[VO(6mpa)_2]$ in 1999.[115]

The mechanism involving $[VO(opt)_2]$ was examined. It is well known that TNF-α is a key factor in the obesity-diabetes link, and elevate expression of TNF-α is observed in the epidermal and subcutaneous fat tissue of ob/ob mice. $[VO(opt)_2]$ treated diabetes in ob/ob mice by improving the impaired glucose tolerance and attenuating the TNF-α induced decrease in IRS-I phosphorylation in adipocytes. Thus, it was proposed that the activity of $[VO(opt)_2]$ is caused by attenuation of the impaired insulin signal transduction through activation of IRS as it is related to the inhibition of PTP1B. Therefore, $[VO(opt)_2]$ is expected to have clinical potential with regard to the treatment of obesity in type 2 diabetes.[124]

Insulin-Mimetic Action Mechanism of Vanadium and Zinc Complexes

The mode of action of vanadium compounds has been intensively examined, and important data have been accumulated with regard to the inhibition of PTP1B activity.[29,151–153] This activity is involved in the activation of the insulin receptor tyrosine kinase as well as the cytosolic nonreceptor tyrosine kinase, direct phosphorylation of IRS1, and the activation of PI3K, leading to GLUT4 translocation.[100,154–158] Suppression of hepatic glucose output via inhibition of key gluconeogenic enzymes is suggested to play an important role in mediating the glucoregulatory effects of vanadium.[159] Additionally, vanadium compounds were reported to inhibit phosphatase and tensin homologue deleted on chromosome 10 (PTEN), and to recover the action of PI3K.[160] In 2005, $[VO(pa)_2]$ and related complexes were shown to stimulate protein kinase B (PKB or Akt) activity at low concentrations without exhibiting cellular toxicity, indicating the activation of insulin signals pathway and translocation of GLUT4 to the surface of cell membranes (Fig. 9).[161]

On the other hand, zinc was examined relevant to a known antioxidant in the immune system,[162] and it was found that zinc supplementation inhibits the development of type 1 DM through inhibition of NF-κB activation.[163] Dietary zinc supplementation was reported to decrease hyperglycemia and hyperinsulinemia in type 2 diabetic mice, suggesting that zinc plays an important role in pancreatic function and peripheral tissue glucose uptake.[164]

Zinc appears to play a role in modulating insulin receptor tyrosine kinase activity in the skeletal muscle of a genetic type 2 DM model mouse, similarly to the action of vanadium.[165] In addition, zinc was proposed to affect carbohydrate metabolism through the insulin receptor, PTP1B, and other related proteins.[166,167] The action sites of vanadium and zinc compounds after the insulin receptor are depicted in detail in Fig. 21, along with recently obtained new results.[161]

The action mechanism for antidiabetic and insulin-mimetic activities of vanadium and zinc in a whole cell have been studied in isolated rat adipocytes.[100,105] Because, both vanadyl and zinc(II) ions and several of their complexes have been known

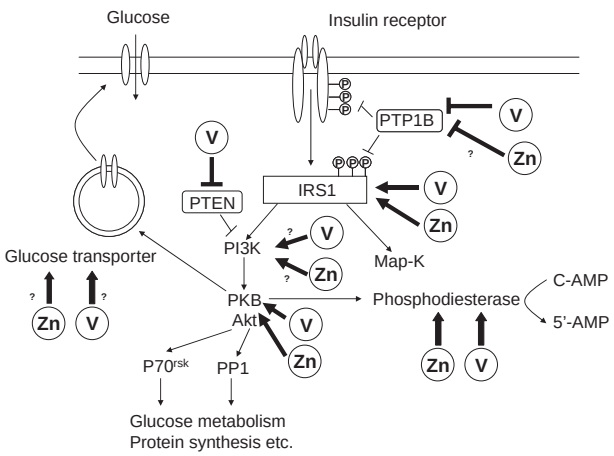


Fig. 21. Potential action sites of vanadium and zinc compounds in the insulin signal cascade. Binding of insulin to its receptor on the surface of the cell results in a conformational change of the receptor, leading to the activations of IRS1, PI3K, and PKB, which in turn translocate the glucose transporter 4 (GLUT4) on the surface of the cell. Thus, glucose is incorporated into the cell. Potential multi action sites of vanadium and zinc compounds, called an "ensemble mechanism," are indicated by arrow (⬅) in the figure. IRS, insulin receptor substrate; PI3K, phosphatidylinositol-3-kinase; PP-1, protein phosphatase-1; PTP1B, protein tyrosine phosphatase 1B; PTEN, phosphatase and tensin homolog.

to be incorporated in the cell,[104,168] the mechanism was examined with respect to the effect of such metal ions and their complexes on the insulin receptor PI3K, a glucose transporter, and phosphodiesterase by using various inhibitors, such as HNMPA-$(AM)_3$ (inhibitor of the tyrosine kinase activity of the insulin receptor via a 95-kDa β-subunit of the insulin receptor),[169] wortmannin (inhibitor of PI3K),[170,171] cytochalasin B (glucose transporter inhibitor),[172] and cilostamide (inhibitor of phosphodiesterase (PDE))[173] were used (Fig. 9).

The following results were obtained:[104,168] (1) vanadyl and its complexes act on at least four sites involving the tyrosine kinase of the insulin receptor, signal transduction glucose transporter (GLUT4) and PDE, in the "ensemble mechanism," (2) zinc(II) ion and its complexes strongly act on GLUT4 and PDE, and (3) $[Zn(pa)_2]$ complex acts on the insulin receptor. These results were observed at the molecular level.[161]

Propose of New Preparations of Vanadium Compounds: New Drug Delivery Systems

Since 1995, simple vanadium compounds, such as vanadium(IV) oxide sulfate ($VOSO_4$) and sodium metavanadate ($NaVO_3$), have been clinically tested in diabetic patients.[174] When orally administered at a dose of 150 mg (3 mmol)/day for 6 weeks, $VOSO_4$ improved the type 2 diabetes state with respect to plasma glucose, HbA_{1c}, and fructosamine levels.[175] The most important point reported in the study was vanadium concentration in the plasma of the subjects. Before $VOSO_4$ administration, subjects showed plasma vanadium levels below $10\,\mu g\,L^{-1}$ ($0.2\,\mu mol\,L^{-1}$), which increased to $104 \pm 18\,\mu g\,L^{-1}$ ($2.0 \pm 0.4\,\mu mol\,L^{-1}$) after the 6-week administration. These results clearly indicate that an increase in plasma vanadium levels correlates to an improvement in the subject's diabetic state. Such significant andiabetic effect from orally administered $VOSO_4$ prompted a study on the administration methods of the compound. The enhancement in the antidiabetic effect of $VOSO_4$ is indeed correlated with an increase in the plasma vanadium level; however, the development of toxicity by increasing the concentration of vanadium should be avoided. For this purpose, two different administration methods have been proposed.

The first method involves coordination compounds of $VOSO_4$ that non-toxic and low molecular weight ligands to enhance the activity of the metal ion. Chelation reduces the polarity of the metal, and thus, the complex is able to permeate through the lipid layer of the cell membrane.[42,101,102,106–108] The second method is based on increasing the bioavailability of $VOSO_4$. For example, the encapsulation of $VOSO_4$ allows the metal ion to be delivered to the desired gastrointestinal sites, where $VOSO_4$ can be absorbed. Because the first method was described in the article, trials for the drug delivery of $VOSO_4$ are discussed in this section.

Enteric-Coated Capsulation. First of all, the absorption processes in the gastrointestinal tract of rats given bolus $VOSO_4$ were examined in terms of time course for vanadyl concentration in the blood of rats. The bioavailability, Fa value, which is defined as an absorption ratio from non-intravenous routes, of $VOSO_4$ was enhanced when this compound was given through ileum, which was followed by the administration of jejunum and stomach (oral administration). After oral administration, $VOSO_4$ is supposed to change to undesirable forms, which are not absorbed from the gastrointestinal tract, in the stomach, where the pH is 1.5–3.5. Thus, $VOSO_4$ given by direct intestinal administration (e.g. into the ileum) is absorbed more thoroughly than those given orally, resulting in a high level of pharmacological activity.[176] Therefore, a drug delivery system composed of $VOSO_4$ and an enteric-coated capsule (ECC), which releases $VOSO_4$ in the ileum where the pH is 6–7, was examined. A mini-gelatine capsule (GC) (diameter 2.5 mm, length 8.5 mm) was filled with solid $VOSO_4$ by using a miniature-capsule filling device. Then, the GC was placed in a hydroxypropylmethyl-cellulose phthalate (HPMCP) solution (solvent: methylene chloride:methanol = 4:1). The solvent was evaporated at 4C, and the $VOSO_4$-containing ECC were prepared. The ECC was administered to rats by single oral administration, and the time courses of vanadyl concentration in the blood were examined by EPR. The delayed appearance of vanadyl concentration after oral administration of ECC compared with the release of GC was observed. The most important fact was that the Fa of the vanadyl species for ECC (9.8%) increased almost twice as much as that associated with either GC (4.0%) or the solution (4.8%).[177] These results indicated that administration of ECC containing $VOSO_4$ improved vanadyl absorption more than either GC or the solution did.[178]

A Vanadyl-Biopolymer Complex. The use of a polymer that is both biodegradable and biocompatible would be effective to enhance drug targeting specificity, lowering systemic drug toxicity, improving treatment absorption rates and providing protection for pharmaceutics against biochemical degradation. Poly-γ-glutamic acid, γ-pga, is a naturally occurring biodegradable and biocompatible polymer, which is non-toxic to humans and the environment. The potential applications of this polymer, therefore, are of interest in a broad range of industrial fields such as food, medicine, and water treatment.[179]

In order to achieve a safer and more effective treat of diabetes, a controlled dosing regimen and dosage standard for the oral administration of $VOSO_4$ or vanadyl complexes must be established. Therefore, the high biological importance of γ-pga made this polymer useful a carrier of vanadyl, i.e. vanadyl-γ-pga [VO(γ-pga)], for a sustained drug delivery system. Thus, the [VO(γ-pga)] complex was prepared in solution.[180] After the characterization of the complex, in vitro insulin-mimetic activity, metallokinetic feature in the blood of rats and in vivo hypoglycemic effect in STZ-rats were examined. In the [VO(γ-pga)] complex, that the vanadyl binds with four oxygen atoms of the carboxyl groups on the side chain of the polymer to form $VO(O_4)$ coordination sphere at around pH 3. The complex was found to have higher insulin-mimetic activity than that of $VOSO_4$, which was evaluated based on the inhibition of FFA release and glucose uptake in the adipocytes. Metallokinetic parameters of vanadyl species after administration of $VOSO_4$ and [VO(γ-pga)] revealed that AUC (area under the curve) and MRT (mean resident time) values in the rats given [VO(γ-pga)] ($2.1\,\mu mol\,min\,mL^{-1}$, and 22.3 min, respectively) were both significantly higher than those of $VOSO_4$ ($0.26\,\mu m\,min\,mL^{-1}$ and 4.4 min, respectively). Considering other parameters, the bioavailability of [VO(γ-pga)] was determined to be much higher than that of $VOSO_4$.

Consequently, the hypoglycemic effects of [VO(γ-pga)] were evaluated in STZ-mice following not only single oral gavage but also daily oral administrations for 16 days. Both results indicated that [VO(γ-pga)] has much higher hypoglycemic effect in type 2 diabetic mice, in which the HbA_{1C} level and the results of OGTT were also improved. From these results, γ-pga is a good carrier for sustained release of vanadyl with an increased bioavailability, and therefore, [VO(γ-pga)] should be a future therapy for type 1 diabetes.[180]

Perspective

After discovering in 1990 that some vanadyl complexes involving $[VO(cysm)_2]$ with $VO(S_2N_2)$ coordination sphere could treat type 1 DM rats on daily oral administrations, a large class of compounds based on vanadyl and zinc complexes have been extensively studied. The accumulated data from animal studies led to a new and surprising concept, in which low molecular weight (approximately 350–400 unit) vanadyl and zinc complexes cause antidiabetic activity on a blood glucose lowering effect, similar to that of insulin with a molecular weight of approximately 6000. As well, since 1995, simple vanadium compounds, such $VOSO_4$ and $NaVO_3$, have been clinically tested in human DM subjects, who showed improvements in their diabetic state. The most important results indicated that plasma vanadium levels of the subject before $VOSO_4$ treatment increased after the treatment for 6 weeks, suggesting a dose-dependency.

These findings involving diabetic model animals and DM subjects show that vanadyl and zinc compounds are useful for treating DM and that they will be clinically useful pharmaceuticals in the future. There are two possible methods to increase the plasma metal levels which enhance the insulin-mimetic and antidiabetic activities of vanadyl and zinc compounds. However, it is important to reduce the dosage of such compounds to avoid unnecessary side effects or toxicity resulting from the metals. The first way is to develop new types of vanadyl and zinc complexes with high bioavailability and without toxicity. The second way is to increase the bioavailability of simple vanadium and zinc compounds. Therefore, introduction of new drug delivery systems, for example, uses of enteric-coated capsules containing simple metal compounds and biodegradable and biocompatible polymers that bind simple metal compounds, are needed.

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