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Coordination Chemistry Reviews 226 (2002) 187–198

COORDINATION CHEMISTRY REVIEWS

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Antidiabetic vanadium(IV) and zinc(II) complexes

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Received 1 April 2001; accepted 15 October 2001

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Abstract

Diabetes mellitus (DM), which develops many secondary complications such as atherosclerosis, microangiopathy, renal dysfunction and failure, cardiac abnormality, diabetes retinopathy and ocular disorders, is classified as either insulin-dependent type 1 or non-insulin-dependent type 2, according to the definition of WHO. Although several types of insulin preparations for type 1 DM and those of synthetic drugs for type 2 DM have been developed and clinically used, they have several problems such as physical and mental pain due to daily insulin injections and defects involving side effects, respectively. In the 21st century, a new class of pharmaceuticals should be introduced. For this reason, metallopharmaceutical compounds containing vanadium and zinc ions are expected to treat both types of DM, by making effective use of unique characteristics of the metals. In this article, the current state of development of insulin-mimetic vanadium and zinc complexes with different coordination modes are reviewed, focusing on the preparations and coordination structures of the complexes and in vitro and in vivo evaluations as well as the possible mechanism.

Keywords: Diabetes mellitus; Insulin-mimetic activity; Vanadium(IV) complexes; Zinc(II) complexes; Structure–activity relationship; Adipocytes

Abbreviations: FFA, free fatty acid; BUN, blood urea nitrogen; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; TCHO, total cholesterol; HbA_{1c}, hemoglobin A_{1c}; cysm, methylcysteinate; ox, oxalate; mal, malonate; sal, salicylaldehyde; tar, (+)-tartrate; pcd, pyrrolidine-*N*-carbodithiolate; opt, 1-oxy-2-pyridinethiolate; pic, picolinate; 3mpa, 3-methylpicolinate; 6mpa, 6-methylpicolinate; 5ipa, 5-iodopicolinate; 4clpa, 4-chloropicolinate; ma, maltolate; ema, ethylmaltolate; GeG, *N*,*N*′-ethylenebis(glycinate) = ethylenediamine-*N*,*N*′-diacetate = EDDA; MeM, *N*,*N*′-ethylenebis(L-methioninate); mGeGm, *N*,*N*′-ethylenebis(sarcosinate); βAeAβ, *N*,*N*′-ethylenebis(β-alaninate); GtG, *N*,*N*′-trimethylenebis(glycinate); VtV, *N*,*N*′-trimethylenebis(L-valinate).

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0010-8545/02/$ - see front matter
PII: S0010-8545(01)00447-7

1. Introduction

Diabetes mellitus (DM) is one of the life style-related diseases as well as one of the most widespread diseases in the world. DM is generally classified as either type 1 insulin-dependent or type 2 non-insulin dependent [1]. Type 1 DM can be controlled only by daily injections of insulin, and type 2 DM is treated by several types of synthetic therapeutics. In the 21st century, the creation of therapeutics with a new aspect is an essential investigation; the development is required of orally active compounds in place of painful insulin injections for type 1 DM, and that of compounds without side effects for type 2 DM. Interestingly, in 1899 before the discovery of insulin and its clinical use to treat DM by Banting and Macleod, in 1921, orally administrated sodium vanadate was reported to improve DM in human diabetes [2]. The in vitro insulin-mimetic effect of vanadium ions was confirmed later in 1979 [3].

Thus vanadium has taken part in DM since the beginning of DM history. However, besides vanadium, other metal ions such as chromium [4], manganese [5], tungsten [6] and molybdenum [6] exhibit insulin-mimetic effects. Therefore, these metal ions are expected to be developed as clinically useful metallopharmaceuticals like platinum-containing *cis*-platin and gold-containing auranofin as an anticancer and oral rheumatoid arthritis drug, respectively [7]. However, the insulin-mimetic effect of these metal ions has not been evaluated under the same conditions. We have reexamined the relative insulin-mimetic activity of metal ions in terms of inhibition of free fatty acid (FFA) release from isolated rat adipocytes treated with epinephrine, which is the method being proposed in our laboratory [8]. As shown in Fig. 1, rather toxic ions, Hg(II), Se(IV) and Cd(II), strongly inhibited FFA release. Following these ions, V(III), V(IV), Zn(II) and Mn(II), exhibited insulin-mimetic activities. Because V(III) is readily oxidized to V(IV) and V(V) at physiological pH and V(IV) is less toxic than V(V), we exclusively used V(IV) in our further study. In addition to V(IV), we extended our work on Zn(II).

In the development of insulin-mimetic agents, we synthesized low molecular weight metal complexes by using naturally occurring ligands and their derivatives intending to enhance the lipophilicity, membrane transport and bioavailability. This article reviews recent progress in the development of insulin-mimetic antidiabetic vanadium(IV) and zinc(II) complexes and their possible mechanisms.

2. Vanadium(IV) complexes

Vanadium with atomic number 23, atomic weight 50.9415 and oxidation states from III to V has a wide variety of biochemical and physiological functions [9]. Among them, an insulin-mimetic antidiabetic effect is the most striking, the effect being provided by the oxidation states of vanadic V(III), vanadyl V(IV) and vanadate V(V). Historically, sodium vanadate was used to treat human DM in 1899 [2], before the discovery of insulin in 1921. Recently, both vanadyl sulfate and sodium vanadate have been examined clinically to find out whether they improve human DM [10–15]. However, the absorption and incorporation of these inorganic salts are generally very low. In addition, the vanadyl state is less toxic to rats than the vanadate states [16], and most vanadium in organs of normal rats treated with vanadate is exclusively present in the

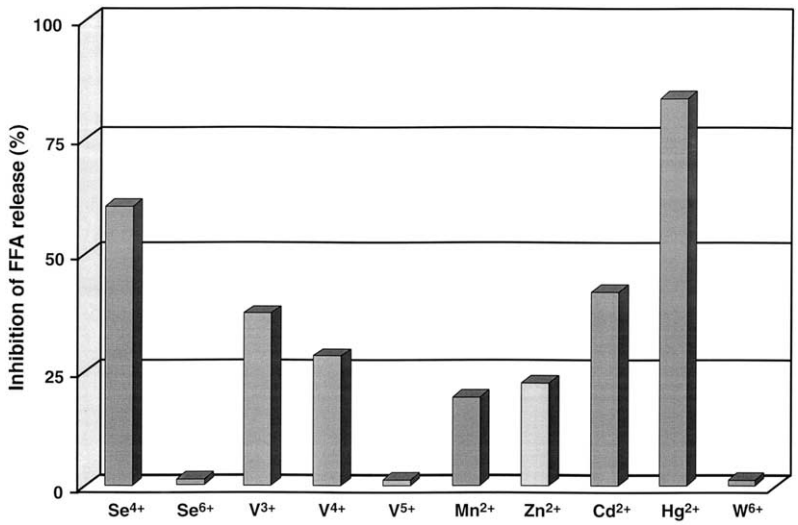


Fig. 1. Inhibitory effects of FFA release from rat adipocytes (2.5×10^6 cells ml^{-1}) treated with epinephrine in the presence of various types of elements (1 mM).

vanadyl form [17,18]. From these observations, we used low molecular weight ligands for vanadyl complexes expecting higher bioavailability and lower toxicity than vanadate in animals.

In this chapter, the recent progresses in developing antidiabetic vanadium complexes, especially focusing on vanadyl complexes, are summarized. Other types of vanadium complexes such as vanadate and peroxovanadates involving vanadyl are reviewed in several volumes [19–24].

2.1. In vitro evaluation of insulin-mimetic activity of vanadium complexes

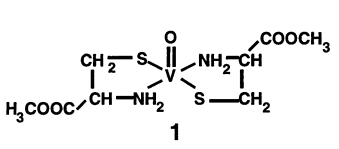
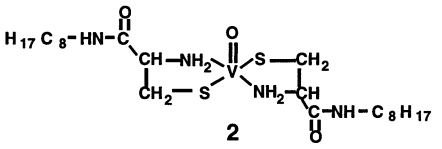
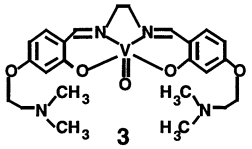
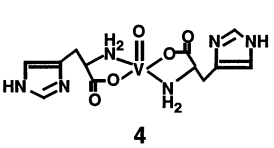
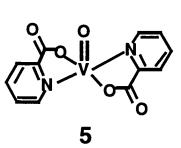
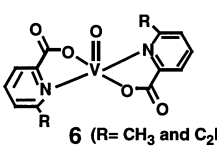
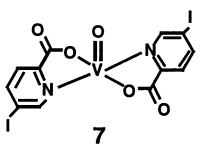
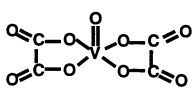
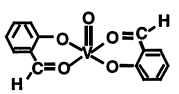
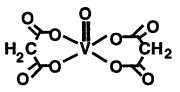
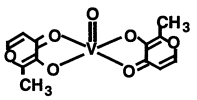
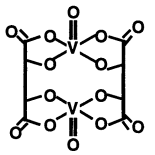
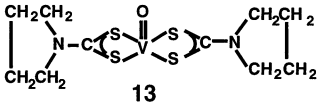
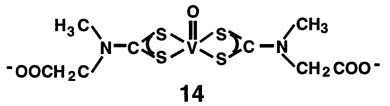
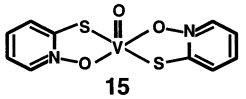
The pharmacologically active state of vanadium was evaluated with respect to the interaction of vanadium ions and isolated Wistar rat adipocytes treated with epinephrine. The following important results were obtained [8,25]. (1) Vanadyl ion enhanced glucose uptake, (2) vanadyl ion suppressed FFA release in the absence of glucose and inhibited FFA release in the presence of glucose, (3) glucose inhibited FFA release and the effect was suppressed by cytochalasin B (Cyt B), an inhibitor of glucose transporter, (4) the suppressed FFA release by vanadyl ion was restored by Cyt B, (5) vanadyl ion was uptaken into adipocytes but vanadate ion was not, and (6) vanadate ion was partially reduced to a vanadyl ion in the presence of glucose and then uptaken into the adipocytes. From these results, the vanadyl state is proposed to be a possible active form of vanadium in mimicking or enhancing insulin action by interacting with the glucose transporter.

On the basis of the results, we proposed a simple and convenient in vitro test system using isolated rat adipocytes treated with epinephrine for evaluating the insulin-mimetic action of vanadyl complexes. Because additional insulin in the adipocytes suppressed the FFA release dose-dependently, a complex which caused dose-dependent suppression of FFA release was proposed to have an insulin-mimetic action in vivo.

2.2. In vivo evaluation of insulin-mimetic antidiabetic activity of vanadyl complexes in animals

In 1990, we first proposed that bis(methylcysteinato) $[VO(cysm)_2]$-, bis(oxalato) $[VO(ox)_2]$-, bis(malonato) $[VO(mal)_2]$-, bis(salicylaldehyde) $[VO(sal)_2]$-, and bis-((+)-tartrato) $[VO(tar)_2]$-oxovanadium(IV) complexes with coordination modes such as $VO(S_2N_2)$ and $VO(O_4)$ exhibited normoglycemic effects in streptozotocin (STZ)-induced type 1 diabetic rats (STZ-rats), when given daily oral administrations (Table 1) [26].

Table 1
Insulin-mimetic vanadyl complexes with different coordination modes

mode	complex
N_2S_2	 1  2
N_2O_2	 3  4  5  6 (R= CH_3 and C_2H_5)  7
O_4	 8  9  10  11  12
S_4	 13  14
S_2O_2	 15

1: Sakurai et al. [26], 2: Cam et al. [75], 3: Junod et al. [76], 4–6: Sakuri et al. [34,35], 6: Sasagawa et al. ($R = C_2H_5$) [77], 7: Takino et al. [38], 8–10: Sakurai et al. [26], 11: McNeill et al. [40], 12: Sakurai et al. [26], 13: Watanabe et al. [29,30], 14: Sakurai et al. [30], 15: Sakurai et al. [31,32].

The order of normoglycemic effect in STZ-rats was $VO(mal)_2 > VO(cysm)_2 > VO(tar)_2 > VO(sal)_2 > VO(ox)_2$. The action of $VO(cysm)_2$ was dose-dependent in the range of 1–10 mg V kg^{-1} body weight. In general, the coordination bond between vanadyl ion as a hard Lewis acid and thiolate as 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 [27]. Nevertheless, the purple and monomeric *trans*-$VO(cysm)_2$ complex was found to form a strong bond of V–S [28].

From these results, we focused on the preparation of vanadyl complexes containing the V–S coordination mode, and evaluated their insulin-mimetic effects both in in vitro adipocyte system and in vivo experiments. The following results were obtained. (1) Bis(pyrrolidine-*N*-carbodithiolato)oxovanadium(IV) $[VO(pcd)_2]$ complex [29,30], was the most effective among six complexes with the $VO(S_4)$ coordination mode, with the effect being dose-dependent in an in vitro system. (2) $VO(pcd)_2$ complex was effective when given daily to STZ-rats by both i.p. injections and oral administrations. (3) Bis(1-oxy-2-pyridinethiolato)oxovanadium-(IV) $[VO(opt)_2]$ [31,32] complex with the $VO(S_2O_2)$ coordination mode exhibited strong insulin-mimetic activity in a dose-dependent manner in an in vitro system and normalized the blood glucose levels in STZ-rats when given daily i.p. injections or oral administrations. (4) In ob/ob mice, an obese type 2 DM animal model, 15 day oral treatment with $VO(opt)_2$ complex resulted in a dose-dependent decrease in the levels of glucose, insulin and triglyceride in the blood [33]. (5) Tumor necrosis factor-α (TNF-α) is a key component of obesity-diabetes link. Indeed, an elevated expression of TNF-α was observed in the epidermal and subcutaneous fat tissue of ob/ob mice. Because $VO(opt)_2$ exerted an antidiabetic effect in ob/ob mice by ameliorating impaired glucose tolerance and attenuated the TNF-α-induced decrease in insulin receptor substrate-1 (IRS-1) phosphorylation in adipocytes, the effect of the complex was concluded to be derived from an attenuation of a TNF-α-induced impaired insulin signal transduction via inhibition of protein tyrosine phosphatase [33]. As a result, the potential clinical use of $VO(opt)_2$ is expected in the treatment of obesity-type 2 DM.

On the other hand, we prepared a new type of vanadyl complex with the $VO(N_2O_2)$ coordination mode in 1995 and found that the bis(picolinato)oxovanadium(IV) $[VO(pic)_2]$ complex has a strong insulin-mimetic effect in an in vitro adipocytes system [34]. This complex was effective in normalizing the blood glucose level of STZ-rats when given by daily i.p. injections or oral administration. The normal glucose level of STZ-rats was maintained for about 30 days with a gain of body weight after the end of oral administration of $VO(pic)_2$ for 14 days. We have tried to examine the structure–activity relationship of antidiabetic vanadyl complexes with the $VO(N_2O_2)$ coordination mode, in which $VO(pic)_2$ was used as a leading compound. As shown in Fig. 2, by introducing an electron-donating group such as a methyl group into the pyridine ring of picolinate ligand, bis(3-methylpicolinato)oxovanadium(IV) $[VO(3mpa)_2]$ and bis(6-methylpicolinato)oxovanadium(IV) $[VO(6mpa)_2]$ were

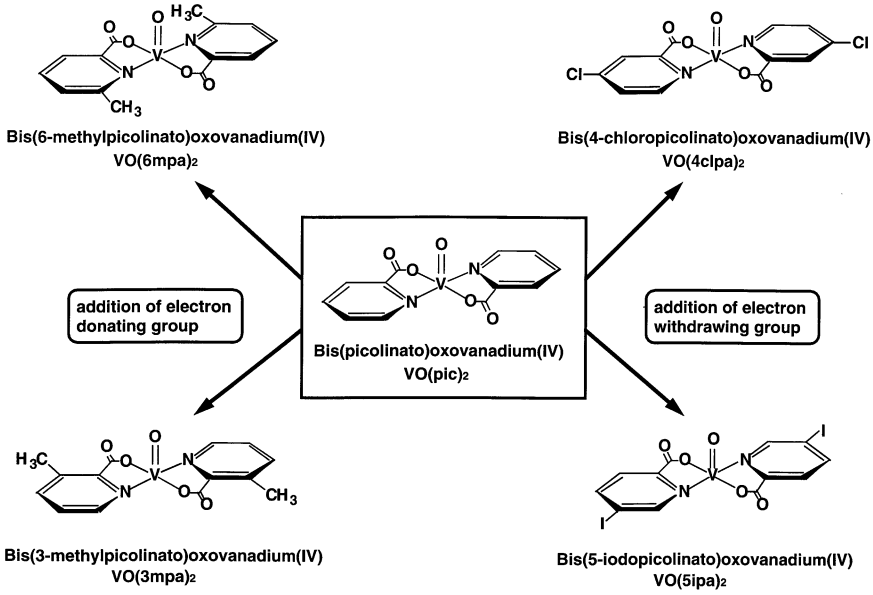


Fig. 2. Chemical structures of $VO(pic)_2$ and its derivatives with electron-donating or -withdrawing groups.

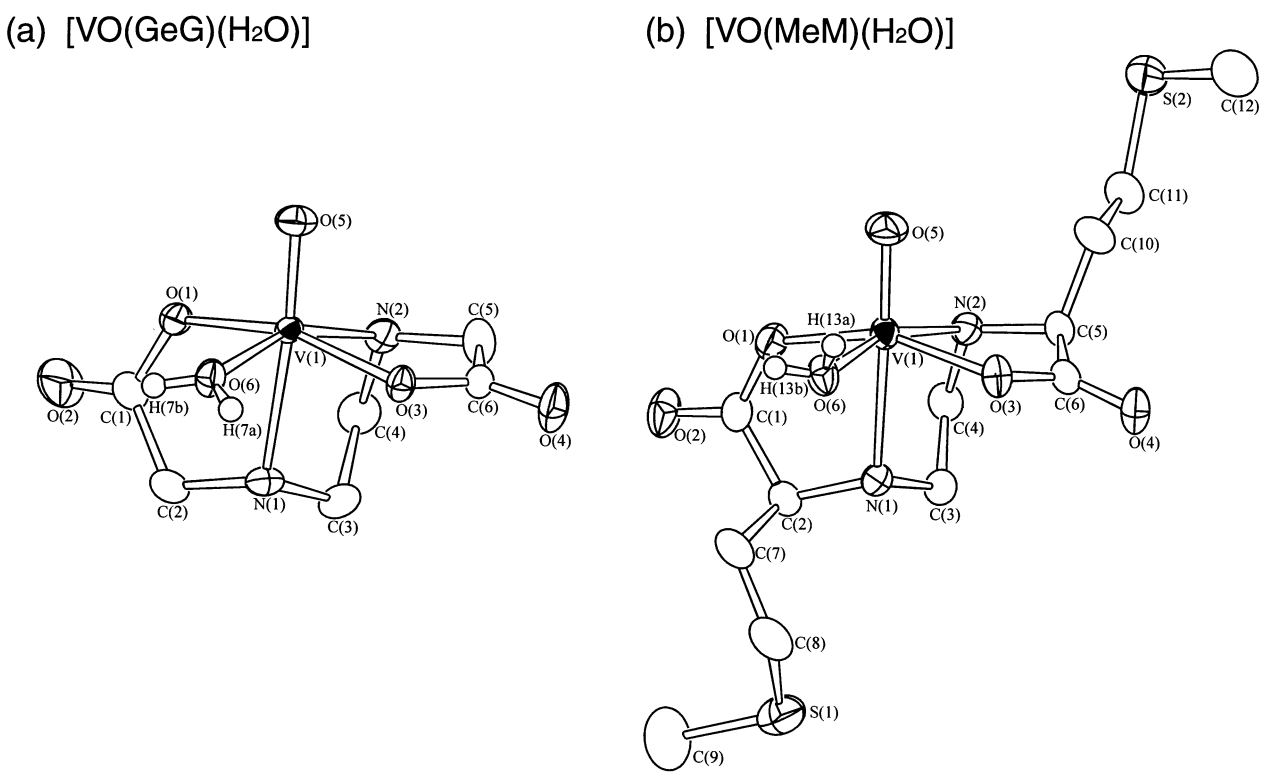


Fig. 3. (a) X-ray structure of [VO(GeG)(H_2O)] (Δ-type) (ORTEP representation; ellipsoids at 50% probability level). The optical isomer (Λ-type) was omitted. Selected bond distances (Å): V(1)–O(1) 2.013(1), V(1)–O(3) 1.985(1), V(1)–O(5) 1.595(1), V(1)–O(6) 2.047(2), V(1)–N(1) 2.330(1), V(1)–N(2) 2.106(2). (b) X-ray structure of [VO(MeM)(H_2O)] (ORTEP representation; ellipsoids at 50% probability level). Selected bond distances (Å): V(1)–O(1) 1.993(2), V(1)–O(3) 1.990(2), V(1)–O(5) 1.603(2), V(1)–O(6) 2.033(2), V(1)–N(1) 2.371(2), V(1)–N(2) 2.141(2). ORTEP representation is based on C.K. Johnson, ORTEP II, Oak Ridge National Laboratory Report, ORNL-TM 5138, 1976 (see Ref. [46]).

prepared [35,36]. On the other hand, by introducing an electron-withdrawing group such as halogen atoms into the picolinate ligand, bis(5-iodopicolinato)oxovanadium(IV) [$VO(5ipa)_2$] and bis(4-chloropicolinato)oxovanadium(IV) [$VO(4clpa)_2$] were prepared [37,38]. The structures were determined by using the usual methods together with the EXAFS spectra [37]. It was revealed that $VO(pic)_2$, $VO(3mpa)_2$ and $VO(5ipa)_2$ complexes have a six-coordinate structure with an additional V–OH_2 bond, while, $VO(6mpa)_2$ and $VOSO_4$ with no coordinated water molecule have a five-coordinate structure. The lack of water may be caused by steric hindrance of the 6-methyl group. The equatorial V–O bond length in $VO(6mpa)_2$ is 202 pm, which is shorter than those (205 pm for all) in the six-coordinate $VO(pic)_2$, $VO(3mpa)_2$ and $VO(5ipa)_2$. On the other hand, the V–N bond length of 229 pm in the five-coordinate complex is longer than those (221–223 pm) in the six-coordinate complexes. Such differences in the coordination structure around vanadyl ion involving H_2O coordination appear to reflect the insulin-mimetic activity of the complexes, the order was $VO(5ipa)_2 > VO(6mpa)_2 > VO(pic)_2 > VO(3mpa)_2 > VO(4clpa)_2$, and in vivo normoglycemic effects in STZ-rats were found to be better in both $VO(5ipa)_2$ and $VO(6mpa)_2$ than other complexes when given by daily i.p. injections [37]. These results suggest that introduction of the methyl group or halogen atom into the picolinate ligand is a useful method to develop more active insulin-mimetic antidiabetic complexes. However, the effect of substituents should be compared at the same position of the ligand. Interestingly, $VO(6mpa)_2$ complex was found to decrease the high blood glucose levels of a hereditary type 2 DM animal, KK-A^y mice by daily i.p. injection or oral administration of the complex [39].

Orally active insulin-mimetic vanadyl complexes with the $VO(O_4)$ coordination mode have been developed (Table 1). The most widely tested bis(maltolato)oxovanadium(IV) [$VO(ma)_2$] [40–42] and bis(ethylmaltolato)oxovanadium(IV) [$VO(ema)_2$] [43] complexes added in drinking water were reported to normalize the high blood glucose levels of STZ-rats, following useful studies of vanadyl complexes with the $VO(O_4)$ coordination mode in 1990 [26]. The results on $VO(ma)_2$ complex are described in detail in Ref. [23]. Other interesting candidate complexes with the $VO(O_4)$ coordination mode have been reported [44,45].

Stereospecific and structure-dependent insulin-mimetic vanadyl complexes have been reported as interesting examples [46–48]. In the newly prepared vanadyl

complexes [VO(XeX)(H_2O)], where XeX = *N*,*N*′-ethylenebis(α-amino acid) (Fig. 3), VO(GeG)(H_2O) containing an *N*-alkylated derivative of glycine inhibited FFA release in a dose-dependent manner, while in contrast, VO(MeM)(H_2O) containing methionine was only slightly effective in an in vitro adipocyte evaluation system [46]. Based on these observations, the insulin-mimetic activities of several vanadyl complexes with the tetradentate XeX ligand containing different L- and D-amino acids were examined [47]. The complexes with Λ-type configuration which contain achiral amino acids or D-amino acids were concluded to exhibit 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). Furthermore, vanadyl complexes with pentadentate ligands were found to have no insulin-mimetic activities [48].

2.3. Organ distribution of vanadium and blood disposition of vanadyl species in rats given vanadyl complexes—neutron activation analysis (NAA) and blood circulation monitoring-electron paramagnetic resonance (BCM-EPR)

When we aim for the clinical use of vanadyl complexes in the future, we have to understand the organ distribution and metallokinetic features of vanadium as well as the complex forms. For these purposes, neutron activation analysis (NAA) was used to determine the total vanadium levels in organs of rats given vanadyl sulfate ($VOSO_4$) or vanadyl complexes with different coordination modes. Vanadium was found in kidney > liver > bone > pancreas in rats treated with $VOSO_4$ [8,25,26], while the metal was detected in bone > kidney > spleen > liver > pancreas in rats treated with vanadyl complexes such as VO(5ipa)$_2$ [38]. Such a difference between ionic vanadyl and vanadyl complexes might suggest toxicity and long-acting character of the complexes, although further detailed study is needed.

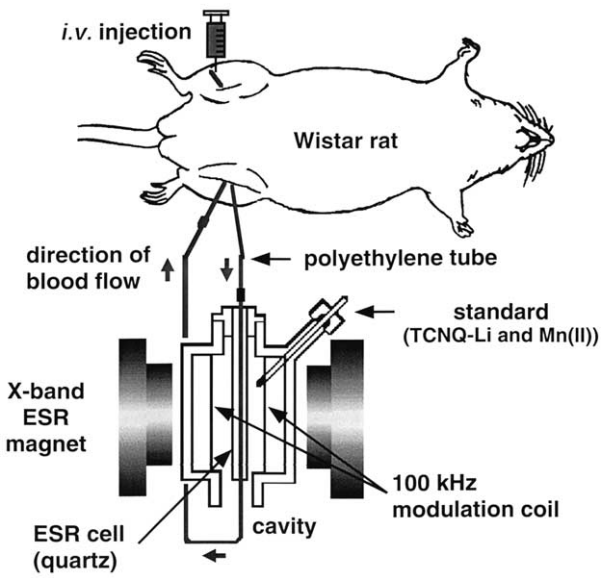


Fig. 4. In vivo blood circulation monitoring-electron paramagnetic resonance (BCM-EPR) method (see Ref. [49]).

To study the metallokinetic features of vanadyl complexes, we applied BCM-EPR, which permits one to know the disposition of paramagnetic species in the blood (Fig. 4) [49]. Vanadyl compounds were given by a single i.v. injection to rats at 37 °C under anesthesia with pentobarbital, and the EPR spectra were measured at room temperature every 30 sec. The disappearance of the EPR signal due to vanadyl species in the blood was plotted against time after the compounds administration, and the data were analyzed by one or two compartment models (Fig. 5). The real-time EPR analysis of vanadyl species revealed that the clearance rate of the vanadyl species from the blood of rats given $VOSO_4$ was higher than those given vanadyl complexes in terms of half-life ($t_{1/2}$), being 5 min in $VOSO_4$-treated rats and 7–30 min in vanadyl complex-treated rats [38]. The slow clearance rate of the vanadyl complexes suggests a high distribution of vanadium in rat organs, which in turn indicates long-term acting normoglycemic effects after their withdrawal from the STZ-rats.

The chemical structures and metabolic pathways in terms of vanadyl states in organs of rats treated with $VOSO_4$ and VO(pic)$_2$ were investigated for the first time using the electron spin echo envelope modulation (ESEEM) method [50,51]. When $VOSO_4$ was administered, ESEEM results strongly suggested the formation of vanadyl-protein (or -peptide) complexes in rat organs, supporting the idea that the administered vanadyl ions are bound with the ε-amine of lysine or the *N*-terminal nitrogen of some proteins [50]. When VO(pic)$_2$ was given to rats, the ESEEM spectra of kidney and liver exhibited a weak signal due to picolinate imine nitrogen, suggesting some picolinate species including both the bispicolinate and a partially decomposed monopicolinate species still exist in the organs as minor species. The picolinate ligand presumably serves to prevent vanadyl ion from being converted into the inactive amine-coordinate species. On the other hand, bone samples exhibited an ESEEM signal due to ^{31}P nuclei, indicating that vanadyl species in bone are incorporated into the hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ matrix. Thus it appears that vanadyl species in bone are released gradually and transported to other organs. Such released vanadyl ions may be transferred to unknown active species to contribute to the insulin-mimetic activity [51].

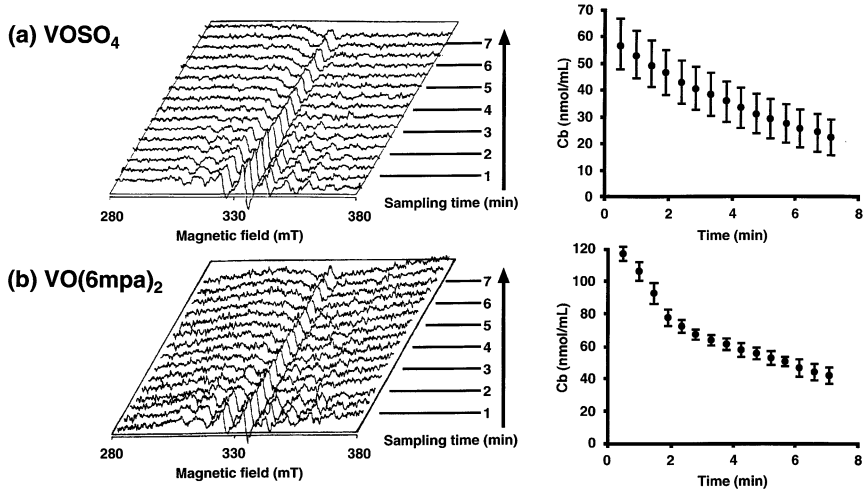


Fig. 5. In vivo BCM-EPR method of vanadyl state. Rats were treated by intravenous injection of: (a) $VOSO_4$ or (b) $VO(6mpa)_2$ at a dose of 0.5 mg V kg^{-1} body weight under anesthesia. EPR spectra were recorded at room temperature (see Ref. [49]).

In conclusion, the observation of the unique properties of vanadium and its complexes contributes to maintaining human health and to developing therapy for the prevention of important diseases like DM in the 21st century.

3. Zinc(II) complexes

Zinc with atomic number 30, atomic weight 65.39 and oxidation state II is an essential element in all living systems and plays a structural role in many proteins and enzymes. It is recognized that transcription factors regulate gene expression and the essential feature is binding to a regulatory protein in the recognition sequence of the gene. Many proteins have been found to have a zinc-containing motif that serves to bind DNA embedded in their structure. In the relevance of zinc to DM, zinc is known to be present in insulin, coordinated by three nitrogen atoms from histidines and three water molecules in an irregular octahedral environment, which is also believed to have a functional structure. Surprisingly, zinc was found to have important physiological and pharmaceutical functions involving insulin-mimetic activity. In 1980, Coulston and Dandona first reported the insulin-mimetic activity of zinc ion [52], in which administration of $ZnCl_2$ to STZ-rats or ob/ob mice normalized their high blood glucose levels. However, they used high doses or long-terms (8 weeks) in zinc(II) ion administration. Following this observation, several research groups attempted to confirm the insulin-mimetic activity of the zinc ion [53–55].

Although zinc(II) ion has been revealed to have an insulin-mimetic activity, zinc complexes have never been examined. Under the circumstance, we have tried to develop insulin-mimetic zinc complexes with various coordination modes around a zinc(II) ion. In this section, the structure, insulin-mimetic activity and blood glucose normalizing effect of zinc complexes are reviewed.

3.1. Preparation and structural analysis of zinc complexes

Zinc complexes are readily prepared by adding $ZnSO_4 \cdot 7H_2O$ to an aqueous solution of lithium or barium salt of the appropriate ligand (generated in situ from ligands and lithium or barium hydroxide) at room temperature. All complexes were purified from hot water or hot methanol solution by recrystallization, identified by elemental analyses as well as other spectral methods, and found to be molecular complexes without a counter ion.

A colorless single crystal of $Zn(ma)_2$ was determined to be *trans*-$[Zn(ma)_2(H_2O)_2][Zn(ma)_2(H_2O)] \cdot (H_2O)_2$ by X-ray structural analysis [56]. Two different geometries around the zinc(II) atom in the complex *trans*-$[Zn(ma)_2(H_2O)_2][Zn(ma)_2(H_2O)] \cdot (H_2O)_2$ were revealed to be in a unit cell, where two maltols and two H_2O molecules coordinate to a zinc(II) by *trans* forms in an octahedral conformation, and two maltols and an apical H_2O molecule coordinate to a zinc(II) in a square pyramidal conformation (Fig. 6(a)). In the colorless single crystal of $[Zn(6mpa)_2(H_2O)] \cdot H_2O$ obtained from a methanol solution [57], the coordination geometry is a distorted trigonal bipyramidal structure (Fig. 6(b)), with the zinc(II) ion being in an equatorial plane (O1, O1′ and O3) with two apical atoms (N1 and N1′).

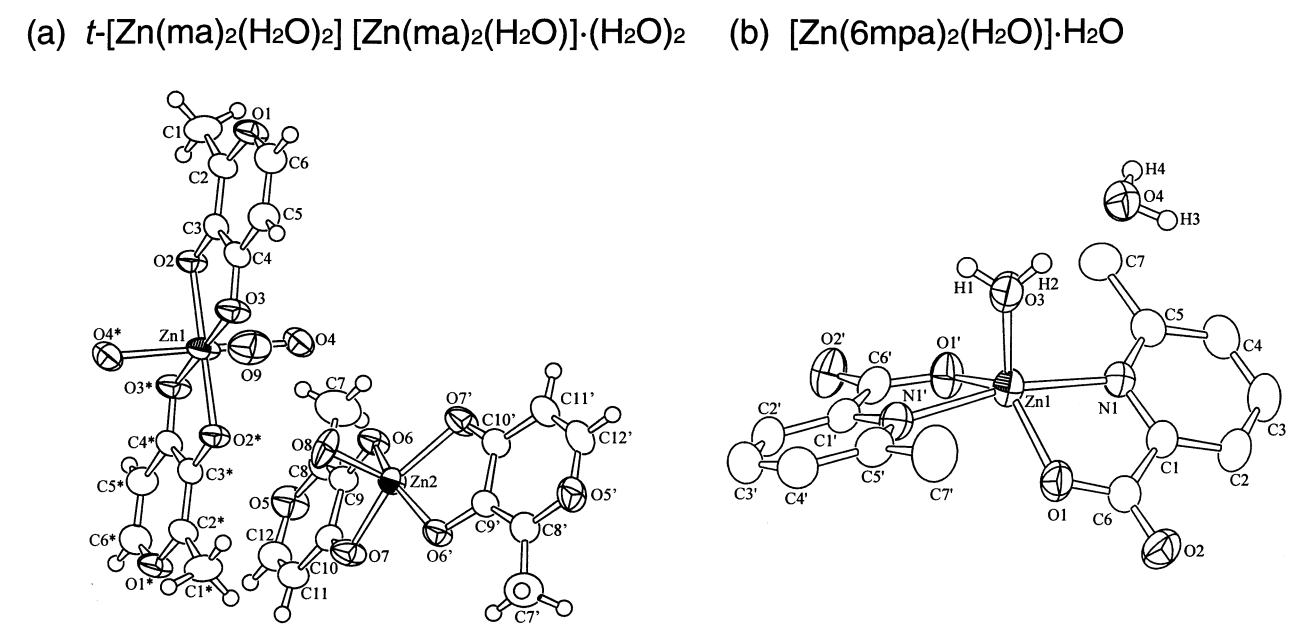


Fig. 6. (a) X-ray structure of t-[Zn(ma)$_2$(H$_2$O)$_2$] [Zn(ma)$_2$(H$_2$O)] · (H$_2$O)$_2$ (ORTEP representation; ellipsoids at 50% probability level). Two crystal water molecules are omitted. Selected bond distances (Å): Zn1–O2, 2.027(2); Zn1–O3, 2.072(3); Zn1–O4, 2.282(3); Zn2–O6, 2.014 (3); Zn1–O7, 2.078(3); Zn2–O8, 2.015(4). (b) X-ray structure of [Zn(6mpa)$_2$(H$_2$O)] · H$_2$O (ORTEP representation; ellipsoids at 50% probability level). All hydrogen atoms on 6mpa are omitted. Selected bond distances (Å): Zn1–O1, 2.000(2); Zn1–O1′, 1.990(2); Zn1–O3, 1.992(1); Zn1–N1, 2.159 (2); Zn1–N1′, 2.151(2). ORTEP representation is as for the caption of Fig. 3 (see Refs. [56,57]).

3.2. In vitro evaluation of insulin-mimetic activity of zinc complexes

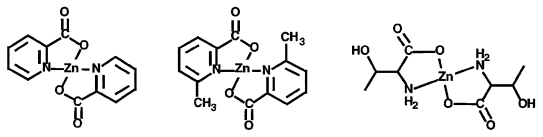
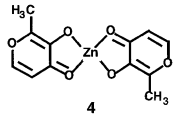
The in vitro evaluation of the insulin-mimetic activity of zinc complexes was performed in the same manner as for vanadyl complexes [8,25]. The zinc complexes with lower over-all stability constants ($\log \beta$) than 10.5 exhibited higher insulin-mimetic activities than those of $ZnSO_4$ and $VOSO_4$ or were comparable to them except for Zn(GtG) ($IC_{50} = 3.18$) (Table 2). On the other hand, zinc complexes with His, GeG, and mGeGm ($\log \beta = 12.05$, 11.22, and 11.83, respectively) with higher $\log \beta$ values than 11.0 showed essentially no insulin-mimetic activity. Furthermore, we found that Zn(ma)$_2$ and Zn(pic)$_2$ complexes which promote absorption of zinc(II) have higher insulin-mimetic activity than free zinc(II) ion (Table 2). In addition, the zinc complexes with L- and D-amino acids, Asn, Pro, Thr, and Val, exhibited similar insulin-mimetic activities to each other (Table 2). Accordingly, the difference in the insulin-mimetic activities of zinc complexes was not observed the absolute configurations of the α-amino acids, but was relevant to their stability in the solution.

Table 2
Estimated IC_{50} values for the free fatty acids (FFA) release from isolated rat adipocytes in the presence of glucose and the over-all stability constants ($\log \beta$) of zinc complexes

Complex	IC_{50} (mM) ($\pm$ S.D. [a])	$\log \beta$	Complex	IC_{50} (mM) ($\pm$ S.D. [a])	$\log \beta$
Zn(L-Asn)$_2$	0.65 (0.03) *	8.55	Zn(D-Asn)$_2$	0.65 (0.09) *	8.55
Zn(L-Pro)$_2$	0.89 (0.07)	9.75	Zn(D-Pro)$_2$	0.89 (0.07)	9.75
Zn(L-Thr)$_2$	0.54 (0.03) **	8.46	Zn(D-Thr)$_2$	0.48 (0.03) **	8.46
Zn(L-Val)$_2$	0.77 (0.08)	8.24	Zn(D-Val)$_2$	0.87 (0.04)	8.24
Zn(L-His)$_2$	None	12.05			
Zn(pic)$_2$	0.64 (0.13) *	9.52	Zn(6mpa)$_2$	0.31 (0.05) **	– [b]
Zn(ma)$_2$	0.59 (0.10) **	10.4			
Zn(GeG)	None	11.22	Zn(MGeGm)	None	11.83
Zn(βAeAβ)	0.82 (0.05)	7.6			
Zn(GtG)	3.18 (0.04)	10.27	Zn(VtV)	0.92 (0.04)	8.63
$VOSO_4$	1.00		$ZnSO_4$	0.81 (0.10)	

[a] Each value is expressed as the mean $\pm$ S.D. for three experiments.
[b] The value could not be obtained because of the precipitations occurred during the course of titration.
* Significance at $P < 0.05$ vs. $ZnSO_4$.
** Significance at $P < 0.01$ vs. $ZnSO_4$.

Table 3
Structures of zinc complexes with blood glucose normalizing effect

	Complex
N_2O_2	 1 2 3
O_4	 4

1: Kojima et al. [78], 2: Yoshikawa et al. [57], 3: Yoshikawa et al. [58], 4: Yoshikawa et al. [56,59].

3.3. *In vivo evaluation of the insulin-mimetic antidiabetic activity of zinc complexes in KK-A^y mice*

KK-A^y mice (type 2 diabetes model animal) received daily i.p. injections of zinc complexes for 2 weeks. Blood samples for analysis of their glucose levels were obtained from the mouse-tail vein, and measured with Glucocard (Arkray, Kyoto). The body weights of the KK-A^y mice who were allowed free access to solid food and tap water were measured daily during administration of the zinc complexes. Throughout the experiments, the intake of solid food and drinking water for each mouse were checked daily. The zinc complexes were dissolved in 5% acacia. Doses of zinc complexes were 3 mg Zn kg^{-1} body weight. The serum concentrations of the BUN, GOT, GPT, and TCHO were determined.

After administration of zinc complexes for 14 days, the mice fasted for 12 h, then a glucose dose of 1 g kg^{-1} body weight was given orally, and blood glucose levels were measured (oral glucose tolerance test).

Because $Zn(ma)_2$, $Zn(pic)_2$, $Zn(6mpa)_2$, and $Zn(Thr)_2$ complexes (Table 3) exhibited higher in vitro insulin-mimetic activity, the in vivo blood glucose lowering effect of these complexes in KK-A^y mice was evaluated [56–59]. When the mice were given these complexes at a dose of 3 mg Zn kg^{-1} body weight, the glucose levels were approximately maintained at about 250 mg dl^{-1} (13.9 mM) for 2 weeks (Fig. 7). During the treatment, the body weight of the KK-A^y mice increased slightly. The BUN level, which indicates the degree of renal disturbance, did not change compared with those of the untreated KK-A^y mice. GOT and GPT levels, which indicate the degree of liver disturbance, were also identical to those of the untreated KK-A^y mice. Thus, it was suggested that these complexes have low toxicities in hepatic and renal functions. On the other hand, the TCHO level was lower than the control KK-A^y mice. Because the measurement of HbA_{1c} has been used as an index of glycemic control in diabetic patients [60,61], the changes of HbA_{1c} in untreated KK-A^y mice and KK-A^y mice treated with these complexes were examined. In untreated KK-A^y mice, the HbA_{1c} level was $8.3 \pm 0.3\%$ after administration. However, the HbA_{1c} levels of KK-A^y mice treated with these complexes were about 6.5% after administration. These results show that the DM of KK-A^y mice treated with these complexes improved glucose tolerance compared with those of the untreated KK-A^y mice.

Because the blood glucose levels of KK-A^y mice treated with these complexes for 2 weeks were improved below 250 mg dl^{-1} (13.9 mM), the animals

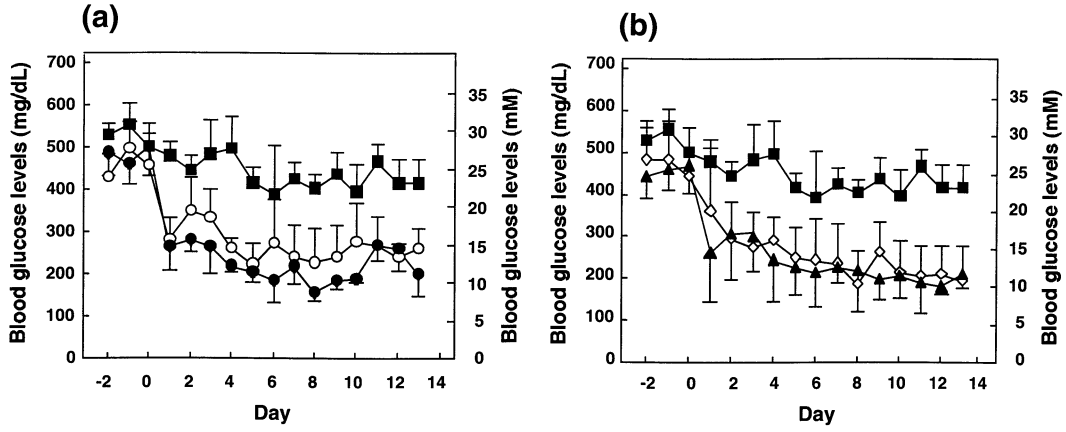


Fig. 7. (a) Changes in blood glucose level (left) in control KK-A^y mice (-■-), KK-A^y mice treated with $Zn(pic)_2$ (-○-), and KK-A^y mice treated with $Zn(6mpa)_2$ (-●-). Hyperglycemic KK-A^y mice received an i.p. injection of 5% acacia ($n = 5$), $Zn(pic)_2$ or $Zn(6mpa)_2$ (3.0 mg Zn kg^{-1} body weights for 2 weeks, $n = 5$ and 4, respectively). Values are means ± S.D.s for four or five mice. (b) Changes in blood glucose level (right) in the control KK-A^y mice (-■-), KK-A^y mice treated with $Zn(Thr)_2$ (-◇-), and KK-A^y mice treated with $Zn(ma)_2$ (-▲-). Hyperglycemic KK-A^y mice received an i.p. injection of 5% acacia ($n = 5$), $Zn(Thr)_2$ or $Zn(ma)_2$ (3.0 mg Zn kg^{-1} body weight for 2 weeks, $n = 5$). Values are means ± S.D.s for five mice (see Refs. [57–59]).

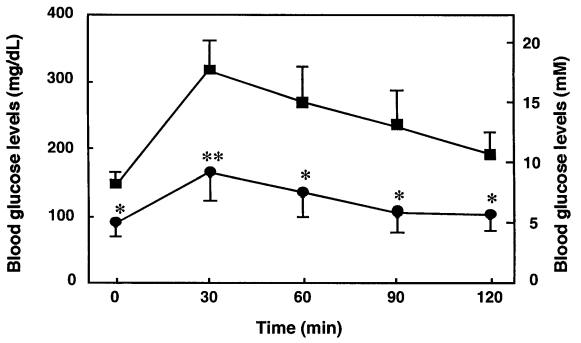


Fig. 8. Oral glucose tolerance tests for the control KK-A^y mice (-■-), KK-A^y mice receiving daily i.p. injections of Zn(6mpa)$_2$ (-●-). Oral glucose tolerance tests were performed on mice who fasted for 12 h and then were given glucose solution orally at a dose of 1 g kg^{-1} body weight. Values are means ± S.D.s for four or five mice. * Significance at $P < 0.05$ vs. control KK-A^y mice. ** Significance at $P < 0.01$ vs. control KK-A^y mice (see Ref. [57]).

received an oral glucose tolerance test. As shown in Fig. 8, blood glucose levels of control KK-A^y mice were elevated to the maximum (300 mg dl^{-1} (16.7 mM)) at 30 min after the glucose administration, and then gradually decreased. In contrast, the blood glucose levels of KK-A^y mice treated with Zn(6mpa)$_2$ complex which has the highest insulin-mimetic activity in an in vitro experiment was also elevated at first, but they were obviously lower when compared with those of the control KK-A^y mice. These results indicate that treatment with these complexes mitigates diabetes in KK-A^y mice by improving glucose tolerance.

In conclusion, the zinc complexes proposed here show good blood glucose lowering effects in a model animal with type 2 DM. Thus, these complexes might contribute to improve type 2 DM when they are developed as clinically useful therapeutics after their non-toxicity, bioavailability, and pharmacokinetics are examined.

4. Mechanism of insulin-mimetic action of vanadium and zinc

Because vanadate behaves like phosphate, the effect of vanadium in biochemistry has been understood to inhibit protein phosphotyrosine phosphatase, which in turn stimulates protein tyrosine phosphorylation. Thus, vanadate was reported to activate autophospohrylation of solubilized insulin receptors [62–65], similar to insulin action. Vanadate also stimulated the tyrosine kinase activity of the insulin receptor β subunit [66,67]. In addition, both vanadate and vanadyl were found to be effective in stimulating glucose metabolism in rat adipocytes [5,62,64,68].

We have proposed that the vanadyl state is a possible active form of vanadium for insulin-mimetic action and for acting on a glucose transporter [8]. Evidence for the proposal comes from the observation that vanadate, which was in turn reduced to vanadyl, restored expression of the insulin-sensitive glucose transporter of skeletal muscles in rats [69] and induced the recruitment of GLUT4 glucose transporter to the plasma membrane of adipocytes [70].

In addition, the effect of vanadium on lipid metabolism has been examined. The adenosine 3′,5′-cyclicmonophosphate (c-AMP)-mediated protein phosphorylation cascade in adipocytes was either activated during diabetes (in vivo) or in the presence of epinephrine (in vitro), and both glucose and vanadyl ion, which were taken up in adipocytes by the vanadyl treatment, led to restored regulation of this cascade in peripheral cells [71–73]. Thus, it was proposed that FFA release from adipocytes is inhibited by vanadyl ion. The suppressed FFA release by a vanadate ion depended on the enhancement of glucose uptake by the metal ion, which was reduced to vanadyl by added glucose. Therefore, vanadate was concluded not to inhibit FFA release in adipocytes in a dose-dependent manner, and the effect in the presence of 1 mg ml^{-1} glucose was completely reversed by 10^{-5} M Cyt B. These effects were not seen in the absence of glucose. Therefore, it was suggested that glucose, which was taken up in adipocytes by either insulin or vanadyl ion, suppressed FFA release [8]. When other inhibitors such as HNMPA-(AM)$_3$ (hydroxy-2-naphthalenylmethylphosphonic acid tris-acetoxymethylester), wortmannin and cilostamide for insulin receptor tyrosine kinase, phasphatidyl inositol 3-kinase and phosphodiesterase, respectively, were tested, cilostamide alone partially restored the inhibited FFA release by vanadyl. On the basis of these observations, we have proposed a possible mechanism, by which vanadyl acts at least on two sites such as glucose transporter and phosphodiesterase in cells to normalize both glucose and FFA levels in diabetic rats, as shown in Fig. 9.

Similar observations were obtained when zinc was used in place of vanadyl. Both metal ions act similarly on the adipocytes.

5. Future aspects

In the 20th century, several metallopharmaceutical compounds such as platinum-containing *cis*-platin and its derivatives as anticancer drugs, gold-containing auranofin as an oral rheumatoid arthritis drug and zinc-containing polaprezinc as an antigastric ulcer drug have been developed and used clinically. These successful developments of metallopharmaceutical compounds have prompted many researchers to provide other types of therapeutic agents using the unique and characteristic properties of metal ions.

The number of patients suffering from DM is increasing. Although many types of insulin pharmaceutical preparations for type 1 DM and synthetic drugs for type

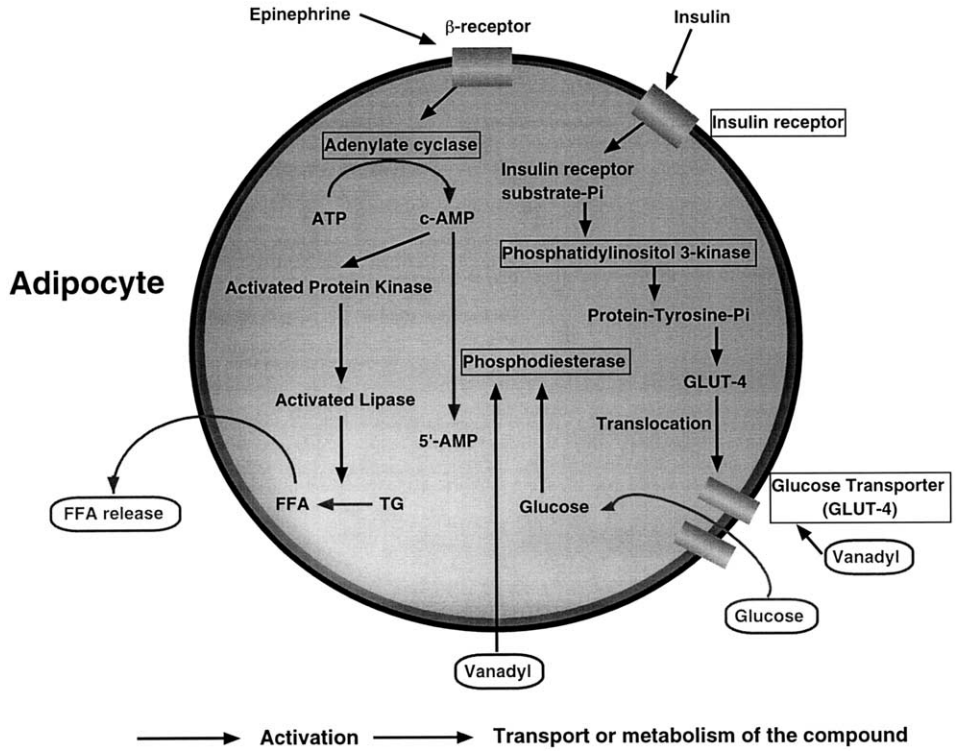


Fig. 9. A possible mechanism of insulin-mimetic vanadyl ion in isolated rat adipocytes.

2 DM have been developed and clinically applied, each preparation so far involves side effects.

In the 21st century, a new method to treat both types of DM is needed. For this reason, several vanadium compounds are being developed for pharmaceutical use to treat or improve both types of DM. In addition to the therapeutic effect of vanadium ion and vanadium complexes, these vanadium compounds have a preventive effect on the onset of STZ-induced diabetes in terms of nitric oxide released from the macrophages [74]. Thus, vanadium is expected not only to treat DM but to prevent DM.

In addition to vanadium complexes, zinc complexes have been proposed to be new candidates in treating type 2 DM. Designing new vanadium complexes require stereochemical considerations for binding the complexes with receptors such as glucose transporter and other enzymes as well as consideration of the redox properties of vanadium.

Designing new zinc complexes on the other hand requires attention to the stability and structural properties under physiological conditions.

Both, new vanadium and zinc complexes, are being studied for potential uses in the future.

Acknowledgements

The studies were supported in part by grants from the Ministry of Education, Science, Sports and Culture of Japan and were carried out in part under the Visiting Researcher Program of the Research Reactor Institute, Kyoto University. K.K. thanks the Kyoto Pharmaceutical University fellowship for a postdoctoral program.

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