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New antidiabetic vanadyl–pyridone complexes: effect of equivalent transformation of coordinating atom in the ligand

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Contents

Abstract

To treat both insulin-dependent type 1 and non-insulin-dependent type 2 diabetic mellitus, two potent antidiabetic vanadyl pyridone complexes, bis(1-oxy-2-pyridinethiolato)oxovanadium(IV), ($VO(opt)_2$) and bis(1-oxy-2-pyridonato)oxovanadium(IV), ($VO(opd)_2$), were proposed on the basis of the results of diabetic model animals by using a concept of equivalent transformation, which has been proved to be effective in changing the chemical property of a compound. Their physico-chemical properties, chemical specications, in vitro insulin–mimetic activity in isolated rat adipocytes, in vivo antidiabetic activity in both type 1 and type 2 diabetic animals, and metallokinetic feature of the vanadyl species in the blood flow of live rats by using blood circulation monitoring-electron paramagnetic resonance (BCM-EPR) were examined. Both complexes exhibited higher in vitro insulin–mimetic activity than $VOSO_4$, and the activity of $VO(opt)_2$ was superior to that of $VO(opd)_2$. However, on oral administration to type 1 diabetic rats, $VO(opd)_2$ was more effective at lower dose than $VO(opt)_2$. The results are supported by chemical specifications and metallokinetic (kinetic of metal disposition) parameters.

Keywords: Vanadyl–pyridone complexes; Insulin–mimetic activity; Antidiabetic activity; Chemical specification; Adipocytes; Blood circulation monitoring-electron paramagnetic resonance (BCM-EPR)

1. Introduction

The concept of equivalent transformation was useful for the development of many tools, that were inspired from figure or shape in nature. For instance, scissor, shovel with a tractor and fan are surely inspired from the figure or shape of beak of birds, flamingo and fully expanded wings of a peacock, respectively. In the world of chemistry, we have an example for the equivalent transformation, two temples in Kyoto, Japan, the Ginkakuji, the surface of the Shogun's villa being spread with silver (Ag), while the another villa, Kinkakuji was covered with brilliant gold (Au), where Ag

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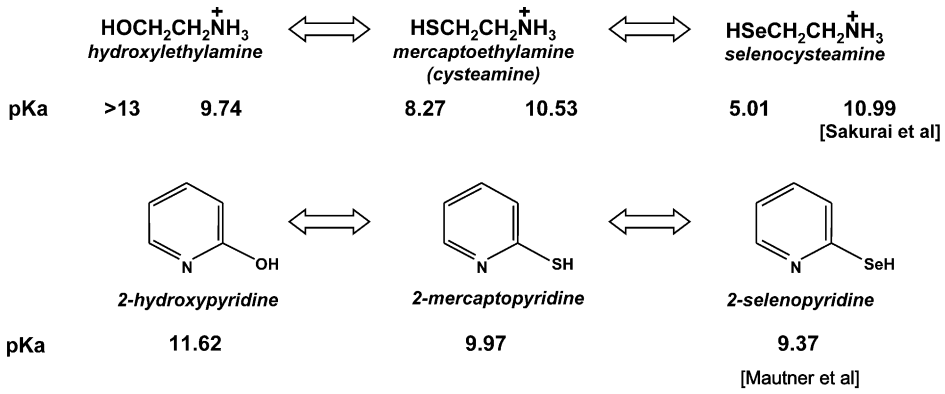
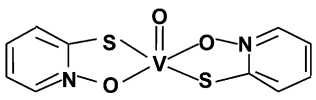


Fig. 1. pK_a values of equivalent transformation compounds.

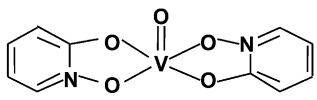
and Au belong to the 11 Group in the periodic table of the elements. Furthermore, the importance of equivalent transformation was indicated, in which when an oxygen atom in the functional group was substituted by sulfur or selenium, the pK_a value of HX-R (X = O, S and Se) of hydroxyethylamine and 2-hydroxypyridine was lowered in this sequence (Fig. 1) [1,2].

Since 1990 [3], many vanadyl complexes with different coordination modes have been proposed to have in vitro insulin–mimetic activity and in vivo antidiabetic blood glucose lowering activity in several types of experimental animals [4–10], based on the results that the simple vanadyl ion shows both insulin–mimetic and antidiabetic activities [11,12]. Among them, in 1999, we proposed a purple vanadyl complex, bis(1-oxy-2-pyridinethiolato)oxovanadium(IV), $VO(opt)_2$, with $VO(S_2O_2)$ coordination mode, which exhibited a high in vitro insulin–mimetic activity and a potent in vivo treating effect of insulin-dependent type 1 diabetes mellitus when given by daily intraperitoneal (i.p.) injection or oral administration [13,14]. $VO(opt)_2$ was prepared and its structure was characterized in 1994 [15]. However, we have neither prepared nor tested the oxygen analogue of $VO(opt)_2$ with a $VO(O_4)$ coordination mode. By using the concept of equivalent transformation and in the hope of developing better vanadyl complexes, we recently prepared the bis(1-oxy-2-pyridonato)oxovanadium(IV), ($VO(opd)_2$), and found that this complex is a better agent to treat a type 1 diabetic experimental animal than $VO(opt)_2$ (Table 1). This article reviews recent progress in the development of insulin–mimetic antidiabetic vanadyl–pyridone

Table 1
Physico-chemical properties of vanadyl–pyridone complexes



$VO(opt)_2$



$VO(opd)_2$

	$VO(opt)_2$			$VO(opd)_2$		
Elemental analysis Calcd found	H (%)	C (%)	N (%)	H (%)	C (%)	N (%)
	2.51	37.60	8.78	2.79	41.84	9.75
	2.61	37.76	8.41	2.76	41.52	9.60
Visible absorption λ (nm) ε (mm^{-1} cm^{-1}) in DMSO	514 (6.6×10^{-2}) 624 (1.9×10^{-2})			516 (2.5×10^{-2}) 578 (2.1×10^{-2})		
ESR in DMSO	g_o A_o (10^{-4} cm^{-1})	1.981	82		1.993	87
	$g_\perp$ $A_\perp$ (10^{-4} cm^{-1})	1.948	156		1.948	168
	$g_\parallel$ $A_\parallel$ (10^{-4} cm^{-1})	1.998	34		2.016	35
Magnetic susceptibility	χ_g (cgs)	3.73×10^{-6}			4.30×10^{-6}	
	μ_{eff}	1.79			1.86	
Infra-red ($\nu_{V=O}$: cm^{-1})		960			984	

complexes by recognizing the concept of equivalent transformation.

2. Usefulness of thiolate ligands to vanadyl ion in the development of antidiabetic vanadyl complexes

According to Pearson's HSAB (hard and soft acids and bases) rule [16], vanadyl ion is classified as a hard acid, and thus this metal ion binds with hard bases such as oxygen and nitrogen ligands more strongly than with soft bases such as a sulfur ligand. Nevertheless, we found in 1990 that vanadyl ion binds fairly strongly with a sulfur-containing ligand, cysteine methyl ester, and forms reasonably stable complex with high insulin–mimetic antidiabetic activity by both daily i.p. and oral administration [11,17]. Following this important observation, we developed insulin–mimetic antidiabetic vanadyl complexes, vanadyl–dithiocarbamate complexes with $VO(S_4)$ coordination mode, in which a hard vanadyl ion is coordinated by four soft sulfur bases [18,19], and vanadyl-1-oxy-2-pyridinethiolate $VO(opt)_2$ complex with $VO(S_2O_2)$ coordination mode [13,14], those also being effective with daily oral administration (Fig. 2).

In the literature, several insulin–mimetic vanadyl complexes with V–S bond involving bis(*O*-aminothiophenolato)-oxovanadium(IV), $VO(thioam)_2$, have been proposed [20], in which ON ligation to vanadyl ion was superior to in vitro insulin–mimetic efficacy to OO and O/NS ligations, irrespective of the vanadium oxidation sate. In addition, after evaluation of the toxicity of vanadyl complexes with respect to short-term cell toxicity (for 36 h), vanadyl compounds showed a tendency to be less toxic than vanadate (+5 oxidation state) compounds, and complexes containing thiol functional ligands were more toxic than others [20].

In the case of $VO(opt)_2$, no mutagenic activity for several mutagenesis test systems were found [14]. Because data on the insulin–mimetic antidiabetic activity of vanadyl complexes with V–S bond(s) in relation to the structure and toxicity are still limited, such evaluation on much more complexes will be needed.

3. Preparation, physico-chemical properties and chemical specification of $VO(opt)_2$ and $VO(opd)_2$ complexes

Using the concept of equivalent transformation, we newly prepared $VO(opd)_2$ similar to the preparation method of $VO(opt)_2$, and measured several physico-chemical properties, as summarized in Table 1, as shown together with the possible structures.

Chemical specification was performed by pH-potentiometric titration and electron paramagnetic resonance (EPR) methods [21]. In both vanadyl–opt and –opd systems, VOA, VOA_2 and a mixed hydroxo species, $VOA_2H_{-1} = VOA_2(OH)$ were formed, where A stands for the fully deprotonated ligands opt^- or opd^- (Table 2 and Fig. 3). Both the N-oxide moiety and the deprotonated hydroxy- or mercapto group coordinated to the vanadyl ion forming 5-membered chelate rings. The ligand opt formed very stable bis-complexes with vanadyl ion at pH 3–6, while opd did so in a wide pH range 3–8, as determined by pH-potentiometric titration. Interestingly, the EPR study indicated that a *cis–trans* isomeric equilibrium strongly shifts in the direction of one of the isomers: *cis* with opd and *trans* with opt. In addition, an equilibrium between 5- and 6-coordinate trans complexes with a solvent molecule (water or DMSO) in the apical position

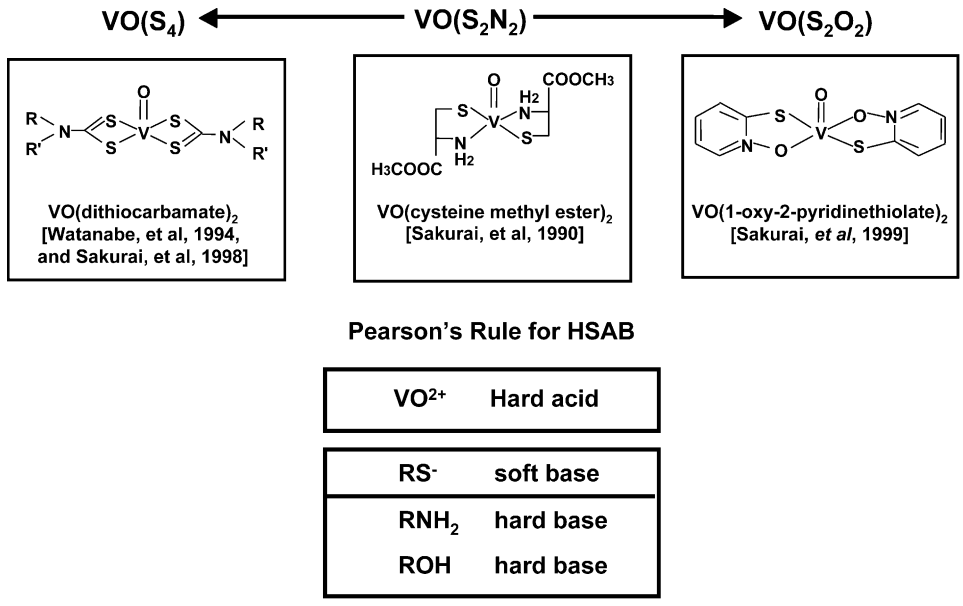


Fig. 2. Antidiabetic vanadyl complexes containing V–S coordination bond.

Table 2
Proton ($\log K$) and oxovanadium(IV) ($\log \beta$) stability constants for the complexes of opt and opd at 25.0±0.1 °C and I = 0.20 M (KCl) in aqueous solution and in DMSO–water (60–40 (w/w)%) mixture

Complex	log K/log β			
	opt		opd	
	Water	DMSO–water	Water	DMSO–water
HA	4.49 (2)	5.73 (2)	5.80 (1)	7.05 (1)
VOA	6.94 (29)	7.72 (56)	8.29 (5)	8.80 (15)
VOA_2	13.28 (22)	14.98 (38)	15.99 (4)	16.87 (9)
VOA_2H_{-1}	6.12 (42)	6.86(39)	6.81 (11)	7.11 (11)

was found, as assumed by the deprotonation of the bis-complex.

4. Comparison of in vitro insulin–mimetic activity and in vivo antidiabetic activity of $VO(opt)_2$ and $VO(opd)_2$ complexes

4.1. In vitro insulin–mimetic activity

In evaluation of the in vitro insulin–mimetic activity of both complexes, we examined the effect of the complexes on FFA release from isolated rat adipocytes, this method being proposed to be simple and convenient without using radioisotopes [8–10,12], in which vanadyl sulfate ($VOSO_4$) was used as a positive control.

As shown in Fig. 4, both $VO(opt)_2$ and $VO(opd)_2$ complexes inhibited FFA release in a dose-dependent manner more strongly than did $VOSO_4$. Then, the inhibitory effects of the complexes on FFA release from the adipocytes were evaluated by the IC_{50} value, which expresses the 50% inhibition concentration of the complex on the FFA release stimulated by epinephrine (adrenaline). The IC_{50} values of $VO(opt)_2$, $VO(opd)_2$, and $VOSO_4$ were estimated to be 0.18, 0.34, and 1.4 mM, respectively, indicating that both $VO(opt)_2$ and $VO(opd)_2$ complexes have stronger insulin–mimetic activity than $VOSO_4$.

4.2. In vivo antidiabetic activity

On the basis of the in vitro results, both $VO(opt)_2$ and $VO(opd)_2$ complexes were given at the same dose to streptozotocin (STZ)-induced type 1 diabetic rats (STZ-rats) by daily i.p. injection and oral administration. When STZ-rats received the dose of 2.5 mg (49 μmol) V kg^{-1} of body weight for the first 7 days, the blood glucose level of the rats decreased to the normal range and remained in the normal range by the subsequent administration of each complex at the dose of 1 mg (19.6 μmol) V kg^{-1} (Fig. 5). Both complexes exhibited almost the same antidiabetic activity by daily i.p. injections. During the administration of both complexes, the body weight of STZ-rats was not decreased but increased slightly.

Then, both complexes were given to STZ-rats by oral administration. When STZ-rats received daily oral administration of $VO(opt)_2$ complex at the dose of 10 mg (196 μmol) V kg^{-1}, the blood glucose level was normalized within 4 or 5 days and it was maintained in the normal range as long as the complex was administered (Fig. 6). During the complex administration, no body weight loss was observed. The equivalent blood glucose normalizing effect was achieved on oral administration of $VO(opd)_2$ complex at lower dose (7.5 mg (147 μmol) V kg^{-1}) than that of $VO(opt)_2$ complex at 10 mg (196 μmol) V kg^{-1}, indicating that $VO(opd)_2$ complex is more effective than $VO(opt)_2$ complex on oral administration. Such differential effects of two vanadyl complexes might relate to the stability and distribution at physiological pH 7.4 of the complex, as indicated by the chemical specification study. Serum parameters in STZ-rats treated with $VO(opt)_2$ and $VO(opd)_2$ complexes by oral administration are summarized in Table 3. Glucose tolerance test (oral glucose loading at 2 g kg^{-1}) revealed that type 1 diabetes was completely improved following oral administration of both complexes for 2 or 3 weeks.

On the other hand, $VO(opt)_2$ complex was recently found to treat an obese type 2 diabetic mouse [22]. Oral administration of $VO(opt)_2$ for 15 days resulted in a dose-dependent decrease in the levels of glucose, insulin, and triglyceride in the blood. Then the action mechanism of the complex was examined. In the epidermal and subcutaneous

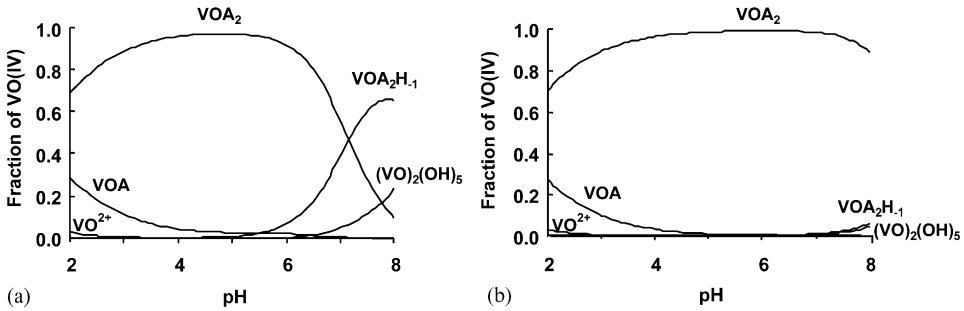
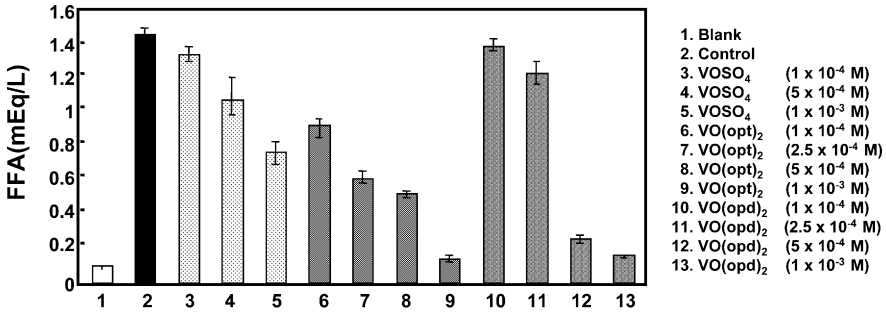


Fig. 3. Specification curves for the complexes in the: (a) VO(IV)–opt; and (b) VO(IV)–opd systems. [VO(IV)] = 1 mM and [ligand] = 2 mM [21].



complex	IC_{50} (mM)	
$VOSO_4$	1.4 ± 0.43	
$VO(opt)_2$	0.18 ± 0.02	*
$VO(opd)_2$	0.34 ± 0.03	*

P<0.01 (n=3) *

Fig. 4. Inhibitory effects of vanadyl sulfate ($VOSO_4$) and vanadyl–pyridone complexes on FFA release from rat adipocytes treated with epinephrine (adrenaline) (a) and 50% inhibitory concentration of the compound on FFA release from isolated rat adipocytes (b) [13,14].

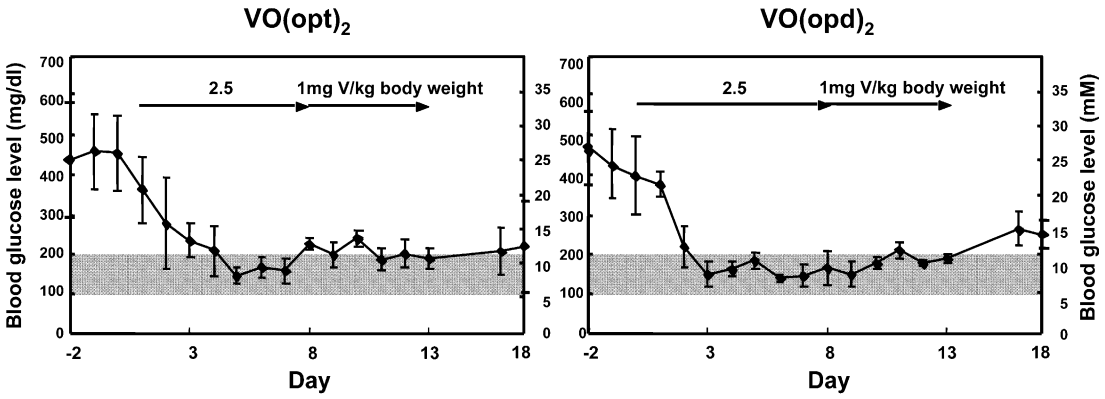


Fig. 5. Comparison of hypoglycemic effect in STZ-rats treated with $VO(opt)_2$ and $VO(opd)_2$ by daily i.p. injections [13,14].

Table 3
Serum parameters in STZ-rats treated with $VO(opt)_2$ or $VO(opd)_2$ complex by oral administration

Complex	Dose (mg V kg^{-1})	*n*	Day	HbA_{1c} (%)	Insulin (mU l^{-1})	Glucose (mg dl^{-1})	BUN (mg dl^{-1})
Saline		6	0	4.5 ± 0.4	4.9 ± 3.0	401 ± 34	15.1 ± 3.2
$VO(opt)_2$	10	6	14	n.d.	2.5 ± 2.0	210 ± 45**	13.6 ± 1.6
$VO(opd)_2$	7.5	3	21	4.0 ± 0.2	0.4 ± 0.2**	188 ± 102**	22.0 ± 3.8*

HbA_{1c}, insulin and BUN were measured after fasting for 12 h. Data are expressed as the means ± SDs. Significance level: *$P<0.05$, **$P<0.01$ vs. 0 day.

fat tissue of ob/ob mice, the expression of TNF-α (tumor necrosis factor-α) was elevated, which in turn decreased in insulin receptor substrate-1 (IRS-1) phosphorylation. Thus, the $VO(opt)_2$ complex was found to attenuate the TNF-α-induced impaired insulin signal transduction through inhibition of protein tyrosine phosphatase [22]. From these results, the $VO(opt)_2$ complex is expected to be of potential clinical use in the treatment of obesity-type 2 diabetes mellitus. On the basis of these results, $VO(opt)_2$ complex has a therapeutic potential for the treatment of both types of diabetes mellitus due to an inhibitory action on protein tyrosine phosphatase. Such an investigation is now underway with the $VO(opd)_2$ complex.

5. Comparison of metallokinetic features of $VO(opt)_2$ and $VO(opd)_2$ complexes as studied by BCM-EPR

For the clinical trial of vanadyl complexes in the future, understanding the pharmacokinetic features of the complexes is essential. For this purpose, information about the fate or metabolism of the complexes will be needed. In

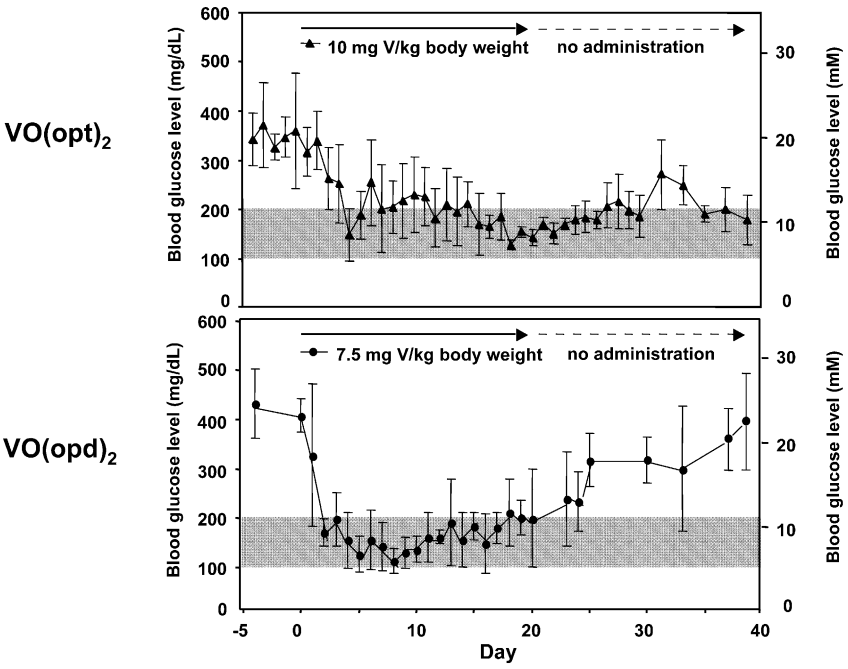


Fig. 6. Comparison of hypoglycemic effect in STZ-rats treated with $VO(opt)_2$ and $VO(opd)_2$ by daily oral administrations [13,14].

fact, we examined the distribution of vanadium and chemical forms of the complexes in several separated organs of animals given different vanadyl complexes by neutron activation analysis (NAA) [11,12,19,23] and electron spin echo envelope modulation (ESEEM) methods [25,26]. However, at present it is quite difficult to study the fate or metabolism of a vanadyl complex in live animals. Then we developed a new technique, which combined both pharmacokinetic analysis and EPR, to know the real time disposition of paramagnetic vanadyl species in the blood flow of a live rat [13, 14, 24, 27, 28]. EPR analysis might allow one to know not only the concentration of vanadyl species but also the chemical forms of a complex in the blood flow of a rat receiving a complex. Both $VO(opt)_2$ and $VO(opd)_2$ complexes were given by a single intravenous (i.v.) injection to rats at 37 °C under anesthesia with pentobarbital, and EPR spectra were measured at room temperature every 30 s. The disappearance of the EPR signal due to vanadyl species in the blood was plotted against time after the complex administration and the data were treated with a two-compartment model by the equations, as shown in Fig. 7. The clearance rate of vanadyl species from the blood flow of rats given $VOSO_4$ was ca. eight times higher than those given two vanadyl complexes in terms of half-life ($t_{1/2}$) (Table 4). The slow clearance rate of the vanadyl complexes suggested a high distribution of vanadium in the organs of rats, which in turn brought about the high blood glucose lowering effect. In fact, the importance of monitoring plasma vanadium levels in exhibiting antidiabetic activity during $VOSO_4$ treatment in human subjects with type 2 diabetes mellitus was recently indicated [29]. After the oral administration of the compound at a dose of 150 mg per day for 6 weeks, the plasma glucose, hemoglobin A_{1c} (HbA_{1c}) and fructosamin levels were improved, and plasma vanadium levels increased to 104 ± 18 $\mu g\, l^{-1}$ after 6 weeks from below 10 $\mu g\, l^{-1}$ before treatment.

The AUC value for $VO(opd)_2$-treated rats increased significantly with respect to the $VO(opt)_2$-treated rats, while CL_{tot} and Vd_{ss} values for the $VO(opd)_2$-treated rats decreased significantly from those of $VO(opt)_2$-treated rats, indicating that vanadyl species in a blood flow after i.v. injection of $VO(opd)_2$ is more directly exposed to the blood as well as to organs than that after injection of $VO(opt)_2$. Such an observation suggests that even after the cessation of $VO(opd)_2$ administration the blood glucose normalization effect is prolonged, as reported on

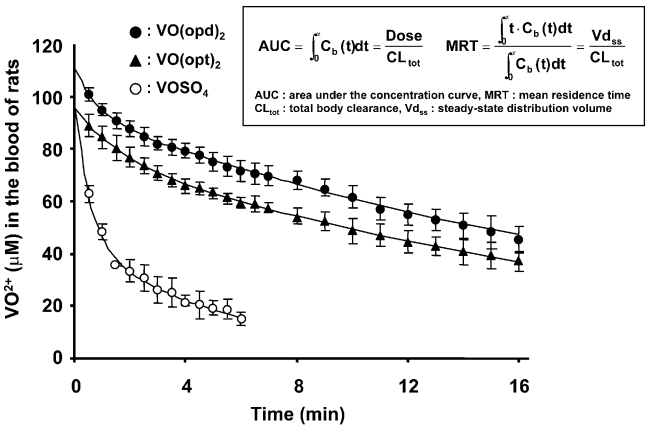


Fig. 7. Clearance curves of vanadyl species in the blood flow of live rats given the vanadyl complexes at a dose of 0.5 mg V kg^{-1} body weight by an intravenous injection under anesthesia, and the equations for the metallokinetic analysis. EPR spectra were recorded at room temperature.

Table 4
Metallokinetic parameters in rats given $VOSO_4$, $VO(opt)_2$, and $VO(opd)_2$ as estimated by BCM-ESR method

	AUC (μmol min ml^{-1})	MRT (min)	CL_{tot} (ml min^{-1} kg^{-1})	Vd_{ss} (ml kg^{-1})	$t_{1/2}$ (β) (min)
$VOSO_4$	0.26 ± 0.04	4.4 ± 0.7	38.9 ± 5.8	167 ± 10	3.0 ± 0.5
$VO(opt)_2$	1.84 ± 0.05*	22.8 ± 2.2*	5.3 ± 0.2*	122 ± 9*	16.0 ± 1.7*
$VO(opd)_2$	2.23 ± 0.25*,**	23.3 ± 2.0*	4.4 ± 0.5*,**	103 ± 3*,**	16.3 ± 1.4*

Data are expressed as the means ± SDs for three or four rats. Rats were treated with vanadyl complexes such as $VOSO_4$ ($m=4$), $VO(opt)_2$ ($n=3$), and $VO(opd)_2$ ($n=3$), V: 0.5 mg kg^{-1} body weight by intravenous injection under anesthesia.
* $P<0.01$ vs. $VOSO_4$.
** $P<0.01$ vs. $VO(opt)_2$.

the bis(6-methylpicolinato)oxovanadium(IV) ($VO(6mpa)_2$) [23]. Our effort on this problem continued.

Thus the BCM-EPR method will provide useful information not only for determination of the dosage plan or time schedule of a complex but also for molecular design of a complex structure.

6. Conclusion

Two oxygen and sulfur analogs of vanadyl–pyridone complex, $VO(opt)_2$ and $VO(opd)_2$, were prepared and their physico-chemical properties, chemical specification, in vitro insulin–mimetic activity in isolated rat adipocytes, in vivo antidiabetic activity in both type 1 and 2 diabetic animals, and metallokinetic characters by BCM-EPR were examined. Although the $VO(opt)_2$ complex exhibited a higher in vitro insulin–mimetic activity, the $VO(opd)_2$ complex was found to be more effective at a lower dose than $VO(opt)_2$ on oral administration to type 1 diabetic rats, the results being supported by chemical specification and metallokinetic parameters.

Both complexes are being studied not only for their mechanism action but also for potential trials in diabetic subjects in the future.

Acknowledgements

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