VII-C Research on the Relationship between Structure of Vanadyl Complex and Insulin-Mimetic Activity

The number of people suffering from diabetes mellitus (DM), one of the life-style related disease, has risen to approximately 14 million, including the figure of potential patients in Japan. DM is classified mainly into two types; insulin-dependent DM (IDDM: type 1) is associated with absolute insulin deficiency and non-insulin dependent DM (NIDDM: type 2) is with relative insulin deficiency. Although NIDDM is treated with several types of synthetic medicines, IDDM is given daily insulin injections to normalize the high blood glucose levels. Vanadium ions and complexes have been found to be active to normalize the high blood glucose level in not only experimental animals but the patients with both type of DM. Then we have developed several types of vanadyl complexes in VO^{2+} state and evaluated their in vitro and in vivo insulinomimetic activities to know the relationship between structure of vanadyl complexes and their insulinomimetic activities.

VII-C-1 Stereospecific and Structure-Dependent Insulin-Mimetic Oxovanadium(IV) Complexes with N,N'-Ethylene-bis-amino Acids

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The structure-insulinomimetic activity relationship of the tetradentate oxovanadium(IV) complexes with N,N'-ethylene-bis-amino acid (XeX) was examined. The complexes [VO(XeX)(H_2O)], where X = G (Gly), mG or Gm (N-methylglycine), L- and D-A (Ala), L- and D-V (Val), L- and D-M (Met), and L- and D-P (Pro), were prepared and structurally characterized by X-ray analysis and CD spectra. The insulin-mimetic activity of the complexes was evaluated in an *in vitro* system in terms of IC_{50} value due to FFA release from isolated rat adipocytes. The *in vitro* insulin-like activities of the complexes were found to depend on the absolute configuration of the complexes and the complexes which contain achiral amino acids or D-amino acids were found to have higher insulin-mimetic activities than the corresponding L-isomers. In addition, the insulin-like activities of the complexes were found to depend on the acid dissociation constants of the amino acids as the ligands, partition coefficients and redox potentials of them.

VII-C-2 A New Type of Orally Active Insulin-Mimetic Vanadyl Complex: Bis(1-oxy-2-pyridinethiolate)oxovanadium(IV) with VO(S_2O_2) Coordination Mode

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[*Chem. Lett.* 913 (1999)]

A new purple vanadyl complex, bis(1-oxy-2-pyridinethiolato)oxovanadium(IV), VO(OPT), with VO(S_2O_2) coordination mode, was prepared by mixing 2-mercaptopyridine-N-oxide or 1-hydroxy-2-pyridine-thione and $VOSO_4$, and characterized by UV, IR and EPR spectra, magnetic susceptibility and partition coefficient. Based on the higher *in vitro* insulin-mimetic activity of VO(OPT) (IC_{50} = 0.19 mM) than that of $VOSO_4$ (IC_{50} = 0.9 mM), the complex was found to be a potent agent for treating insulin-dependent diabetes mellitus in rats when given by daily intravenous injection or oral administration.

VII-C-3 Evidence for the Improvement of Noninsulin-Dependent Diabetes Mellitus in KKA^y Mice with Daily Oral Administration of Bis(6-methylpicolinato)oxovanadium(IV) Complex

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A vanadyl complex, bis(6-methylpicolinato)oxo-vanadium(IV), VO(6MPA), with VO(N_2O_2) coordination mode, was found to exhibit a normoglycemic effect on KKA^y mice with hereditary noninsulin-dependent diabetes mellitus with daily oral administration.

VII-C-4 In vivo Coordination Structural Changes of a Potent Insulin-Mimetic Agent, Bis(picolinato)oxovanadium(IV), Studied by Electron Spin-Echo Envelope Modulation Spectroscopy

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Bis(picolinato)oxovanadium(IV) [$VO(pic)_2$] is one of the most potent insulin-mimetic vanadium complexes. To probe coordination structural changes of this complex in vivo and provide insights into the origin of its high potency, an electron spin-echo envelope modulation (ESEEM) study was performed on organs (kidney, liver and bone) of $VO(pic)_2$- and $VOSO_4$-treated rats. Kidney and liver samples from both types of rats exhibited a ^{14}N ESEEM signal that could be attributed to equatorially coordinating amine nitrogen.

The relative intensity of the amine signal was larger for the organs of the rat treated with the less potent $VOSO_4$, suggesting that this amine coordination inhibits the insulin-mimetic activity. The spectra of kidney and liver from the $VO(pic)_2$-treated rat contained a weak signal due to the picolinato imine nitrogen. This suggests that some picolinato species (including both the bis-picolinato and a partially decomposed monopicolinato species) still exist in the organs as a minor species, where the proportions of the picolinato species to the total amount of the EPR-detectable $V^{IV}O$ species are estimated as 8–16% in the kidney and 12–24% in the liver. The picolinato ligand presumably serves to prevent VO^{2+} from being converted into the inactive amine-coordinated species. Bone samples from both types of rats exhibited an ESEEM signal due to ^{31}P nuclei. The VO^{2+} in bone is therefore most likely incorporated into the hydroxyapatite $Ca_{10}(PO_4)_6(OH)$ matrix, which is consistent with the hypothesis that the bone-accumulated VO^{2+} is gradually released and transported to other organs as is Ca^{2+}. No ^{14}N signals were observed, even in the bone samples of the $VO(pic)_2$-treated rats, indicating that vanadium uptake by bone requieres complete decomposition of the complex.

VII-C-5 Role of Vanadium in Treating Diabetes

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[*J. Trace Elem. Exp. Med.* **12**, 393 (1999)]

Since insulin-dependent diabetes mellitus (IDDM), which causes many severe secondary complications, is characterized by hyperglyceria due to absolute deficiency of insulin, the diseases is controlled by daily injection of insulin. Therefore, the development of insulin replacements or mimetics upon oral administration is an important investigation. Recent studies indicate that vanadium, which is proposed to be one of essential trace elements in animals and humans, relates to both glucose and lipid metabolisms, and the metal in turn shows insulin-mimetic effect. Thus several types of vanadium complex have been proposed to be insulin mimetics. In 1990, we proposed first vanadyl-cysteinate complex, which normalized the blood glucose level of IDDM rats on oral administration. On the other hand, simple vanadium compounds such as vanadyl sulfate and sodium vanadate have been reported to be useful to treat human non-insulin-dependent diabetes mellitus (NIDDM). Based on the observations, we have developed several types of vanadyl complexes with different coordination modes such as $VO(O_4)$, $VO(N_4)$, $VO(S_4)$, $VO(O_2N_2)$, $VO(S_2N_2)$ and $VO(O_2S_2)$, and found that vanadyle-methylpicolinate complex with long acting character and low toxicity is the most effective to treat IDDM as well as NIDDM rats, when administered orally. The mechanism was also studied with respect to the pharmacokinetic analysis and vanadium distribution in animals.

VII-C-6 Ternary Complex Formation between VO(IV)-picolinic Acid or VO(IV)-6-Methyl-picolinic Acid and Small Blood Serum Bioligands

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In order to assess the role of the low molecular mass bioligands of blood serum in vanadium binding, a study was made of the interactions of the complexes formed in the VO(IV)-picolinic acid and VO(IV)-6-methyl-picolinic acid systems with various low molecular mass constituents of blood serum, such as oxalate, lactate, citrate and phosphate. The speciation of VO(IV) in these ternary systems and also in the binary VO(IV)-picolinic acid and VO(IV)-6-methylpicolinic acid systems was studied by pH-potentiometry at 25 °C and at an ionic strength I = 0.2 M (KCl). The binding modes of the complexes formed were determined by spectral (electronic absorption and EPR) methods. Picolinic acid and 6-methylpicokinic acid were found to form mono and bis complexes through the pyridine nitrogen and carboxylate oxygen, but the presence of the methyl group in 6-methylpicolinic acid surprisingly decreases the stability of its complexes significantly. The results obtained on the ternary systems reveal that mixed ligand complex formation is favoured in these systems, especially with citrate, and must therefore be taken into account in the speciation description of VO(IV) in blood serum.

VII-D-7 An Orally Active Antidiabetic Vanadyl Complex, Bis(1-oxy-2-pyridinethiolato)oxo-vanadium (IV), with $VO(S_2O_2)$ Coordination Mode; *In vitro* and *In vivo* Evaluation in Rats

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According to Pearson's HSAB (hard and soft acids and bases) rule, the vanadyl ion is classified as a hard acid. However, vanadyl-cysteine methyl ester and dithiocarbamate complexes with $VO(S_2O_2)$ and $VO(S_4)$ coordination modes, respectively, that contain bonds with a combination of hard acid (VO^{2+}) and soft base (sulfur) have been found to form stable complexes and exhibit insulin-mimetic activities in *in vitro* and *in vivo* evalutions. Based on such observations, a purple bis(1-oxy-2-pyridinethiolato)oxovanadium(IV) (VO(OPT)) complex with $VO(S_2O_2)$ coordination mode was prepared and found to have a strong insulin-mimetic activity in *in vitro* evalution, which followed *in vivo* effectiveness on intraperitoneal injection and oral administration. Then, we examined the real-time ESR analysis of vanadyl species in the blood of live rats given VO(OPT) by use of the blood circulation

monitoring-ESR method. The clearance of vanadyl species from the blood in terms of half-life ($t_{i/2}$) was determined as 15 min in VO(OPT)-treated rats, while $t_{i/2}$ of $VOSO_4$-treated rats was 5 min, indicating the long-term acting character of VO(OPT). On the basis of the results, VO(OPT) with $VO(S_2O_2)$ coordination mode is proposed to be a potent orally active insulin-mimetic complex in treating insulin-dependent diabetes mellitus in experimental animals.

VII-C-8 Interaction of Vanadyl Complexes with Biological Systems: Structure-Insulinomimetic Activity Relationship of Vanadyl-Picolinate Complexes

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Since bis(picolinato)oxovanadium(IV), ($VO(PA)_2$), complex was found in 1995 to have good insulinomimetic activities, the structure-activity relationship was examined to develop more active complexes. By introducing an electron-donating group such as methyl group into the picolinate ligand, bis(3- and 6-methylpicolinate)oxovanadium(IV) ($VO(3MPA)_2$ and $VO(6MPA)_2$) were prepared. By introducing an electron-withdrawing group such as a halogen atom into the ligand, bis(5-iodo- and 4-choloro-picolinato)oxovanadium(IV) ($VO(5IPA)_2$ and $VO(4ClPA)_2$) were prepared. The structure of the complexes was characterized by EXAFS, ESR, IR and absorption spectra.

In vitro insulionmimetic activity of the complexes were in the order of $VO(5IPA)_2 > VO(6MPA)_2 > VO(PA)_2 > VO(3MPA)_2 > VO(4ClPA)_2$. Among four best complexes, both $VO(5IPA)_2$ and $VO(3MPA)_2$ were found to be potent agents to treat the hyperglycemia of streptozotocin (STZ)-induced diabetic rats. On the basis of the results, introduction of halogen atom or methyl group into the picolinate ligand was indicated to be a useful method to design more active insulinomimetic complexes.

VII-C-9 Synthesis of New Vanadyl Complexes of Hydroxyazine-Type Heterocycles and Their Insulin-Mimetic Activities

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Four kinds of vanadyl complexes of hydroxyazine-type heterocycles were synthesized. Bis(1,2-dihydro-4,6-dimethyl-2-oxo-1-pyrimidinolato)-and bis(1,2-dihydro-2-oxo-3,5,6-trimethyl-1-pyrimidinolato)-oxovanadium(IV) complexes showed higher insulin-mimetic activity than vanadyl sulfate as a positive control.

VII-C-10 Speciation of Insulin-Mimetic VO(IV)-Containing Drugs in Blood Serum

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The biospeciations of three potential insulin-mimetic VO(IV) compounds, $VO(maltolate)_2$, $VO(picolinate)_2$ and $VO(6\text{-}Me\text{-}picolinate)_2$, in blood serum were assessed via modelling calculations, using the stability constants reported in the literature for the binary insulin-mimetic complexes and their ternary complexes formed with the most important low molecular mass binders in the serum: oxalic acid, citric acid and phosphate. The binding capabilities of two high molecular mass serum proteins, albumin and transferrin, were also taken into account.