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Aqueous Chemistry of the Vanadium^{III} (V^{III}) and the V^{III}–Dipicolinate Systems and a Comparison of the Effect of Three Oxidation States of Vanadium Compounds on Diabetic Hyperglycemia in Rats

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The aqueous vanadium(III) (V^{III}) speciation chemistry of two dipicolinate-type complexes and the insulin-enhancing effects of V–dipicolinate (V-dipic) complexes in three different oxidation states (V^{III}, V^{IV}, and V^{V}) have been studied in a chronic animal model system. The characterization of the V^{III} species was carried out at low ionic strength to reflect physiological conditions and required an evaluation of the hydrolysis of V^{III} at 0.20 M KCl. The aqueous V^{III}–dipic and V^{III}–dipic-OH systems were characterized, and complexes were observed from pH 2 to 7 at 0.2 M KCl. The V^{III}–dipic system forms stable 1:2 complexes, whereas the V^{III}–dipic-OH system forms stable 1:1 complexes. A comparison of these complexes with the V–pic system demonstrates that a second ligand has lower affinity for the V^{III}, presumably reflecting bidentate coordination of the second dipic^{2-} to the V^{III}. The thermodynamic stability of the $[V^{III}(dipic)_2]^-$ complex was compared to the stability of the corresponding V^{IV} and V^{V} complexes, and surprisingly, the V^{III} complexes were found to be more stable than anticipated. Oral administration of three V–dipicolinate compounds in different oxidation states {$H[V^{III}(dipic)_2H_2O]\cdot 3H_2O$, $[V^{IV}Odipic(H_2O)_2]\cdot 2H_2O$, and $NH_4[V^{V}O_2dipic]$} and the positive control, $VOSO_4$, significantly lowered diabetic hyperglycemia in rats with streptozotocin-induced diabetes. The diabetic animals treated with the V^{III}– or V^{IV}–dipic complexes had blood glucose levels that were statistically different from those of the diabetic group. The animals treated with the V^{V}–dipic complex had the lowest blood glucose levels of the treated diabetic animals, which were statistically different from those of the diabetic group at all time points. Among the diabetic animals, complexation to dipic increased the serum levels of V after the administration of the V^{V} and V^{IV} complexes but not after the administration of the V^{III} complex when data are normalized to the ingested dose of V. Because V compounds differing only in oxidation state have different biological properties, it is implied that redox processes must be important factors for the biological action of V compounds. We observe that the V^{V}–dipic complex is the most effective insulin-enhancing agent, in contrast to previous studies in which the V^{IV}–maltol complex is the most effective. We conclude that the effectiveness of complexed V is *both ligand and oxidation state dependent*.

Introduction

Whether the insulin-enhancing effects associated with different oxidation states of vanadium (V) can be distinguished is important for the mode of action of V compounds. Our interest in this question led us to examine the speciation of the vanadium(III)−dipicolinate (V^{III}−dipic) system at a lower ionic strength that more closely resembles the physiological environment in blood. The aqueous chemistry of vanadium(III) (V^{III}) complexes has been investigated far less than the corresponding aqueous chemistry of vanadium(IV) (V^{IV}) and vanadium(V) (V^{V}).[1−4] Potentiometric titrations and

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electrochemistry have been used to investigate the hydrolysis of V^{III} in aqueous solutions, which is difficult because of the redox and hydrolytic reactivity of V^{III} salts and complexes.[3−8] The reported studies are limited to very few experimental conditions, focusing on high ionic strength environments and the acidic environment of the tunicates.[3−5,7] In these studies, a range of different mononuclear, dinuclear, trinuclear, and tetranuclear species have been identified. In this work, we will report the aqueous V^{III} chemistry and the effects of a series of V^{III}−, V^{IV}−, and V^{V}−dipicolinate complexes on diabetic hyperglycemia.

Oral administration of V compounds alleviates the symptoms of diabetes; however, a number of factors limit the potential use of V as a therapeutic agent. The poor absorption of V compounds into the circulation and the small therapeutic window in vivo are two of these factors.[9,10] Because of the poor absorption of V into the blood from the gastrointestinal (GI) tract,[11] the effective oral use of V compounds requires higher doses of V to reach therapeutically useful levels. Unfortunately, efficacious doses often reach near-toxic levels.[12] Enteric coating of V compounds has been pursued to lower V toxicity.[13] The organic ligands used increase the absorption of V tissue uptake and compound mobility, thereby effectively reducing the required dosage.[14] Vanadium administered orally as vanadate not only lowers diabetic hyperglycemia and hyperlipidemia in the diabetic rat, but it also alleviates secondary complications.[15,16] Many drugs used to treat diabetes, including insulin, can cause hypoglycemic episodes that are a major problem for the diabetic patient. Oral administration of V compounds in animal model systems, however, does not cause clinically significant hypoglycemia.[9]

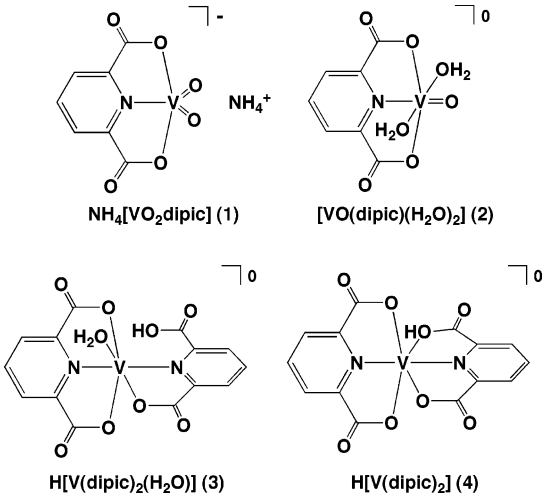
It has been shown that the insulin-enhancing properties of V complexes are caused by different classes of V compounds ranging from elaborate to very simple systems.[1,17−19] The first class consists of the simple salts vanadate [obtained as orthovanadate (VO_4^{3-}) and metavanadate (VO_3^-)] and vanadyl sulfate ($VOSO_4$). The second class of compounds consists of oxovanadium(IV) coordination complexes. This class is the most well-studied class of compounds, and it includes bis(maltolato)oxovanadium {[$VO(malto)_2$]; more commonly known as BMOV} and bis(ethylmaltolato)-oxovanadium (more commonly know as BEOV), which has completed phase 1 clinical trials.[17,18] In the maltol system, it was found that the V^{V} compound is not effective in lowering diabetic hyperglycemia,[20] whereas the V^{III}−maltol complex is effective when administered acutely.[21] A few V^{V} complexes[22−25] and V^{III}−hydroxypyrone and pyridinone complexes[10] have been reported as having glucose lowering effects. Some V^{III} complexes have been shown to lower diabetic hyperglycemia after acute treatment, but they are not usually considered as potential therapeutic agents because they readily oxidize to V^{IV} and V^{V} compounds under physiological conditions.[10] Administration of the bis(5-iodopicolinato)oxovanadium(IV) complex has also been shown to alleviate diabetic hyperglycemia in the rat.[26] Direct comparison of these results, which are obtained from many laboratories, is hampered by the use of different doses of V, different routes of administration, and the fact that the Wistar rat is not an inbred strain. In addition, a range of other complexes have been examined in tissue culture studies.[27] An important problem that remains to be understood concerns the role of the oxidation state of the V compound and how the metal oxidation state affects the insulin-enhancing properties of complexes.

If V compounds that only differ in oxidation state have different biological properties, this will demonstrate that oxidation processes are important factors in the biological action of the V compound. Comparing the effectiveness of a series of V^{III}, V^{IV}, and V^{V} complexes has not been previously attempted with chronic administration in animals.

(1) Crans, D. C.; Smee, J. J.; Gaidamauskas, E.; Yang, L. *Chem. Rev.* **2004**, *104*, 849−902.
(2) Rehder, D. *Coord. Chem. Rev.* **1999**, *182*, 297−322.
(3) Meier, R.; Boddin, M.; Mitzenheim, S.; Kanamori, K. *Met. Ions Biol. Syst.* **1995**, *31*, 45−88.
(4) Kanamori, K.; Ookubo, Y.; Ino, K.; Kawai, K.; Michibata, L. *Inorg. Chem.* **1991**, *30*, 3832−3836.
(5) Pajdowski, L. *Rocz. Chem.* **1963**, *37*, 1351−1361.
(6) Pajdowski, L. *Rocz. Chem.* **1963**, *37*, 1363−1377.
(7) Pajdowski, L.; Jezowska-Trzebiatowska, B. *J. Inorg. Nucl. Chem.* **1966**, *28*, 433−442.
(8) Pajdowski, L.; Jezowska-Trzebiatowska, B. *J. Inorg. Nucl. Chem.* **1966**, *28*, 443−446.
(9) Willsky, G. R.; Goldfine, A. B.; Kostyniak, P. J. *ACS Symp. Ser.* **1998**, *711*, 278−296.
(10) Melchior, M.; Rettig, S.; Liboiron, B.; Thompson, K.; Yuen, V.; McNeill, J.; Orvig, C. *Inorg. Chem.* **2001**, *40*, 4686−4690.
(11) Fugono, J.; Yasui, H.; Sakurai, H. *J. Pharm. Pharmacol.* **2001**, *53*, 1247−1255.
(12) Caravan, P.; Gelmini, L.; Glover, N.; Herring, F. G.; Li, H.; McNeill, J. H.; Rettig, S. J.; Setyawati, I. A.; Shuter, E.; Sun, Y.; Tracey, A. S.; Yuen, V. G.; Orvig, C. *J. Am. Chem. Soc.* **1995**, *117*, 12759−12770.
(13) Fugono, J.; Yasui, H.; Sakurai, H. *J. Pharm. Pharmacol.* **2002**, *54*, 611−615.
(14) Thompson, K. H.; Battell, M.; McNeill, J. H. In *Advances in Environmental Sciences and Technology, Vanadium in the Environment Part Two: Health Effects*; Nriagu, J. O., Ed.; John Wiley & Sons: New York, 1998.
(15) Heyliger, C. E.; Tahiliani, A. G.; McNeill, J. H. *Science* **1985**, *227*, 1474−1477.
(16) Lohr, J. W.; Bennett, M. I.; Pochal, M. A.; McReynolds, J.; Acara, M.; Willsky, G. R. *Res. Commun. Chem. Pathol. Pharmacol.* **1991**, *72*, 191−202.
(17) Thompson, K. H.; Liboiron, B. D.; Sun, Y.; Bellman, K. D. D.; Setyawati, I. A.; Patrick, B. O.; Karunaratne, V.; Rawji, G.; Wheeler, J.; Sutton, K.; Bhanot, S.; Cassidy, C.; McNeill, J. H.; Yuen, V. G.; Orvig, C. *JBIC, J. Biol. Inorg. Chem.* **2003**, *8*, 66−74.
(18) Thompson, K. H.; McNeill, J. H.; Orvig, C. *Chem. Rev.* **1999**, *99*, 2561−2571.
(19) Rehder, D. *Inorg. Chem. Commun.* **2003**, *6*, 604−617.
(20) Yuen, V. G.; Caravan, P.; Gelmini, L.; Glover, N.; McNeill, J. H.; Setyawati, I. A.; Zhou, Y.; Orvig, C. *J. Inorg. Biochem.* **1997**, *68*, 109−116.
(21) Melchior, M.; Rettig, S.; Liboiron, B.; Thompson, K.; Yuen, V.; McNeil, J.; Orvig, C. *Inorg. Chem.* **2001**, *40*, 4686−4690.
(22) Crans, D. C.; Yang, L.; Jakusch, T.; Kiss, T. *Inorg. Chem.* **2000**, *39*, 4409−4416.
(23) Crans, D. C. *J. Inorg. Biochem.* **2000**, *80*, 123−131.
(24) Kanamori, K.; Nishida, K.; Miyata, N.; Okamoto, K. i.; Miyoshi, Y.; Tamura, A.; Sakurai, H. *J. Inorg. Biochem.* **2001**, *86*, 649−656.
(25) Goldwasser, I.; Li, J.; Gershonov, E.; Armoni, M.; Karnieli, E.; Fridkin, M.; Shechter, Y. *J. Biol. Chem.* **1999**, *274*, 26617−26624.
(26) Takino, T.; Yasui, H.; Yoshitake, A.; Hamajima, Y.; Matsushita, R.; Takada, J.; Sakurai, H. *J. Biol. Inorg. Chem.* **2001**, *6*, 133−142.
(27) Rehder, D.; Costa Pessoa, J.; Geraldes, C. F. G. C.; Margarida, M.; Castro, C. A.; Kabanos, T. A.; Kiss, T.; Meier, B.; Micera, G.; Pettersson, L.; Rangel, M.; Salifoglou, A.; Turel, I.; Wang, D. *J. Biol. Inorg. Chem.* **2002**, *7*, 384−396.

The structure and reactivity of the V^V complexes are most commonly governed by the *cis*-dioxovanadium (VO_2^+) coordination chemistry.[1] The structure and reactivity of the V^{IV} complexes are governed primarily by the oxovanadium (VO^{2+}) chemistry.[1] In contrast, the chemistry of V^{III} is not governed by oxocationic effects.[1] However, the aqueous chemistry of each of these ions is very complex, with hydrolytic and redox reactions taking place even in the simplest systems. Characterizing the speciation of V^{III} ions and complexes in aqueous solution is particularly experimentally demanding and is required to interpret biological studies under physiological conditions.[1]

Because it is possible that V^{III}, V^{IV}, and V^V complexes convert to the same species under physiological conditions, these three complexes might thus exhibit similar effects. Surprisingly, our studies suggest that the metal oxidation state affects the processing and mode of action of the complexes after the chronic oral administration of the V−dipic complexes. In this work, we present studies demonstrating the effects of three V−dipicolinate complexes {$NH_4[V^VO_2dipic]$ (**1**), $[V^{IV}Odipic(H_2O)_2]\cdot 2H_2O$ (**2**), and $H[V^{III}(dipic)_2H_2O]\cdot 3H_2O$ (as **3** or **4**)} and the dipic ligand alone in Wistar rats with streptozotocin (STZ)-induced diabetes. We show that there is a difference in compound efficacy as the V oxidation state in the complex changes. These studies challenge the proposal that the role of the ligand is simply to transport the metal ion into the cell[28] and imply that the role of the oxidation state of the metal ion may be as important as that of the ligand. As part of this work, we have characterized the aqueous chemistry of V^{III} to gain information on the speciation and stability of the $[V^{III}(dipic)_2]^-$ complex to better understand the comparative effects of V^{III}−, V^{IV}−, and V^V−dipic complexes.



Experimental

Materials and Methods. The VCl_3 was obtained from Merck Chemical Company, and the other sources of V (mainly $NaVO_3$ and $VOSO_4$) were obtained from Aldrich. The dipicolinic acid was obtained from Aldrich, and the 4-hydroxydipicolinic acid (chelidamic acid) was acquired from TCI America. The purities of dipicolinic acid and the 4-hydroxydipicolinic acid were checked by the Gran method for the potentiometric studies.[29] The complexes **3**,[30] **2**,[31] and **1**[22,32] were prepared as reported previously.

Preparation of Stock Solutions for Studies of Aqueous V^{III}. The V^{III} stock solution was prepared from VCl_3 by dissolving it in deoxygenated 0.1 M HCl. All solutions were maintained under a deoxygenated argon atmosphere. We carefully removed the $V^{IV}O$ present by bubbling purified H_2 through the solution for 2−3 days in the presence of a Pd sludge (prepared from $K_2[PdCl_4]$ using Zn in HCl). We filtered the stock solution using Schlenk techniques and stored it under an atmosphere of strictly deoxygenated argon. The V^{III} stock solution was monitored for Pd content, and none was found by inductively coupled plasma-atomic emission spectrometry (ICP-AES).

The V^{III} concentration was determined by reacting an aliquot of the V^{III} solution with an oxygen-free IO_3^- solution and measuring the iodine liberated with thiosulfate as described by Furman and Garner.[33] We confirmed the total V content using several methods including ICP-AES, spectrophotometric determination in the form of $V^{IV}O$, and permanganate titration. Very little $V^{IV}O$ (<0.5%) was found in the V^{III} solution used for the equilibrium studies.

Because V^{III} hydrolyzes even below pH = 2, we determined the hydrogen ion concentration of the stock solution by pH-potentiometry using the appropriate Gran function.[29] We determined the H^+ concentration after the complete oxidation of a sample by air to V^{IV}, taking into consideration the liberated protons according to eq 1.

$$4[V(H_2O)_6]^{3+} + O_2 \rightarrow 4[VO(H_2O)_5]^{2+} + 4H^+ + 2H_2O \quad (1)$$

Potentiometric Measurements: V^{III}−Dipic and V^{III}−Dipic-OH Systems. The stability constants of the proton and V^{III} complexes of the ligands were determined by pH-potentiometric titration of 10.0-mL samples at an ionic strength of 0.20 M KCl and at 25.0 ± 0.1 °C. During preparation of the V^{III}-containing solutions to be titrated, the solution without the metal ion was purged with argon for 20 min in a glass vessel with an NS-joint for the electrode and for the addition of the V^{III}-containing solution. We added the V^{III} solution into the vessel using a buret equipped with a stainless steel syringe at positive argon pressure. During titration, both the magnetic stirrer and the argon bubbles were used for stirring. The ligand concentration varied in the range of 1−4 mM and the metal-to-ligand ratios ranged from 1:1 to 1:4. A total of 125 or 196 titration points were used for the joint evaluation of the potentiometric data. All the titrations were performed with a carbonate and oxygen-free KOH solution of known concentration (0.20 M) over the pH range of 2−11 or until precipitation was observed under a purified argon atmosphere. A maximum 10-min waiting time was allowed at each titration point. Only data points obtained in less than 10 min were used in the calculation. The reproducibility of the titration points included in the evaluation was within 0.005 pH units throughout the entire pH range investigated.

The pH was measured with a Radiometer pHM 84 instrument using a Metrohm combined electrode with an NS-joint (type 6.0233.100) calibrated for hydrogen ion concentration according

(28) Thompson, K. H.; Orvig, C. *Dalton* **2000**, 2885−2892.

(29) Gran, G. *Acta Chem. Scand.* **1950**, *4*, 559−577.
(30) Chatterjee, M.; Maji, M.; Ghosh, S.; Mak, T. C. W. *J. Chem. Soc., Dalton Trans.* **1998**, 3641−3645.
(31) Bersted, B. H.; Belford, R. L.; Paul, I. C. *Inorg. Chem.* **1968**, *7*, 1557−1562.
(32) Wieghardt, K. *Inorg. Chem.* **1978**, *17*, 57−64.
(33) Furman, S. C.; Garner, C. S. *J. Am. Chem. Soc.* **1950**, *72*, 1785−1789.

to Irving et al.[34] A pK_w value of 13.76 ± 0.01 was determined and was used for the calculations. Concentration stability constants ($\beta_{pqr} = [M_pH_qA_r]/[M]^p[H]^q[A]^r$) were calculated with the aid of the PSEQUAD computer program.[35] The uncertainties [3 standard deviations (SDs)] in the stability constants are given in parentheses in the tables. The accepted fittings [i.e., the average difference in the calculated and experimental titration curves expressed in the volume (cm^3) of the titrant] were below 0.01.

UV−Vis Spectroscopic Measurements. Absorption spectra were recorded with a Hewlett-Packard HP 8453 spectrophotometer under an atmosphere of purified argon. Aliquots taken from the sample to record the spectrum were discarded after the measurement. Aqueous solutions of the V^{III}−ligand systems at ratios from 1:1 to 1:4 with a V^{III} concentration of 0.001−0.004 M and a pH range of 0.7−9.0 were studied by UV−Vis spectroscopy. Supportive information on the stability constants of the major complexes was obtained from UV−Vis absorbance measurements from 300 to 800 nm at different pH values with the aid of the PSEQUAD[35] computer program. In a few samples, the 0.20 M KCl-based ionic strength was partially or completely replaced by HCl, and the pH values were calculated to be in the 0.7−1.4 range based on the HCl content.

Hydrolysis of V^{III}. We studied the hydrolysis of V^{III} in aqueous solution at 25.0 °C and at an ionic strength of 0.20 M (KCl) using combined pH-potentiometric and spectroscopic techniques. Potentiometric titrations with metal ion concentrations of 0.0005−0.008 M were carried out starting at pH 1.9 until precipitation occurred at pH 4.5. The formation and decomposition of $[V_2(OH)_2]^{4+}$, which exhibits a well-documented[3−5,7] characteristic band at 430 nm, was monitored independently by UV−Vis spectrophotometry. This allowed us to calculate the stability constant of the dimeric species using spectroscopic data with the aid of the PSEQUAD[35] computer program. Jointly evaluating the data at different $c_{V(III)}$, while keeping the stability constant determined for $[V_2(OH)_2]^{4+}$ from UV−Vis spectrophotometry fixed, made it possible to obtain the full speciation from pH 1.9 to 4.5 in the H^+−V^{3+} system.

Animal Protocol. Previously published procedures for the induction of diabetes with STZ in male Wistar rats and subsequent animal care from a protocol approved by the University of Buffalo IACUC were used.[36,37] The protocol is in compliance with state and federal regulations. Diabetes was induced in the rats by administering 60 mg/mL of freshly prepared STZ injected intravenously in saline at a dose of 60 mg/kg body weight. Overall, this study was a repeated measures, parallel design animal experiment with the following groups of rats: normal (N), $n = 13$; diabetic (D), $n = 25$; N treated with $VOSO_4$ (N/$VOSO_4$), $n = 8$; D treated with $VOSO_4$ (D/$VOSO_4$), $n = 30$; D treated with H_2dipic (D/H_2-dipic), $n = 6$; D treated with **3**, $n = 8$; D treated with **2**, $n = 7$; and D treated with **1**, $n = 5$. A few of the diabetic animals, both treated and untreated, did not survive the 28-day treatment period. In each temporal group of animals there were always some N, D, and D/$VOSO_4$ animals as controls. Four days after STZ injections, blood glucose levels were determined from a drop of tail blood with an Accu-Chek blood glucose monitor as previously described.[36,37] Animals with blood glucose levels over 17 mM were considered diabetic and were randomly assigned to one of the treated or untreated groups. Untreated N animals were matched in age and weight. The treatment protocol started 5 days after STZ injection and consisted of adding compounds to the drinking water daily, which varied from 0.5 to 2.0 mg/mL. The amount was changed no more than twice in week one to obtain the desired final treatment level. We monitored the actual ingested millimoles of metal per kilogram per day for the animals using the concentration of the complex or salt in the drinking water, the amount of fluid consumed, and the weight of the animal. The dose of metal was calculated as the amount of ingested metal per kilogram per day. Oral treatment with the compounds in the drinking water was done for 28 days.

We determined blood glucose levels twice per week using an Accu-Chek monitor and determined urine ketones and glucose once per week using Ketodiastix. Animals were weighed daily. An animal appearing to be dehydrated, as monitored by loss of weight or by physical examination, was rehydrated subcutaneously with 10−30 mL per day of lactated Ringer's bicarbonate solution (0.84%). Blood samples were obtained after the rats were killed. Serum was obtained by low-speed centrifugation of the samples after clotting had occurred.

Determination of Serum Vanadium. We determined serum levels of elemental V by graphite furnace atomic absorption spectroscopy (GFAAS) using a Perkin-Elmer (Norwalk, CT) 41102 (with Zeeman correction) spectrophotometer as reported previously.[38] Because the tissue matrix interferes with GFAAS analysis, both the standards and the samples underwent pretreatment procedures before V analysis. Standards and samples (100 mL) were dried at 55 °C for approximately 48 h. After drying, they were heated in a CEM microwave ashing system (model # 300), which resulted in complete ashing of the sample. The ashed samples were reconstituted in 100 mL of 0.05% sulfuric acid with the aid of a 5-min incubation in a bath sonicator (Branson Laboratories model # 200). Sample V concentration was determined against a standard curve that was analyzed on the same day as the samples. The standards with concentrations of 0, 25, 50, 100, 250, 500 and 1000 ng/mL were prepared by adding known amounts of a commercially available V stock (1000 mg/mL, Perkin-Elmer) to sheep serum. All standard curves showed good linearity in this range, with correlation coefficients (r^2) of 0.98 or greater. One quality control sample with a concentration of 100 ng of V/mL was analyzed with every 10 samples. A limit of detection (LOD) was calculated as three times the SD of 10 determinations of a whole-blood blank sample. The LOD was calculated to be 10.2 ng/mL V in serum.

Statistical Analysis. We analyzed data obtained at individual time points in the biological studies using a one way analysis of variance with Dunnett's or Bonferroni's multiple means testing. Significance was taken as $p \leq 0.05$.

Results and Discussion

Hydrolysis of V^{III}. The hydrolysis of V^{III} in aqueous solution has been studied in detail by Meier and co-workers[3] at high ionic strength (1.0 M). However, speciation is very sensitive to ionic strength, and because V^{III} has a high tendency to hydrolyze, any investigations under conditions different from those used previously require reevaluation of the speciation of the hydrolysis parameters. We determined the hydrolysis of V^{III} in aqueous solution at 25.0 °C and at

(34) Irving, H. M.; Miles, M. G.; Pettit, L. D. *Anal. Chim. Acta* **1967**, *38*, 475−488.
(35) Zekany, L.; Nagypal, I. *Computational Methods for the Determination of Stability Constants*; Plenum: New York, 1985.
(36) Yang, L.; Crans, D. C.; Miller, S. M.; la Cour, A.; Anderson, O. P.; Kasnynski, P. M.; Godzala III, M. E.; Austin, L. D.; Willsky, G. R. *Inorg. Chem.* **2002**, *41*, 4859−4871.
(37) Johnson, T. M.; Meisler, M. H.; Bennett, M. I.; Willsky, G. R. *Diabetes* **1990**, *39*, 757−759.
(38) Goldfine, A. K.; Patti, M.-E.; Zuberi, L.; Goldstein, B. J.; LeBlanc, R.; Landaker, E. J.; Jiang, Z. Y.; Willsky, G. R.; Kahn, C. R. *Metab., Clin. Exp.* **2000**, *49*, 400−410.

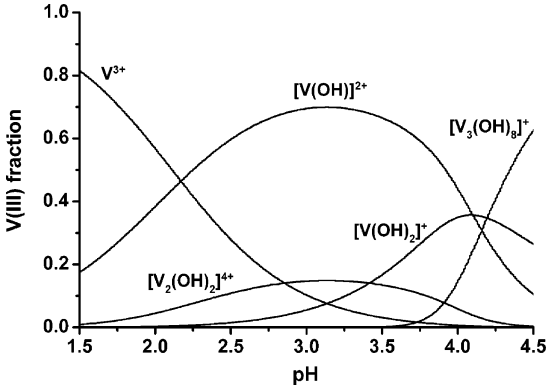


Figure 1. Distribution curves for V^{III} hydrolysis; $c_{V(III)} = 4$ mM.

an ionic strength of 0.20 M (KCl) using a combination of pH-potentiometry and UV−Vis spectroscopy. The dimeric species $[V_2(OH)_2]^{4+}$ has an intense absorption band at 430 nm,[3−5,7] and its formation and decomposition are thus well-defined in the pH range of 1.5−4.5. Because $[V(OH)]^{2+}$ and $[V_2(OH)_2]^{4+}$ both liberate one proton per equivalent of metal ion, they have the same pH effect. The evaluation of the overall system required an independent determination of the $[V_2(OH)_2]^{4+}$, which was then fixed and used to determine the monomeric species in the subsequent calculations.

The V^{III} titration curves were best fitted with a data set similar to one found by Meier et al. at higher ionic strength.[3] Our model also required contributions of $[V(OH)]^{2+}$, $[V_2(OH)_2]^{4+}$, $[V(OH)_2]^{+}$, and $[V_3(OH)_8]^{+}$ as shown in the concentration speciation diagram of soluble hydroxo V^{III} species (Figure 1). Despite several attempts, we did not find any indication of significant formation of a tetrametric species $[V_4(OH)_{12}]^0$. According to Meier et al.,[3] $[V_4(OH)_{12}]^0$ is formed as a major complex above pH 4.0 at $c_{V(III)} = 5$ mM and is assumed to be the soluble form of $V(OH)_3$. At 5 mM we observed opalescence of the solution at pH = 4.30 and no indication of the tetramer. This is consistent with the speciation changes in the V^{IV} and V^{V} systems, in which higher ionic strength and high concentration support the formation of species with larger nuclearity.[39,40] Formation of the chloro complex was found to be negligible at these conditions, consistent with earlier studies at high ionic strength.[3] Overall, the values of the stability constants for the soluble hydroxo complexes of the V^{III} system (Table 1) are reasonably consistent with those reported by Meier and co-workers, when the lower ionic strength is considered.[3]

Dipic Ligand Protonation Processes. To evaluate the V^{III} complexes, a reevaluation of ligand properties is preferable. The protonation constants calculated for pyridine-2,6-dicarboxylic (H_2dipic) and 4-hydroxypyridine-2,6-dicarboxylic acid (H_2dipic-OH) are listed in Table 2. The log *K* values are consistent with published data obtained at the same or similar experimental conditions.[22,41]

Evaluation of the V^{III}−Pyridine-2,6-dicarboxylate System: V^{III}−Dipic Complexes. The V^{III}−dipic system was titrated from pH <1.9 to 8.0 at a 1:4 ratio, to pH 7.0 at a 1:2 ratio, or to pH 4.6 at a 1:1 ratio in which precipitation occurred in all cases. The ligand concentration was 2−4 mM, and the V^{III} concentration was in the range of 1−4 mM. Because complex formation occurred at acidic pH, and no free metal ion was observed at the beginning of the pH-potentiometric titrations, the complex formation was also monitored by UV−Vis spectroscopy. At a 1:1 ratio, this allowed for the calculation of the stability constant for $[V\text{dipic}]^+$ below pH 1.5. Keeping this value constant during the evaluation of the potentiometric data allowed for the calculation of the speciation profile from pH 1.5 to 7. Full speciation from potentiometry alone is also possible because the equilibrium system can be fixed to the formation of the hydroxo species $[V_3(OH)_8]^+$ at high pH. However, this series of data is not precise as a consequence of slow equilibria and the formation of precipitates in the pH range in which $[V_3(OH)_8]^+$ forms and is more uncertain than the analysis done using the combined spectroscopic and potentiometric data. These studies served to confirm the results listed in Table 2.

From the titration data, which consisted of different metal-to-ligand ratios (1:1, 1:2, 1:3, and 1:4), it is clear that complex formation starts well below pH = 2, and no extra base consumption occurs until the pH reaches 5 in the samples with at least a 2-fold excess of ligand. The fact that these

Table 1. Compositions, Notations, and Formation Constants (log β) for the H^+−V^{III} System[a]

(*p*,*q*,*r*)	notation	log β (± 3SD)	ref
(1,−1,0)	$[V(OH)]^{2+}$	−2.17(2)	this work
(2,−2,0)	$[V_2(OH)_2]^{4+}$	−2.76(3)[b]	this work
(1,−2,0)	$[V(OH)_2]^{+}$	−6.27(3)	this work
(3,−8,0)	$[V_3(OH)_8]^{+}$	−21.96(4)	this work
(1,−1,0)	$[V(OH)]^{2+}$	−2.60	3
(2,−2,0)	$[V_2(OH)_2]^{4+}$	−3.59	3
(1,−2,0)	$[V(OH)_2]^{+}$	−6.83	3
(3,−8,0)	$[V_3(OH)_8]^{+}$	−23.3	3
(4,−12,0)	$[V_4(OH)_{12}]^0$	−33.80	3

[a] [I = 0.20 M (KCl), 25.0 °C] [b] Calculated from spectrophotometric data.

Table 2. Compositions, Notations, Formation Constants (log β), and Acidity Constants (pK_a) for the H^+−dipic^{2-}, H^+−dipic-O^{3-}, H^+−V^{III}−dipic^{2-}, and H^+−V^{III}−dipic-O^{3-} Systems[a]

(*p*,*q*,*r*)	notation	log β (± 3SD)	pK_a
(0,1,1)	Hdipic$^-$	4.56(1)	4.56
(0,2,1)	H_2dipic^0	6.60(1)	2.04
(0,1,1)	dipic-OH^{2-}	10.71(1)	10.71
(0,2,1)	Hdipic-OH$^-$	13.90(3)	3.19
(0,3,1)	H_2dipic-OH^0	15.4(1)	∼1.5
(1,0,1)	$[V\text{dipic}]^+$	9.45(5)[b]	4.82
(1,−1,1)	$[V(\text{dipic})OH]^0$	4.63(9)	
(1,1,2)	$[V(\text{dipic})_2H]^0$	18.02(7)	2.84
(1,0,2)	$[V(\text{dipic})_2]^-$	15.18(5)	6.66
(1,−1,2)	$[V(\text{dipic})_2OH]^{2-}$	8.52(6)	
(1,1,1)	$[V(\text{dipic-OH})]^+$	19.57(8)[b]	4.34
(1,0,1)	$[V(\text{dipic-O})]^0$	15.23(1)	5.26
(1,−1,1)	$[V(\text{dipic-O})OH]^-$	9.97(1)	
(1,2,2)	$[V(\text{dipic-OH})_2]^-$	33.28(4)	4.38
(1,1,2)	$[V(\text{dipic-OH})(\text{dipic-O}^-)]^{2-}$	28.90(6)	

[a] [I = 0.20 M (KCl), 25.0 °C] [b] Calculated from data obtained by spectrophotometry.

(39) Crans, D. C. *Comments Inorg. Chem.* **1994**, *16*, 1−33.
(40) Tracey, A. S.; Jaswal, J. S.; Angus-Dunne, S. J. *Inorg. Chem.* **1995**, *34*, 5680−5685.
(41) Jakusch, T.; Jin, W.; Yang, L.; Kiss, T.; Crans, D. C. *J. Inorg. Biochem.* **2003**, *95*, 1−13.

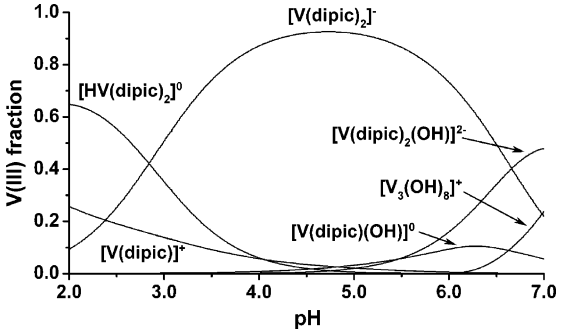


Figure 2. Distribution curves for the V^{III}−dipic system; L:M = 2:1, c_{dipic} = 4 mM.

latter titration curves (1:2, 1:3, and 1:4 ratios) overlap each other and the titration curve from the free ligand at pH ≈ 5 suggests the formation of a very stable complex containing two dipic ligands, which prevent the hydrolysis of the aqueous V^{III} system. Combining the titrations with spectrophotometric data results in the stability constants (log β) of the complexes listed in Table 2 and illustrated in the speciation diagram shown in Figure 2.

The predominant species in the pH range of 2.0−3.0 are a 1:1 complex, [Vdipic]$^+$, and a 1:2 complex, [HV(dipic)$_2$]^0. The proton in the 1:2 species presumably resides on one of the carboxylate groups, as either complex **3** or **4**, because no significant change is observed in the absorbance spectrum upon complex protonation. Indeed, [V(dipic)(Hdipic)]^0 has been isolated from concentrated aqueous solutions of complex and characterized in the solid state.[30] When the pH is increased, the anionic 1:2 complex [V(dipic)$_2$]$^-$ is formed and remains the major species until the pH is neutral. At a 1:4 metal-to-ligand ratio in which [V(dipic)$_2$]$^-$ is the only species, analysis of the spectrophotometric data yielded a stability constant value of 15.2(1) for this complex, which is consistent with that obtained from potentiometry (Table 2). The two dipic ligands maintain the V^{III} in solution beyond pH 4−4.5 in which the simple salt begins to precipitate from concentrated solutions and thus documents the stability of these complexes.

Some resistance to the formation of the 1:2 complex was noted. The ratio of the stepwise stability constants of the 1:1 and 1:2 complexes, log(K_1/K_2) = 3.72, is significantly higher for [V(dipic)$_2$]$^-$ than the statistically expected value of 0.6 and documents the resistance toward the formation of the 1:2 complex. For comparison, the ratio of the stepwise stability constants of the 1:1 and 1:2 complexes, log(K_1/K_2) = 0.40, was found for the V^{III}−pic system.[42] A similar pattern was observed in the corresponding V^{IV}−dipic system.[22] This was previously attributed to the decrease in the coordination denticity of the ligand and/or considerable structural rearrangement of the coordinating donors.[22] Although the two dipic ligands are generally presumed to be coordinated in a tridentate manner through the {O,N,O}-moieties, these observations suggest that perhaps the two ligands may not be chelated equally. This is further supported by the comparison of the stability of the 1:2 complex of the bidentate picolinate ligand pic$^-$ with that of the dipic system. The similar formation constant (log K_2) values, log K[V(pic)$_2$] 5.88[42] vs log K[V(dipic)$_2$] 5.73, indicate very similar binding affinity, and accordingly, similar coordination modes for the two systems would be reasonable.

Above pH 5.0, further base consumption processes take place, indicative of the formation of new species. The titration curves can best be fitted with the simultaneous formation of [V(dipic)OH]^0 and [V(dipic)$_2$OH]$^{2-}$. In the neutral complex [V(dipic)OH]^0, a coordinated water molecule can lose a proton and upon the addition of one more ligand can form the 1:2 hydroxo complex [V(dipic)$_2$OH]$^{2-}$. Alternatively, in both [V(dipic)OH]^0 and [V(dipic)$_2$OH]$^{2-}$ an OH$^-$ can displace one of the coordinated carboxylate moieties. Above pH 7, the hydrolysis of the metal ion becomes significant, and depending on the metal-to-ligand ratio, precipitation occurs.

Comparison of the V^{III}−dipic system with the V^{IV}−dipic and V^{V}−dipic systems was undertaken as described in detail below. To further understand the specific electronic properties of the V^{III}−dipic system, a speciation study was carried out on the V^{III}−dipic-OH system. The corresponding V^{IV} and V^{V} systems have previously been investigated, and some differences had become apparent because of the ability of the hydroxyl or O$^-$ group to pump electrons into the aromatic ring and thus strengthen the donor properties of the ligand.

The V^{III}−4-Hydroxypyridine-2,6-dicarboxylate System: V^{III}−Dipic-OH Complexes. Evaluation of the titration curves obtained in the V^{III}−dipic-OH system at different metal ion-to-ligand ratios yielded a speciation profile with 1:1 complexes as the major species. The formation of the 1:1 complexes was simultaneously monitored by UV−Vis spectroscopy. Because 1:2 complexes were the major species in the V^{III}−dipic system, this is a major change presumably resulting from the OH-substitution on the dipic ligand. This substitution changes the electronic properties of the ligand system, particularly at a pH in which the OH group is deprotonated. The shapes of the titration curves are different from those of the H$_2$dipic/dipic^{2-} system, which is indicative of the hydrolysis of V^{III}; at 1 mM of V^{III} and 2 mM of H$_2$-dipic-OH, the [V^{III}dipic-OH]$^+$ complex was the major species from the beginning of the titration (below pH 2) until approximately pH 4, at which point a neutral complex ([V^{III}-dipic-O$^-$]^0) became the major species. In addition, even at high excess ligand the 1:1 complexes remain the major species. At neutral pH an anionic complex became the most prominent species, and this anionic complex is formally described as [V^{III}dipic-O$^-$(H)$_{-1}$]$^-$ but is more clearly described as [V^{III}(dipic-O$^-$)OH]$^-$, which is the notation that will be used in this work. The combination of the 1:1 complexes and the mono- and dianionic 1:2 complexes ([V^{III}-(dipic-OH)$_2$]$^-$ and [V^{III}(dipic-O$^-$)dipic-OH]$^{2-}$) improved the fit significantly. The calculated constants are listed in Table 2, and the concentration distribution curves of the complexes present in the V(III)−dipic-OH system are depicted in Figure 3. Analysis of the independent spectrophotometric data at a 1:1 ratio yielded stability constant values of 15.2(1) for [V^{III}-

(42) Buglyo, P.; Nagy, E. M.; Sovago, I. *Pure Appl. Chem.* in press.

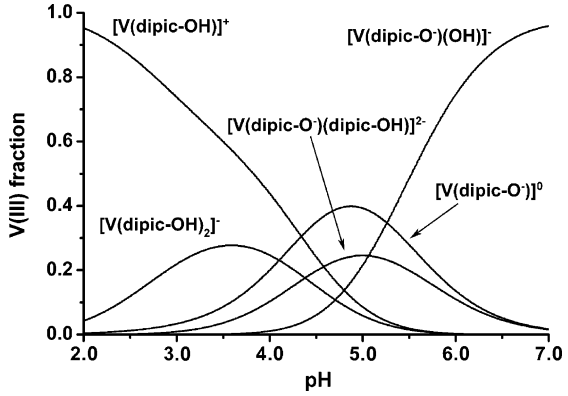


Figure 3. Distribution curves for the V^{III}−dipic-OH system; L/M = 2:1, $c_{dipic\text{-}OH}$ = 4 mM.

dipic-O^-]^0 and 9.7(2) for [V^{III}(dipic-O^-)OH]$^-$, consistent with the data obtained by potentiometry (Table 2).

The complexes [Vdipic-OH]$^+$ and [V(dipic-$OH)_2$]$^-$ are observed in the pH range of 2.0−3.5. The structure of the 1:1 complex, [Vdipic-OH]$^+$, is likely to involve tridentate coordination of the ligand as observed in the reported X-ray structures of 1:1 complexes of dipic-type ligands with V in several oxidations states.[30,31,43,44] Similar species have been proposed in solution studies of VO^{2+}, VO_2^+, or other 3*d* metal ions.[41,45]

When the pH was increased, the OH group of the dipic−OH ligand [pK_a(dipic-OH) = 10.75][41] coordinated to the V^{III} ion deprotonates. The p*K* values of the 1:1 and 1:2 species are p*K*{[Vdipic-OH]$^+$} = 4.34 and p*K*{[V(dipic-$OH)_2$]$^-$} = 4.36, respectively. The acidity of the coordinated ligand compares well with those obtained previously (VO^{2+}: 4.09, VO_2^+: 3.70, Cu^{2+}: 4.86).[41,45] The tridentate dipic-like coordination to the metal ion decreases the electron density of the aromatic ring, making the OH group significantly more acidic than that in the free ligand. The p*K* value of [V(dipic-$OH)_2$]$^-$ for the deprotonation of the OH group is the same as that of the 1:1 species, which is consistent with at least one of the dipic-OH ligands in the [V(dipic-$OH)_2$]$^-$ complex coordinating to the V^{III} ion in a tridentate manner. We conclude, that two likely structural possibilities (see structures **3** and **4** above) exist for [V(dipic-$OH)_2$]$^-$. One involves the coordination of two dipic ligands in a tridentate manner as was reported for the [V(dipic)$_2$]$^-$ complex anion and for other transition metal complexes.[30,36,46] Alternatively, the dipic-type ligand may be coordinated in both a tridentate and a bidentate manner. The latter is supported by the very high log{*K*[V(dipic-OH)]/*K*[V(dipic-$OH)_2$]} = 5.86 value for the [V(dipic-$OH)_2$]$^-$ species. Structural precedent for this type of complex exists.[47] Thesmall value for the equilibrium [V(dipic-OH)]$^+$ + H_2dipic-OH = [V(dipic-$OH)_2$]$^-$ + 2H$^+$ (log *K* = −1.69) also indicates that the second dipic-OH^{2-} ligand is not coordinated as tightly, which would be consistent with a bidentate chelation mode.

Deprotonation of the chelated dipic-OH^{2-} ligand results in a chelated dipic-O^{3-} ligand and the complexes [Vdipic−O^-]^0 and [V(dipic-O^-)dipic-OH]$^{2-}$. The former is a major species at pH 4.5−5, and the latter is a minor component from pH 4−6. The deprotonation of the OH substituent increases the electron donating ability of the ligands and as a result strengthens the V^{III}−dipic interaction. Increased electron donation of the pyridine N atom can, in part, disfavor the coordination of a *second* ligand to the [V(dipic-O^-)]^0 species, which, in conjunction with the other protonated forms of the 1:1 complex, is found to be the minor species in solution. It is likely that the second dipic-OH^{2-} ligand coordinates as a bidentate ligand and thus does not enjoy as much stability as does the first dipic ligand, which chelates to V^{III}. Indeed the greater stability of the V−dipic-OH system over V−pic systems[42] is consistent with the first dipic ligand being coordinated in a tridentate manner.

As the pH is increased to the range of 5.0−7.0, the observed base consumption in the titration can be fitted well with the formation of [V(dipic-O^-)OH]$^-$. This species undergoes no color change, and it is possible that one of the water molecules loses a proton and maintains the same donor atom environment as the complex at more acidic conditions. Above pH 7.0, precipitation was observed during the titration at the 1:1 ratio of metal ion-to-ligand. Attempts to use ligands in excess resulted in very slow deprotonation processes in which the solutions failed to reach pH equilibrium within 10 min. No further evaluation of the system was thus attempted.

Comparison of V^{III}−, V^{IV}−, V^{V}−Dipic and V^{III}−, V^{IV}−, V^{V}−Dipic-OH Speciation. Upon the administration of any compound to an animal, the compound will be subjected to various aerobic and anaerobic environments. Which V−dipic complex is actually present in the stomach during absorption and under the reductive cellular physiological conditions may determine the ultimate fate of each V−dipic compound and its biotransformation and processing. Should any variation in the biological effects be observed, this may point to an aspect of the compound's chemical properties that is important to understanding where and how the compounds act in the biological system.

Previously, the stabilities of V^{IV}−dipic and V^{V}−dipic complexes were compared.[41] In the presence of 2-fold and 4-fold excess dipic ligands, the V^{IV}−dipic system proved to be more stable than the V^{V}−dipic system and extended the stability of the complexes by ∼1 pH unit. In contrast, when the stabilities of V^{IV}−dipic-OH and V^{V}−dipic-OH systems were compared, the corresponding difference was only observed at a 4-fold excess of ligand; at a 2-fold excess of ligand the V^{IV}−dipic-OH and V^{V}−dipic-OH systems were of similar stability. However, in all cases some V^{IV}−dipic and V^{IV}−dipic-OH molecules were intact in the neutral pH

(43) Crans, D. C.; Yang, L.; Alfano, J. A.; Chi, L. H.; Jin, W.; Mahroof-Tahir, M.; Robbins, K.; Toloue, M. M.; Chan, L. K.; Plante, A. J.; Grayson, R. Z.; Willsky, G. R. *Coord. Chem. Rev.* **2003**, *237*, 13−22.
(44) Nuber, B.; Weiss, J.; Wieghardt, K. *Z. Naturforsch.* **1978**, *33B*, 265−267.
(45) Anderegg, G. *Helv. Chim. Acta* **1963**, *46*, 1011−1017.
(46) Laine, P.; Gourdon, A.; Launay, J.-P. *Inorg. Chem.* **1995**, *34*, 5129−5137.
(47) Crans, D. C.; Zhang, B.; Gaidamauskas, E.; Keramidas, A.; Willsky, G. R.; Roberts, C. R. Manuscript under revision.

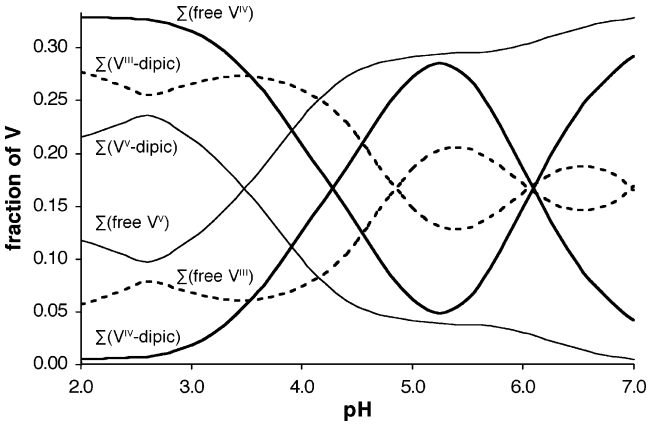


Figure 4. Simulated distribution curves for the $V^{V}-VO^{IV}-V^{III}-$dipic system; $c_{\text{metal ion}} = 2$ mM, $c_{\text{dipic}} = 3$ mM.

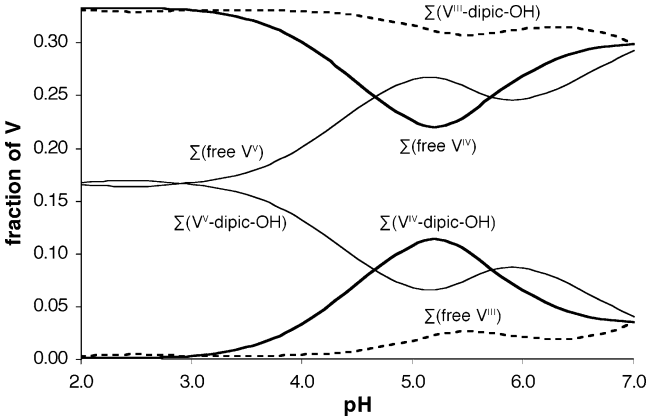


Figure 5. Simulated distribution curves for the $V^{V}-VO^{IV}-V^{III}-$dipic-OH system; $c_{\text{metal ion}} = 2$ mM, $c_{\text{dipic-OH}} = 3$ mM.

range. Given the anticipated higher reactivity of V^{III} complexes, these complexes would not be expected to possess greater stability than the V^{IV} complexes. However, because some V^{III} complexes were recently found to have insulin-enhancing properties, whereas others showed no activity,[10] a comparison of the fundamental thermodynamic stability of these compounds is important.

Using the stability constants listed in Table 2 and those previously reported,[22,41] we will consider a "gedanken experiment" in which we will test which vanadium ion the dipic ligands prefer to bind to in the absence of redox chemistry between the complexes. The formation constants were used to calculate the speciation under conditions in which equimolar concentrations of aqueous V^{III}, V^{IV}, and V^{V} (2 mM) were in the presence of 3 and 6 mM of dipic and dipic-OH ligands, respectively. The simulated speciation diagrams for complexes formed in the 2:2:2:3 systems are shown in Figures 4 (dipic) and 5 (dipic-OH). These two metal-to-ligand ratios were chosen to examine the affinity of the ligand for the metal ion in each oxidation state at the 1:1 ratio of overall metal ion-to-ligand and at a lower ratio in which the ligand selectivity between the three different oxidation states becomes apparent. This calculation assumes that no electron transfer takes place between the V ions, and this assumption is reasonable under some physiological conditions. One important difference in the ligand affinity for V^{III}, $V^{IV}O$, and $V^{V}O_2$ is that the most stable complex of V^{III} is a 1:2 complex, that of $V^{IV}O$ is both a 1:1 and a 1:2 complex, and that of $V^{V}O_2$ is a 1:1 complex. The stability differences are rather small as was observed previously in a comparison of the $V^{IV}O$ and $V^{V}O_2$ systems.[22,41]

In Figure 4, the calculated speciation curve shows that the summed amount of the V^{V} complexes is comparable to that of the V^{III} complexes below pH 3. When the pH is increased, the complexed V^{V} rapidly decreases, and the ligand coordinates mostly to V^{III}. From pH 4 to 6, the V^{IV} species become the major species, but above pH 6 most of the ligand is again found in the V^{III} complex. In the 2:2:2:6 system, the V^{III} complex prevails and overcomes the V^{IV} complex (data not shown). This suggests that H_2dipic, Hdipic^{-}, and dipic^{2-} in the presence of V^{III}, V^{IV}, and V^{V} will form complexes with the V^{III} first. Only if additional dipic ligand is present will substantial concentrations of the V^{IV} and V^{V} complexes form. In the case in which 6 mM of dipic ligand is present in the system, the V^{III} complex forms, and essentially no free V^{III} is observed from pH 2 to 7. As anticipated from the previous studies, both the V^{IV} and V^{V} complexes form, with the latter forming slightly more as the pH approaches 7.[41]

The preference for the formation of V^{III} complexes is even more pronounced for the dipic-OH ligand. As shown in Figure 5, the 1:1 V^{III} complex is the major species over the whole pH range studied, even in the presence of only 3 mM ligand. Some V^{V} complexes are present below pH 4 in which, interestingly, no V^{IV} complex forms. In the pH range of 5−6, dipic-OH formed more complexes with V^{IV} than it did with V^{V}. In this system, even more so than in the dipic system, the V^{III} complex is the most stable complex over the pH range studied. This difference is particularly apparent as the pH increases and presumably can be attributed to the deprotonation of the OH group and stabilization of the anion.[41]

The most surprising result from this comparison is the apparent high stability of the V^{III} complexes. Although these considerations do not take into account reactions with oxygen or possible redox reactions among complexes, the analysis demonstrates the high stability of the V^{III} complexes when the magnitude of the stability constants is taken into consideration. As a result, these V^{III} complexes are more resistant against oxidation than anticipated, which would be consistent with the shift in the corresponding V^{IV}/V^{IIII} redox potentials. Although most V^{III} complexes are known to readily oxidize in the presence of oxygen, the yellow color of the $V^{III}-$dipic solutions left in air did not quickly convert to the colorless oxidized solutions. Thus, the fact that the $V^{III}-$dipic systems do not rapidly oxidize would be consistent with the expectation that the V^{III} complexes have some lifetime after ingestion by the animal in our studies, consistent with a similar observation in a previous study with V^{III} complexes.[10]

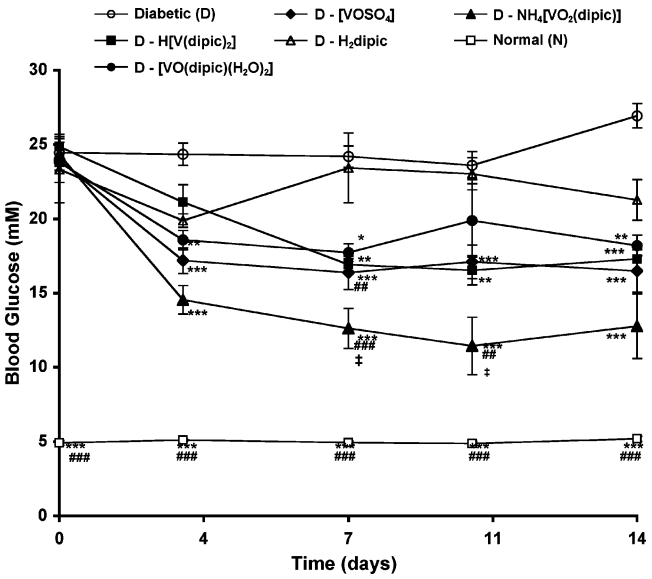


Figure 6. The effect of various oxidation states of vanadium−dipicolinate on hyperglycemia in Wistar rats with STZ-induced diabetes. Compounds were administered in drinking water, and blood glucose was measured as described in the experimental section for N ($n = 13$) (□), untreated D ($n = 25$) (○), H_2dipic-treated D ($n = 6$) (△), $VOSO_4$-treated D ($n = 30$) (◆), **3**-treated D ($n = 8$) (■), **2**-treated D ($n = 7$) (●), and **1**-treated D ($n = 5$) (▲) animals. Data are presented as the mean standard error of the mean (SEM). ***$p \leq 0.001$ vs the D animals, **$p \leq 0.01$ vs the D animals, *$p \leq 0.05$ vs the D animals. #$p < 0.05$ vs the ligand-treated D animals, ###$p < 0.001$ vs the ligand-treated D animals. ‡$p > 0.05$ vs the N animals, which means it is statistically indistinguishable from normal.

Effects of 3, 2, and 1 on Blood Glucose Levels in Rats with STZ-Induced Diabetes. The Wistar rat with STZ-induced diabetes was used to study the antidiabetic effects of V compounds.[48] Vanadyl sulfate ($VOSO_4$), which lowers diabetic hyperglycemia in rats, was used as a positive control for metal efficacy in alleviating symptoms of diabetes. Blood glucose values over time for D animals, and D animals treated with the three V−dipic compounds were determined and compared with blood glucose values from N rats, D rats treated with ligand alone, and D rats treated with $VOSO_4$ salt alone, as shown in Figure 6, for the first 14 days of the 28 day experiment.

The complexes showed different overall effects on blood glucose as a function of the oxidation state. Complexes in all oxidation states showed their effectiveness in lowering diabetic hyperglycemia after day 3. Treatment with both V^{III} and V^{IV}−dipic complexes gave blood glucose levels that were significantly lower ($p < 0.01$) than those seen in the STZ-induced diabetic rat for days 7 to 14, as shown in Figure 6. The V^{V}−dipic-treated animals had significantly lower blood glucose compared to the D animals ($p \leq 0.001$) at all time points shown in Figure 6. From days 3 to 14 the blood glucose levels in the V^{III} and V^{IV}−dipic complex-treated animals were not statistically different from those in the D animals treated with dipic ligand alone. However, compared to the dipic ligand-treated D animals, the V^{V}−dipic-treated D animals showed significantly lower blood glucose levels on days 7 and 10, whereas the $VOSO_4$ treated D animals showed statistically lower values for blood glucose at day 7.

These results show that the three dipic complexes cause different effects on diabetic hyperglycemia. The V^{V}−dipic complex seems to be the best at lowering hyperglycemia because it lowered blood glucose more than any compound tested and was the only dipic complex to cause statistically significant lowering of blood glucose in D animals compared to ligand-treated animals. Compared to the simple salt $VOSO_4$, the V^{V}−dipic complex lowered blood glucose levels closer to N levels at more time points. In addition, only treatment with V^{V}−dipic and not with $VOSO_4$ caused blood glucose levels to be significantly different from those in animals treated with the dipic alone at all time points. These results support previous studies by showing that organic V derivatives have increased efficacy compared to $VOSO_4$.[1,14]

Dosing and Serum Levels in D Rats Treated with Complexes 3, 2, and 1. The study was continued for two more weeks to reach a steady state in dosing before the determination of serum V levels. The average blood glucose levels for each treatment group in this two-week period are shown in Table 3. Note that in the last two weeks of the experiment, the treatments with **3** and **2** were less effective overall as blood glucose levels rose, consistent with some adaptation to the V compounds. During this period, only the V^{V}−dipic complex and $VOSO_4$ lowered blood glucose levels significantly compared to the D animals ($p \leq 0.001$). The parameters in Table 3 include fluid consumed for days 16−28, average doses administered for days 16−28, average V in serum, ratio of average V in serum to average dose administered for days 16−28, and average blood glucose for days 16−28. The concentration of V ingested from the drinking water was calculated for the last two weeks of treatment. The dose ingested in millimoles of V per kilogram per day was monitored from the concentration of V in the drinking solution and the amount of fluid consumed as described in the experimental section (Table 3). The serum levels of V were used as an approximate measurement of the bioavailability of V after entry through the GI system. This measurement provides a convenient way to compare how different V compounds enter the circulation after oral administration.

After killing the animals at day 28, we determined the concentration of V in the serum. Over the final two-week period, the V^{III}, V^{IV}, and V^{V}−dipic-treated animals ingested 1.25 ± 0.04, 0.60 ± 0.08, and 0.59 ± 0.10 mmol of V/kg/day, respectively. The $VOSO_4$-treated animals ingested 0.85 ± 0.08 mmol of V/kg/day. These average daily dose differences were not statistically significant. The $VOSO_4$ animals are included in this study as a positive control to show that this group of animals is responding to oral V therapy. Comparisons of the effects of the administration of V^{IV} and V^{V} salts have been previously reported by others.[49]

There were differences in the amount of fluid consumed over the last two weeks in the animals treated with the

(48) Cam, M. C.; Brownsey, R. W.; McNeill, J. H. *Can. J. Physiol. Pharmacol.* **2000**, *78*, 829−847.

(49) Domingo, J.; Gomez, M.; Sanchez, D.; Llobet, J.; Corbella, J.; Keen, C. *Trace Elem. Electrolytes* **1994**, *11*, 16−22.

Table 3. Effect of Oral Treatment with V Compounds of Differing Oxidation States on Fluid Consumed, Daily Dose of V or Ligand, Serum V Level, Ratio of Average Serum to Average Dose, and Average Blood Glucose Levels[a−c]

group/treatment	*n*	fluid consumed days 16−28 (mL/rat/day)	daily dose of V or ligand days 16−28 (mmol of V or ligand/kg/day)[d]	serum V level (nmol/mL)[d]	ratio of serum V to daily V dose days 16−28	average blood glucose days 16−28 (mmol/L)[d]
N/H_2O	13	48 ± 2***	NA	NA	NA	4.90 ± 0.1***
D/H_2O	25	237 ± 7	NA	NA	NA	26.7 ± 0.08
N/$VOSO_4$	8[e]	20 ± 3***,++	0.45 ± 0.05	14.1 ± 1.0	32.7 ± 5.3+++	3.97 ± 0.12***,++
D/$VOSO_4$	30[e]	43 ± 5***,++	0.85 ± 0.08	11.4 ± 1.1	13.5 ± 1.3	16.3 ± 1.32***
D/H_2dipic	6	183 ± 23**	2.67 ± 0.17+++	NA	NA	22.8 ± 1.62
D/V^{III}−dipic	8	116 ± 6***	1.25 ± 0.04	11.2 ± 1.0	8.9 ± 0.7	21.5 ± 0.86
D/V^{IV}−dipic	7	51 ± 16***,++	0.60 ± 0.08	13.1 ± 0.9	22.8 ± 1.4	20.7 ± 1.26
D/V^{V}−dipic	5[e]	15 ± 4***,++	0.59 ± 0.10	14.2 ± 3.4	26.2 ± 4.0+	15.5 ± 2.74***

[a] Data expressed as mean ± SEM. [b] NA = not applicable. [c] ***Indicates $p \leq 0.001$ compared with diabetics; **indicates $p \leq 0.01$ compared with diabetics; +++indicates $p \leq 0.001$ compared with D/$VOSO_4$; +indicates $p \leq 0.05$ compared to D/$VOSO_4$; ++ indicates $p \geq 0.05$ compared to N (i.e. not statistically different from N using this test). [d] Conversion factors: 1.00 mmol of V = 50.9 mg of V; 1.00 nmol of V = 50.9 ng of V; 1.00 mmol of glucose = 180.2 mg of glucose; 1.00 mM glucose = 18.02 mg/dL glucose. [e] From the D/$VOSO_4$ group, 13 randomly selected rats had serum V determined; for the N/$VOSO_4$ group, 7 animals had serum V determined; and for the D/V^{V}−dipic group, 4 animals had serum V levels determined. Therefore, for the serum V levels and the ratio of serum V to daily V dose calculations, only these animals are included.

various V−dipic complexes. During the final two weeks (days 16−28), all groups, including the N/$VOSO_4$ and D/$VOSO_4$ drank significantly different amounts of water than did the D rats, as seen in Table 3 ($p \leq 0.001$). Interestingly, the D animals treated with $VOSO_4$, **2**, and $[V^{V}O_2dipic]^-$ consumed amounts of fluid that could be distinguished from that consumed by the N animals, whereas the amount consumed by the D animals treated with **3** could not be distinguished from that consumed by the N animals. Of all the V−dipic-treated rats, V^{III}−dipic-treated rats drank the most water, hence ingesting the highest average dose (days 16−28). This suggests that perhaps the V^{III}−dipic complex is more palatable than the other V complexes.

The presence of ligand appears to increase the amount of V found in the serum of some of the D animals treated with the V−dipic complexes compared to those treated with $VOSO_4$. The actual amount of V in the serum of the treated groups was not statistically different between groups. To account for the differing amounts of V ingested by the oral administration, we used the ratio of daily dose of ingested V to serum V as our estimated measurement of the bioavailability of V in the animal after its passage through the animal's GI tract. This ratio was significantly higher for data obtained from the V^{IV}- and V^{V}-treated rats compared to that obtained from rats treated with $VOSO_4$ or V^{III}−dipic. Therefore, we conclude that both the V^{IV}− and V^{V}−dipic-treated animals showed better absorption of V into serum than V^{III}−dipic-treated animals. The ratio of average serum V to average daily dose for V^{V}−dipic-treated animals was statistically significant at $p \leq 0.05$ compared to D/$VOSO_4$ (26.2 ± 4.0 vs 13.5 ± 1.3, respectively). V^{IV}−dipic-treated animals showed better bioavailability of the V ion (ratio of 22.8 ± 1.4) than those treated with $VOSO_4$ (ratio of 13.5 ± 1.3); however this was not significantly different. On the basis of these data, there is experimental support for the hypothesis that the H_2dipic ligand aided V^{IV} and V^{V} metal ion movement through the GI tract. Because the V^{III}−dipic complex showed poor mobility through the GI tract (ratio of 8.9 ± 0.7 serum V to daily V dose), the ligand had little or no effect on the bioavailability of this complex. The absorption of the V^{V}−dipic was the greatest among the D animals treated with V complexes and was also the complex that lowered the elevated blood glucose of diabetes the most. The ratio of serum V to average daily dose of V in the N animals (32.7 ± 5.3) was similar to that of the D animals treated with V^{IV}−dipic or V^{V}−dipic complex and very significantly different ($p < 0.001$) from the bioavailablility of V in D animals treated with $VOSO_4$. The differential appearance of V in the blood stream in N and D treated animals shows that $VOSO_4$ is being processed differently in N and D animals.

In summary, the V^{V}−dipic-treated animals ingested less V than the $VOSO_4$-treated animals but showed better bioavailability as measured by the ratio of V levels in serum to the dose of V ingested. In addition, the V^{V}−dipic-treated animals showed levels of lowering elevated blood glucose (days 16−28) similar to those of the $VOSO_4$-treated animals. These results suggest that the V^{V}−dipic compound was more effective than $VOSO_4$.

Interpretation of the Biological Studies in Consideration of the Compounds' Chemistry. This study of the effects of three V−dipic complexes was designed to examine whether the metal's oxidation state will have an effect on the V compounds' insulin-enhancing properties. We tested the effects of a V compound that would be oxidized (the V^{III} compound), a V compound that would be reduced (the V^{V} compound), and a V compound that would be redox inert (the V^{IV} compound) in a reducing cellular environment. We found that the V^{V} compound was the V−dipic compound that lowered blood glucose the most and was the only V−dipic compound that demonstrated a statistically significant lowering of the elevated glucose levels compared to that of the ligand. Of the D animals treated with the V−dipic complexes, the animals treated with the V^{V}−dipic showed the largest quantity of V in the serum. This is particularly interesting because this complex would be characterized as the least stable and most labile of the series of complexes (except for its high acid stability). Thus, this compound's acid stability or its redox chemistry may be key to the ability of the V^{V} complex to lower the elevated glucose levels in the diabetic Wistar rat during oral treatment.

The V^{III} complexes are generally expected to be very reactive and are anticipated to rapidly convert to V^{IV} and perhaps even V^{V} complexes.[50,51] In the event such interconversions readily took place, the effects of the V^{III}, V^{IV}, and V^{V} complexes should have been similar. Because we showed in this work that only the effects of the V^{V} compound were statistically distinguishable from the effect of the ligand alone in lowering diabetic hyperglycemia, varying effects exist between the compounds when the V ion is in different oxidation states. Because the color of the V^{III}−dipic complexes in aqueous solution remained for more than a day in the presence of oxygen, it is possible that the V^{III}−dipic complexes will remain intact for a significant time period after ingestion, and immediate insulin-enhancing responses will not be invoked. In a study of an acute animal model system with selected V^{III} complexes it was found that some of these complexes were effective, whereas others were not. These findings are consistent with the fact that some of these V^{III} complexes exhibited greater stability than we anticipated based on literature reports.[10] These considerations and our studies are consistent with the oxidation state of the V complex affecting processing and mode of action.

There is ample evidence that V compounds are a part of cellular redox chemistry in biological systems. Although vanadate and V^{V} compounds are generally believed to be reduced intracellularly by glutathione (GSH),[1,52,53] some controversy is associated with this expectation[1,47] because other cellular reductants are likely involved in the redox chemistry of V compounds.[1] Although some V^{V} compounds will be reduced by cellular GSH, the compounds' lifetimes are likely to be more than sufficient to facilitate distribution and action of the compound. If redox conversions are not important for action of the V compounds, one could anticipate that the V^{IV}−dipic complex would be most effective in a reducing intracellular environment because the V^{IV}−dipic complex would not need redox processing before action. Both the V^{III}−dipic and the V^{V}−dipic compounds will undergo some redox processes following oral compound administration. The V^{III}−dipic should be oxidized following cellular uptake, presumably by metabolites (i.e., aldehydes), O_2, H_2O_2, and other cellular oxidants; this processing would assist the cellular antioxidants. The V^{V}−dipic compound should be reduced following cellular uptake and would deplete the cellular antioxidants. Thus, the fact that V^{V}−dipic is the most effective of the three dipic V complexes tested is counterintuitive to the observations that oxidative damage promotes diabetes and diabetic complications.

The differential effects observed during the chronic oral administration of V complexes with different oxidation states can be due to biological interactions in the GI tract, at sites of accumulation of V, and/or at the unknown site of action of V. Full pharmacokinetic studies using different means of administration in conjunction with chronic oral administration will help to address this point. The studies presented in this work were based on oral chronic administration of V−dipic complexes to rats with STZ-induced diabetes, and our observations include the bioavailability of V in the circulation as well as the action of the complexes in lowering diabetic hyperglycemia. We have carried out additional studies to address whether the effects are due to the processing of the complexes in the GI tract or to the action of the compounds directly administered into the circulation, which will be described in greater detail.[54] Others have repored pharmacokinetic and distribution studies for other V complexes using other administration routes.[11,26,55] These studies have demonstrated that bone and kidney tissue are major repositories of V. Direct absorption into the circulation of V administered directly to the latter part of the small intestine (ileum) increases the amount of V accumulated in the blood. This increase is possibly due to the bioprocessing occurring earlier in the GI tract, which interferes with transport across biological membranes into the circulation.

The strongest insulin-enhancing ability of V^{V}−dipic compared to V^{III}− and V^{IV}−dipic complexes may suggest that avenues other than oxidative processes are also critical in these initial stages of compound processing. Previous acute studies with V^{IV}− and V^{V}−malto-type complexes found only a statistically significant effect with the V^{IV} complexes. This ligand-specific difference in the biological effect could arise because $[VO_2(malto)_2]^-$ is not transported to the site of processing or because it is important that the V^{V} complex is stable at acidic pH (the greatest stability for the $[VO_2(malto)_2]^-$ complex is in the neutral pH range). Another factor that may be involved is differential transport of compounds. Precedence for differential transport has been reported for V^{IV} and V^{V} compounds in red blood cells.[56] Evidence exists that at least for some complexes the ligand dissociates from the metal ion, which should affect recognition by biological transport proteins.[11,28] In summary, the data presented in this paper support the hypothesis that the redox chemistry of V compounds and the role of the ligand are important to the insulin-enhancing action of these compounds. Others have implicated the specific inhibition of protein tyrosine phosphatases in the alleviation of diabetes by V compounds.[28] Our studies suggest that *both ligand and V oxidation state effects are also important in the alleviation of the symptoms of diabetes*, which is a multifactorial disease.

Conclusions

The hydrolysis of the V^{III} system was characterized at low ionic strength. Potentiometric studies were supported by UV−Vis studies that provided firm evidence for the dinuclear

(50) Vilas Boas, L. V.; Costa Pessoa, J. In *Comprehensive Coordination Chemistry. The Synthesis, Reactions, Properties & Applications of Coordination Compounds*; Wilkinson, G., Sir, Gillard, R. D., McCleverty, J. A., Eds.; Pergamon Press: New York, 1987; Vol. 3, pp 453−583.
(51) Cotton, F. A.; Wilkinson, G.; Murillo, C. A.; Bochman, M. *Advanced Inorganic Chemistry*, 6th ed.; Wiley-Interscience: New York, 1999.
(52) Cantley, L. C., Jr.; Aisen, P. *J. Biol. Chem.* **1979**, *254*, 1781−1784.
(53) Macara, I. G.; Kustin, K.; Cantley, L. C., Jr. *Biochim. Biophys. Acta* **1980**, *629*, 95−106.
(54) Willsky, G. R.; McNeil, J.; Crans, D. C. Manuscript in preparation.
(55) Setyawati, I. A.; Thompson, K. H.; Yuen, V. G.; Sun, Y.; Battell, M.; Lyster, D. M.; Vo, C.; Ruth, T. J.; Zeisler, S.; McNeill, J. H.; Orvig, C. *J. Appl. Physiol.* **1998**, *84*, 569−575.
(56) Yang, X.; Wang, K.; Lu, J.; Crans, D. C. *Coord. Chem. Rev.* **2003**, *237*, 103−111.

species $[V_2(OH)_2]^{4+}$. The speciation of the V^{III} system was used to calculate the stability constants for complexes formed from two different dipic-type ligands, H_2dipic and H_2dipic-OH. With dipic^{2-}, the major complexes have a 1:2 stoichiometry in contrast to the V^{IV} and V^{V} systems. When the stability of all these complexes was simulated in the absence of oxygen, the V^{III} complex was most stable. Comparing the stability constant for the 1:1 and 1:2 complexes, the difference is larger than expected and consistent with the second ligand binding less strongly. We propose that these complexes contain two dipic ligands and that one of these ligands binds in a tridentate manner, whereas the other binds in a bidentate manner. These differences were also observed for the dipic-OH system; however, in the V^{III}−dipic-OH system more vanadium is complexed than in the parent dipicolinate system. Indeed, at pH 7 very little free V^{III} is observed in the speciation studies.

The effectiveness of a series of V−dipic complexes in three different oxidation states on lowering hyperglycemia by oral administration and the resulting amount of V in serum was compared. The ligand has a modest effect on lowering hyperglycemia but does not lower blood glucose as does the best V−dipic compound. Complexation of V to the dipic ligand improved bioavailability as measured by the amount of V in serum. Although the potential redox bioprocessing of V complexes has been recognized as important in insulin-enhancing activity, this position has not previously been substantiated in a controlled chronic administration study.

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