A New Orally Active Insulin-Mimetic Vanadyl Complex: Bis(pyrrolidine-*N*-carbodithioato)-oxovanadium(IV)

Hiromi Watanabe, Masami Nakai, Kyoko Komazawa, and Hiromu Sakurai*

Department of Analytical Chemistry and Bioinorganic Chemistry, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607, Japan

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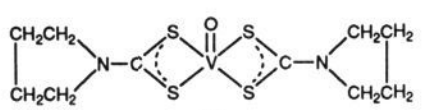
The main abnormality in insulin-dependent diabetes mellitus (IDDM) is hypergycemia due to deficiency of insulin,[1] but there are many serious secondary complications, such as atherosclerosis, microangiopathy, renal disfunction and failure, cardiac abnormality, diabetic retinopathy, and ocular disorders. At present severe diabetes can be controlled only by daily injections of insulin, so the development of compounds that cause insulin replacement or insulin mimetics on oral administration would be very useful.[2]

The finding in 1980 that vanadate (+5 oxidation state of vanadium) has an insulin-like effect[3] stimulated research on insulin-mimetic vanadium compounds. The blood glucose level of rats with streptozotocin (STZ)-induced diabetes (STZ-rats) was found to be normalized by addition of sodium orthovanadate to their drinking water.[4,5] Later, vanadium ions[6-13] and vanadium complexes[14-16] that are effective on oral administration to diabetic rats have been reported. We have been trying to develop new insulin-like vanadyl (+4 oxidation state) complexes that are less toxic[17] than vanadate and are present only in the active form in rat cells and tissues[18] using sulfur ligand–vanadyl complexes[19] such as vanadyl–cysteine methyl ester complexes, which normalize the blood glucose level of STZ-rats[15] when given orally.

Previously we reported that vanadyl sulfate potentiates glucose incorporation into isolated rat adipocytes[12] and suppresses release of free fatty acids (FFA) from adipocytes treated with epinephrine,[20] mimicking the effects of insulin. For evaluating the insulin-mimetic action of a vanadyl complex, we developed an *in vitro* test system using isolated rat adipocytes[20] treated with epinephrine, in which the effect of a vanadyl complex is compared with that of insulin by measuring their effects on FFA release. As addition of insulin to adipocytes inhibits the release of FFA dose-dependently, a complex that causes dose-dependent inhibition of FFA release is expected to have an insulin-mimetic action *in vivo*. Among the various vanadyl complexes with V–O, V–N, and V–S coordination modes tested,[21] the gray-green bis(pyrrolidine-*N*-carbodithioato)oxovanadium(IV) complex ($C_{10}H_{16}N_2OS_4V$, V-P) was found to show the strongest insulin-mimetic activity.



V-P

* To whom correspondence should be addressed.

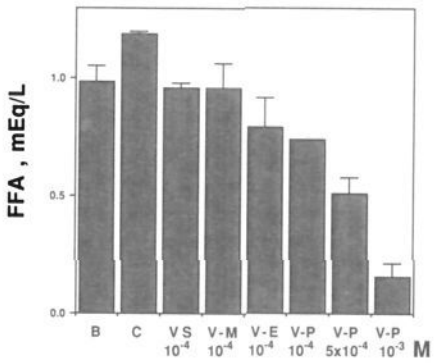


Figure 1. Inhibitory effects of vanadyl complexes on free fatty acid (FFA) release from rat adipocytes treated with epinephrine. Rat adipocytes were prepared as reported.[12,21] B: Blank, adipocytes were treated with saline for 30 min and then incubated for 3 h at 37 °C. C: Control, adipocytes were treated with saline for 30 min and then incubated with 10^{-5} M epinephrine for 3 h at 37 °C. V S, V-M, and V-E: Adipocytes were treated with 10^{-4} M vanadyl sulfate, bis(*N*,*N*-dimethyldithiocarbamato)oxovanadium(IV) and bis(*N*,*N*-diethyldithiocarbamato)oxovanadium(IV), respectively, and then incubated with 10^{-5} M epinephrine for 3 h at 37 °C. V-P: Adipocytes were treated with 10^{-4}, 5×10^{-4}, and 10^{-3} M V-P complex and then incubated with 10^{-5} M epinephrine for 3 h at 37 °C. Values are means ± SD for three experiments.

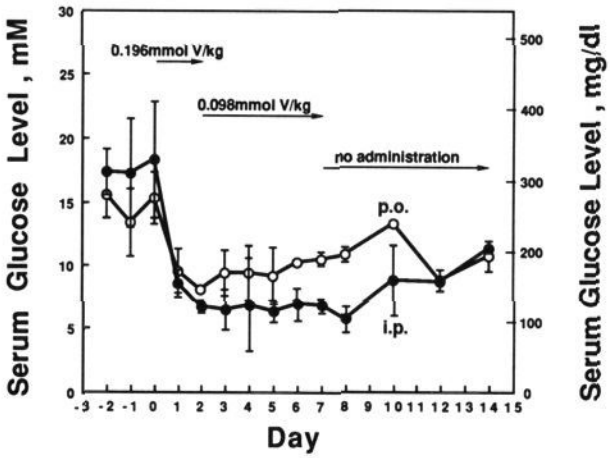


Figure 2. Basal serum glucose levels in STZ-rats receiving daily oral or ip treatments with V-P (0.196 mmol/kg of body weight for the first 2 days and then 0.098 mmol/kg of body weight and then no treatment).[25] Values are means ± SD for four rats.

In 1965, almost quantitative (>96%) preparation of V-P in ethanol by mixing ammonium pyrrolidine-*N*-carbodithioate and vanadyl sulfate in 2:1 molar ratio was reported,[23] but the complex was incompletely characterized. We characterized it by visible absorption, infrared (IR) absorption, and electron paramagnetic resonance (EPR) spectrometries at room and liquid nitrogen temperatures.[24]

Figure 1 shows the inhibitions of FFA release from isolated rat adipocytes treated with epinephrine in the absence of glucose by vanadyl sulfate (VS), bis(*N*,*N*-dimethyldithiocarbamato)oxovanadium(IV) (V-M), bis(*N*,*N*-diethyldithiocarbamato)oxovanadium(IV) (V-E), and V-P complexes. At concentrations of 10^{-4} M, V-P was the most effective, and its effect was dose-dependent in the concentration range 10^{-4}–10^{-3} M.[21] Thus we selected V-P for *in vivo* tests on STZ-rats.

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Figure 2 shows the changes in the basal serum glucose level in male Wistar rats with STZ-induced diabetes on daily intraperitoneal injection (ip) or oral administration (po) of V-P.[25] When the V-P complex was given at a dose of 10 mg (0.196 mmol) of vanadium(V)/kg of body weight for the first 2 days, the serum glucose level decreased to the normal range within 2 or 3 days, and it was maintained in the normal range by daily administration of 5 mg (0.098 mmol) of V/kg. In STZ-rats in which serum glucose level was normalized within 1 week on administration of V-P, the level remained normal for 1 week after the end of treatment and then gradually increased. The results indicated that V-P was more effective on intraperitoneal injection than on oral administration.

Normalization of the serum glucose level of STZ-rats by treatment with V-P was associated with normalization of the serum FFA level (control nondiabetic rats, 0.38 ± 0.06 mequiv/L serum; STZ-induced diabetic rats, 1.12 ± 0.12 mequiv/L; STZ-rats treated orally with V-P for 1 week, 0.42 ± 0.05 mequiv/L) as predicted from the *in vitro* tests. We found a good linear relationship between serum glucose and FFA levels.[26] Thus, monitoring of both serum glucose and FFA levels seems necessary for evaluating the degree of diabetes.

This work showed that the easily prepared complex V-P was very effective for normalizing both the serum glucose and FFA levels in STZ-rats. The present study also indicated the value of an *in vitro* system using isolated rat adipocytes for predicting the *in vivo* effects of test complexes. We are now studying the mechanism of action V-P complex.

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(21) Isolated rat adipocytes were prepared as described in ref 12. Serum glucose, FFA, and insulin were measured by the *o*-toluidine method[22] and with an NFFA Wako Kit and Insulin Wako B Kit, respectively.
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(24) Physical parameters of V-P are as follows: λ max, 600 ^{sh}nm (ϵ = 38.0 M^{-1} cm^{-1}) and 759 nm (ϵ = 47.8 M^{-1} cm^{-1}) in pyridine; $\nu_{V=O}$, 995 cm^{-1} (KBr disk); EPR parameters in pyridine g_0 = 1.979, A_0 = 77.2 × 10^{-4} cm^{-1}, $g_\parallel$ = 1.953, $A_\parallel$ = 162.6 × 10^{-4} cm^{-1}, $g_\perp$ = 1.992, $A_\perp$ = 44.6 × 10^{-4} cm^{-1}. Anal. Calcd for $C_{10}H_{16}N_2OS_4V$: C, 33.4; H, 4.49; N, 7.79. Found: C, 33.1; H, 4.48; N, 7.78. V-P is paramagnetic in the solid state, showing a very broad EPR signal due to VO^{2+}. Other dithiocarbamate–vanadyl complexes show quite similar parameters to those of V-P. V-P is slightly soluble in numerous organic solvents such as pyridine, dimethyl sulfoxide, and dimethylformamide, but insoluble in water, methanol, ethanol, and ether. V-P is oxidized under air in solution but in the solid state it is stable under an inert gas atmosphere. V-P in 5% acacia suspension is also stable.
(25) V-P was suspended in 5% acacia. A dose of 70.7 mg (0.196 mmol) of V-P/kg of body weight corresponds to 10 mg (0.196 mmol) of V/kg of body weight. Diabetes was induced in four Wistar rats, weighing 180–200 g, by a single iv injection of freshly prepared streptozotocin (STZ) (60 mg/kg of body weight) in 0.1 M citrate buffer, pH 5.0. The STZ-rats received an oral or ip dose of V-P (0.196 mmol/kg of body weight for the first 2 days and then of 0.098 mmol/kg body weight for the next 5 days) daily at about 11 a.m. after determination of their serum glucose levels. The body weights of the STZ-rats were measured daily during administrations of V-P. On days 0, 7, and 14 of the experiments, the body weights of the rats treated orally with V-P were 243 ± 8, 250 ± 1, and 280 ± 10 g, while those of rats treated ip with V-P were 240 ± 9, 170 ± 8, and 220 ± 1 g, respectively. The mean serum glucose level of control nondiabetic rats was 114.6 ± 5.1 mg/dL = 6.35 ± 0.28 mM (n = 4).
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