We have investigated the interaction of bis(acetylacetonato)oxovanadium(IV) (VO(acac)₂) with bovine serum albumin (BSA) by EPR and angle-selected electron nuclear double resonance, correlating results with assays of glucose uptake by 3T3-L1 adipocytes. EPR spectra of VO(acac)_2 showed no broadening in the presence of BSA; however, electron nuclear double resonance titrations of VO(acac)₂ in the presence of BSA were indicative of adduct formation of VO(acac)₂ with albumin of 1:1 stoichiometry. The influence of VO(acac)₂ on uptake of 2-deoxy-D-[1-¹⁴C]glucose by serum-starved 3T3-L1 adipocytes was measured in the presence and absence of BSA. Glucose uptake was stimulated 9-fold in the presence of 0.5 mM VO(acac)₂, 17-fold in the presence of 0.5 mM VO(acac)₂ plus 1 mM BSA, and 22-fold in the presence of 100 nM insulin. BSA had no influence on glucose uptake, on the action of insulin, or on glucose uptake in the presence of VOSO₄. The maximum insulin-mimetic effect of VO(acac)₂ was observed at VO(acac)₂:BSA ratios less than or equal to 1.0. Similar results were obtained also with bis(maltolato)oxovanadium(IV). These results suggest that the enhanced insulin-mimetic action of organic chelates of VO²⁺ may be dependent on adduct formation with BSA and possibly other serum transport proteins.

In the past several years the clinical potential of vanadium compounds in the treatment of type II diabetes has changed from low to high because of the introduction of an organic chelate of oxovanadium(IV) known as KP-102 into phase I trials (1). Studies in both laboratory animals and in humans have now convincingly demonstrated the lowering effects of vanadium compounds on blood glucose levels (17–20), we would argue that the organic moiety of VO²⁺ elicits maximal enhancement of insulin-mimetic action of VO²⁺ ion. While pH-dependent speciation of organic chelates observed on the basis of EPR spectra has been ascribed to rearrangements of the organic ligand moieties and displacement by solvent molecules (9–11), it is not known whether these equilibria influence insulin-mimetic action. Furthermore, the physiologically active form of chelated VO²⁺ in the bloodstream is not established.

An important observation made by Chasteen and co-workers (12) shows that VO²⁺, when given as VOSO₄ by gastric intubation to laboratory rats, distributes itself in circulating plasma between the two major isoforms of the serum transferrins in proportion to the amount administered. Although serum albumin and transferrin bind VO²⁺ tightly in the micromolar range (13–16), it is difficult to ascribe the enhanced glucose-lowering capacity of organic VO²⁺ complexes simply to the “stripping” out of the VO²⁺ ion from its chelate ligand environment to form protein-bound VO²⁺ in the blood stream. Such action would be likely to render organic VO²⁺ complexes no more potent in glucose-lowering capacity than VOSO₄ itself. Since the serum transport proteins albumin, transferrin, and transthyretin bind a variety of organic ligands, e.g. fatty acids, steroids, and thyroxine hormone as carrier molecules in circulating blood (17–20), we would argue that the organic moiety of VO²⁺ chelates likely also facilitates binding to serum transport proteins. For this reason in these initial studies, we have investigated the potential of organic chelates of VO²⁺, namely VO(acac)₂, compound b in Fig. 1, to form adducts with serum albumin of defined stoichiometry. We have also analyzed the spectroscopic properties of VO(acac)₂ by EPR and ENDOR spectroscopy to determine the stoichiometry of the organic ligand bound to VO²⁺ as a function of pH. To investigate whether serum proteins have an influence on the insulin-mimetic action of VO²⁺ chelates, we have compared glucose transport, measured as the uptake of 2-deoxy-D-[1-¹⁴C]glucose by 3T3-L1 adipocytes stimulated by VO(acac)₂, in the absence and presence of albumin. The results disprove previous interpretations of the pH-dependent speciation of these organic chelates (10, 11) and demonstrate that there is no change in the stoichiometry or coordination geometry of the bound organic ligand at low pH. Not only does VO(acac)₂ form a tightly bound adduct with albumin of 1:1 stoichiometry, but also a ratio of VO²⁺:chelate to protein of ≤1.0 elicits maximal enhancement of insulin-mimetic activity in cultured 3T3-L1 adipocytes.

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Structural Origins of the Insulin-mimetic Activity of Bis(acetylacetonato)oxovanadium(IV)*

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Insulin-mimetic Action of VO²⁺

EXPERIMENTAL PROCEDURES

Reagents—Vanadyl sulfate hydrate and bis(acetylacetonato)oxovanadate(IV) were purchased from Aldrich. Crystalline bis(2-malato)oxovanadate(IV) was a gift from Professor C. Orvig of the University of British Columbia. Crystalline N-(2-hydroxyethyl)iminodiacetato-oxovanadate(IV) was a gift from Professor D. C. Crans of Colorado State University. Insulin, deoxy-2-glucose, differentiation reagents, and fatty acid-free serum albumin were obtained from Sigma. 2-deoxy-2-[1-14C]glucose (56 mCi/mmol) was supplied by ICN (Costa Mesa, CA). All other reagents were of analytical reagent grade, and deionized distilled water was used throughout. Spectroscopic grade methanol was obtained from Aldrich. Absolute ethanol was obtained from Aaper Chemical Co. (Shelbyville, KY). Deuterated water (99.8 atom % ²H₂O) and [²H₄]methanol (99 atom % ²H) were obtained from Cambridge Isotope Laboratories (Woburn, MA).

Cell Culture and Experimental Treatment—A protocol was developed to avoid the presence of serum or albumin while measuring the influence of insulin and insulin-mimetic compounds on the uptake of 2-deoxy-2-[1-14C]glucose by 3T3-L1 adipocytes. 3T3-L1 fibroblasts were maintained and differentiated into adipocytes as reported previously (21). Cells were used 4–9 days after completion of the differentiation protocol when >95% of the cells contained lipid droplets. Prior to insulin stimulation, or treatment with VO²⁺ compounds, cells were washed twice with phosphate-buffered isotonic saline at 37 °C and serum-starved for 2.5 h at 37 °C in 1 ml/well KRBH with 5 mM glucose and 25 mM HEPES, pH 7.4. The basal and insulin-stimulated rates of glucose transport in adipocytes treated in this manner were identical to cells serum-starved in KRBH/glucose plus 0.5% BSA and similar to previous results when Dulbecco’s modified Eagle’s medium plus 0.5% fetal bovine serum was used (22). The serum starvation medium was then removed, the cells were washed two times with phosphate-buffered isotonic saline (37 °C), and the adipocytes were placed in 0.5 ml/well KRBH containing insulin, the VO²⁺ compound desired (accordingly in the absence or presence of BSA), or no insulin-mimetic compound. After 30 min at 37 °C, 20 μl 2-deoxy-2-[1-14C]glucose (300 cpm/pmol) was added to all wells. After 5 min at room temperature, the assay was terminated by addition of 50 μl of 1000 mM 2-deoxy-D-glucose and washing the cells three times with phosphate-buffered isotonic saline on ice. Adipocytes were collected in 0.5 ml of distilled water, and 2-deoxyglucose uptake was determined by liquid scintillation counting.

Metabolic Assays—Concentrated stock solutions of VO(acac)₂, VO-(malto)₂, VO(acac)₂, and VO(3-ethyl-acac)₂ were prepared by dissolving vanadyl sulfate hydrate in a small volume of absolute ethanol followed by dilution so that the final solution contained 150 mM sodium chloride buffered to pH 7.4 with 10 mM HEPES. The buffered solution had been previously purged with nitrogen gas. Aliquots of the buffered solution were added to the wells in the presence or absence of BSA. (The ethanol content of incubation mixtures was no greater than 1%.) Before use, BSA had been exhaustively dialyzed against HEPES-buffered saline at pH 7.4 containing 10 mM EDTA followed by dialysis against HEPES-buffered saline without EDTA to remove possible contaminant vanadium (23). No vanadyl species could be detected in the BSA solution by EPR. Concentrated stock solutions of VO₅O₄ were prepared by dissolving vanadyl sulfate hydrate in a small volume of H₂O under a nitrogen atmosphere, and aliquots were added to a solution of BSA in buffered isotonic saline to result in 1 mM final concentration of each. This solution was then used for suspension of adipocytes for glucose uptake measurements.

EPR and ENDOR Studies—Stock solutions of VO(acac)₂ for acid-base titrations monitored by EPR were made by dissolving the crystalline compound in methanol or in nitrogen-purged water. EPR spectra of aqueous or methanol solutions of VO(acac)₂ at ambient room temperature were recorded with a high-sensitivity, single-frequency X-band spectrometer. For titrations of VO(acac)₂ in aqueous solution, small aliquots of concentrated HCl or NaOH were added. Before and after spectral recording, the pH was measured with a Radiometer PHM82 standard pH meter equipped with a glass electrode to ensure that the pH of the solution had not changed. The pH meter was generally calibrated with two standard pH solutions bracketing the pH of the test solution. The volume change after addition of concentrated acid or base was less than 1%.

Acid-base titrations of VO(acac)₂ in methanol were carried out by two different methods. Equivalent results were obtained with both methods. Either small aliquots of concentrated aqueous HCl or NaOH were added to the methanolic solution, the volume of the aqueous component remaining <1% of the total volume. To completely avoid possible effects of the acid component, aliquots of methanol saturated with dry, gaseous HCl or NH₃ were added to the methanolic solution of VO(acac)₂ to alter [H⁺]. The pH was measured with a glass electrode prior to and after spectral recording according to Bates (24), allowing adequate time for equilibration of the electrode. The nominal pH reading was converted to pH⁺ according to the relationship pH⁺ = pOH⁻ = pH − δ, where pH is the nominal reading of the electrode and δ has the value of 2.34 for 100% methanol as solvent (24).

EPR and ENDOR spectra were recorded with an X-band Bruker ESP 300E spectrometer equipped with a cylindrical TE₀₁₀ cavity, an Oxford Instruments ESR910 liquid helium cryostat, and Bruker ENDOR digital accessory as described previously (25, 26). The 300E spectrometer was equipped with a complete computer interface (Bruker ESP3220 data system) for spectrometer control and data acquisition and processing. Typical experimental conditions for ENDOR measurements were: temperature, 20 K; microwave frequency, 9.45 GHz; incident microwave power, 64 microwatts (full power, 640 milliwatts at 0 dB); rf power, 50–70 watts; rf modulation frequency, 12.5 kHz; and rf modulation depth, 10–20 kHz. The static laboratory magnetic field was not modulated for ENDOR. EPR spectra were simulated with use of the program WINEPR2.11 (Bruker Instruments, Inc., Bellerica, MA) as described previously (27).

RESULTS AND DISCUSSION

The pH Dependence of the EPR Spectrum of VO(acac)₂—Fig. 2 compares representative EPR spectra of VO(acac)₂ at ambient room temperature in aqueous solution with those observed in methanol. The absorption intensity of the EPR spectrum of the S = 1/2 oxovanadium(IV) ion in solution at ambient temperatures is distributed over eight components due to the hyperfine coupling of the unpaired electron with the (I = 7/2)⁵¹V nucleus. While the centrally located components of the different spectral species overlap heavily with each other over the titratable pH range, the low field and high field components are separated from each other at extremes of protonic activity.

In aqueous solutions we have observed the reversible formation of four spectrally distinct species, labeled A–D, while in methanol only three were observed. Vertical lines have been drawn, therefore, in Fig. 2 at low field and high field positions identifying each species. For both solvent systems, it is seen that the
vertical lines for species B and B’ in aqueous and methanol systems, respectively, identify a weak shoulder that is not part of the spectrum for species A or A’. It is seen in Table I that species A–D and A’–C in aqueous and methanol solutions, respectively, differ from each other primarily on the basis of $A_0$ values. Comparable observations have been made earlier by others for VO(acac)$_2$ (11) and VO(malto)$_2$ (9). The values of the spectroscopic parameters $g_0$, $g_{\perp}$ and $A_0$, $A_\perp$ for these species obtained from frozen solution spectra, summarized in Table I, are nearly identical, indicating very similar structural environments of the oxovanadium(IV) ion. Corresponding values for VO(acac)$_2$ have not been reported from frozen solution spectra by others. Our results for VO(acac)$_2$ from frozen solution spectra are comparable to those reported for VO(malto)$_2$ (9).

In Table I it is seen that the $g$- and $A$-values for species A, B, and C in aqueous solution are within experimental measurement identical to the $g$- and $A$-values of species A’, B’, and C’ in methanol solution, respectively. Only in aqueous solution is the fourth species D observed. Its spectrum yields $g$- and $A$-values identical to those of the [VO(H$_2$O)$_5$]$^{2+}$ ion (9, 26, 28). However, no previous study has shown the equivalence of species A, B, and C in aqueous solution to A’, B’, and C’, respectively, in methanol. Cranis and co-workers (10, 11) have stated that for VO(acac)$_2$ freshly prepared in aqueous solutions the conversion of species A to species C is time-dependent, requiring up to 11 days at ambient temperature. The spectral changes have, therefore, been interpreted to reflect kinetically sluggish, time-dependent alterations in coordination geometry and displacement of an equatorial organic ligand by water. With freshly prepared, unbuffered solutions of species A, we have similarly observed this phenomenon after 11 days but find that the change in spectra was accompanied by a corresponding decrease in pH. On the other hand, direct adjustment of the pH by considering the summed total of species $A(B)$ distinct from species C. The titration curves, therefore, were calculated as a function of the composition of $A(B)$, C, and D in aqueous solution and, correspondingly, $A’(B’)$ and $C’$ in methanol. In aqueous solution, as illustrated in Fig. 3, one ionization was observed with a $pK_a$ value of $-3.1$, governing the reversible interconversion between species $A(B)$ and C, while the second ionization with a $pK_a$ value of $-1.7$ governs the equilibrium between species C and D. In methanol only one ionization was observed as a function of protonic activity with a $pK_a$ value of $-5.1$, governing the interconversion between species $A(B)$ and $C’$.

The spectral speciation of VO(acac)$_2$ (10, 11) and of VO(malto)$_2$ (9) has been previously noted by others on the basis of EPR spectra; however, the values of ionization constants governing their interconversion have not been estimated, and the spectroscopic equivalence of species A, B, and C for VO(acac)$_2$ in
aqueous solutions and of A', B', and C' in methanol solutions has not been demonstrated hitherto. While the molecular origins of the two ionizations in aqueous solution are under further investigation in this laboratory through use of perdeuterated VO(acac)$_2$, the results do demonstrate that the metallo-organic chelate VO(acac)$_2$ is stable in aqueous solutions of pH ~2. This observation, together with its expected greater lipophilicity, undoubtedly underlies in part its enhanced insulin-mimetic activity when introduced orally to laboratory animals compared with that of VO(SO$_4$)$_2$.

ENDOR Characterization of VO(acac)$_2$—Isomerizations of the organic ligand, replacement of carbonyl oxygen atoms by solvent molecules, and other similar structural changes have been attributed to underlie the pH-dependent spectral speciation of VO(acac)$_2$ (10, 11). However, the values of the spectroscopic parameters in Table I extracted from spectra can be related only to the average elemental composition of the equatorial donor-ligand atoms with appropriate changes in vanadium-oxygen covalency (29). The spectroscopic effect of a solvent oxygen atom is essentially indistinguishable from that of a carbonyl or hydroxyl oxygen. Angle-selected ENDOR, therefore, becomes the method of choice to assign the coordination geometry of the acetylacetone ligand.

The underlying principles of angle-selected ENDOR of VO$^{2+}$ have been described in earlier studies from this laboratory (25–33) and are only briefly summarized here. When $H_0$ is set to the $-7/2\text{ }$ component of the EPR absorption spectrum and is, therefore, parallel to the symmetry axis or the V=O bond of the complex, a proton located along the symmetry axis gives rise to a parallel hyperfine (hf) resonance coupling (A$_h$) while A$_v$ or the perpendicular hf coupling is observed for a proton in the molecular x, y plane. On the other hand, when the field is set to the $-3/2\text{ }$ absorption feature, an axial proton gives rise to a perpendicular hf coupling A$_h$, and the equatorial proton gives rise to a combination of parallel and perpendicular hf couplings. The combination of parallel and perpendicular hf couplings is observed only for a proton in the equatorial plane for the $-3/2$ setting of $H_0$. On the other hand, only an axial proton gives rise to a single pair of ENDOR features when $H_0$ is set to the $-3/2$ absorption feature. This variation in the resonance pattern, dependent on the orientation of the magnetic field $H_0$ with respect to magnetic axes in the molecule, is the essence of angle-selected ENDOR as first observed by Rist and Hyde (34).

The detection of ENDOR features of the VO$^{2+}$ ion is heavily dependent on the nearby solvent environment and the quality of glass formation (25–33). For this reason, because of the near identity of the g- and A-values of species A, B, and C to those of A', B', and C', respectively, we have collected ENDOR spectra of species A' and C' only in methanol because of its glass-forming properties. Water does not form a glass upon freezing, and therefore, broadening of ENDOR lines occurs because of variations in the crystalline field, particularly for small molecule VO$^{2+}$ complexes, preventing detection of ENDOR absorptions. Fig. 4 illustrates the proton ENDOR spectra of VO(acac)$_2$ in perdeuterated methanol. Only nonexchangeable, covalently attached hydrogens in the acetylacetonate ligand are detected by ENDOR under these conditions. With $H_0$ set to the $-3/2\text{ }$ component of the EPR spectrum, both A$_h$ and the A$_v$ hf couplings are observed for species A' formed at high pH$^+$ and for species C' formed at low pH$^+$ as defined by the spectra in Fig. 2. On the other hand, with $H_0$ set to the $-7/2\text{ }$ component of the EPR spectrum, only the A$_h$ hf couplings were detected. This pattern of resonances is seen only when the ENDOR-detected hydrogens are in the equatorial plane. This result, therefore, indicates that there is no change in geometry of the bound acetylacetonate ligand with change in [H$^+$]. Since the peak-to-peak amplitudes and line widths of the resonance features for both A' and C' species are identical within experimental measurement, the ENDOR spectra also indicate that there is no change in stoichiometry of bound acetylacetonate ligand with change in pH$^+$. These observations were confirmed by collecting ENDOR spectra of VO(acac)$_2$ in [D$_2$]methanol (data not shown). No resonance features characteristic of equatorial OH groups (30) could be observed for species A' or C' that would have resulted from displacement of an acetylacetonate oxygen by a methanolic hydroxyl group according to the equilibria proposed (10, 11). Also since the ENDOR spectra for both species A' and C' have their origin only in equatorial hydrogens covalently attached to the organic ligand, proposed isomerizations of the ligand into axial positions (10, 11) are excluded. The molecular origins of the ionizations described in Figs. 2 and 3, therefore, must derive from changes in the covalency of vanadium-oxygen interactions induced through protonation-dependent changes of outer sphere solvent molecules hydrogen bonded to the equatorial carbonyl oxygens or to the vanadyl oxygen.

Fig. 5 compares proton ENDOR spectra of VO(acac)$_2$ in deuterated aqueous buffer in the presence of BSA. The spectra were collected with $H_0$ set to both the $-3/2$ and $-7/2\text{ }$ components of the EPR spectrum of VO(acac)$_2$. While the underlying ENDOR features of the acetylacetonate ligand remained unchanged indicating that the organic chelating ligand was not displaced, there are additional features near the Larmor frequency that have their origin in protein residues. Since exchangeable protons on the serum albumin molecule will have been substituted by deuterons from the solvent, the new resonance features can be ascribed only to covalent hydrogens of nearby amino acid residues. The ENDOR splittings of these
resonance features arise from amino acid hydrogens over a 5–10-A distance from the vanadium nucleus (25, 30–33); they, therefore, clearly indicate binding of the organic chelate to the protein molecule. The plot in Fig. 6 shows that binding occurs to form an adduct of 1:1 stoichiometry. Because the underlying features of the acetylacetonate ligand are observed in the ENDOR spectra in Fig. 6, the spectra indicate that the VO(acac)$_2$ complex remains intact upon binding to the protein. The vanadium hf components in the EPR spectra of VO(acac)$_2$ showed no broadening in the presence of BSA. Comparable observations have been reported by others (35). Since unresolved ligand hf broadening measurably adds to the EPR line width only for hydrogens covalently attached to equatorial donor-ligand atoms (36), we conclude that the oxovanadium(IV) moiety is bound axially to the protein either through a residue hydrogen bonded to the axial water molecule or through a residue directly coordinated to the vanadium ion, having displaced the axial water. At present we cannot distinguish between these two possibilities.

**Measurement of the Insulin-mimetic Action of VO(acac)$_2$**—Albumin is often added to cell culture medium as a neutral, protectant macromolecule (cf. Ref. 7); therefore, we have used serum-starved, differentiated 3T3-L1 adipocytes for metabolic assays to avoid albumin as a complicating factor in the assay medium as described under “Experimental Procedures.” Cells were washed twice with phosphate-buffered isotonic saline (37 °C) and serum-starved for 2.5 h in KRBH lacking BSA or serum. The medium was removed, and cells were incubated for 30 min in KRBH with the indicated additions prior to measurement of glucose transport rates. On this basis, we were able to separate the intrinsic influence of a VO$^{2+}$ compound alone on the uptake of 2-deoxy-D-$[1-^{14}C]$glucose from that in the presence of added BSA. Comparative effects are summarized in Table II for four VO$^{2+}$-containing systems.

As shown in Table II, added BSA had no influence on the action of insulin or on the basal rate of glucose uptake. However, not only did the addition of BSA have a pronounced effect on the insulin-mimetic activity of VO(acac)$_2$, but also this varied according to the molar ratio of added BSA to VO$^{2+}$ chelate. Fig. 7 compares in histogram form the uptake of 2-deoxy-D-$[1-^{14}C]$glucose as a function of the VO(acac)$_2$:BSA molar ratio. It is seen that the near maximal influence of BSA on the insulin-mimetic effect of VO(acac)$_2$ occurs at a VO(acac)$_2$:BSA ratio of $\sim 1.0$. Addition of VO(acac)$_2$ in excess of this ratio resulted in a decrease in the enhancement of glucose uptake. Since the results of ENDOR titrations in Fig. 5 indicate that the VO$^{2+}$ ion is not removed from its organic chelate environment, we believe that the lower activity observed at VO(acac)$_2$:BSA ratios $\geq 1.0$ is due to the intrinsically lower activity of the free, unbound portion of VO(acac)$_2$. The vanadium hf components in the EPR spectra of VO(acac)$_2$ and VO(acac)$_2$ ratios are:

The corresponding spectra of VO(acac)$_2$ were measured in 0.1 M NaCl.

The vanadium hf components in the EPR spectra of VO(acac)$_2$ are shown in Fig. 6 (with VO(acac)$_2$ resonance contributions subtracted) as a function of increasing albumin concentration relative to VO$^{2+}$. The solid line was calculated for saturation of a protein with ligand for one binding site. The intersection point of the asymptotes indicates a binding stoichiometry of $\sim 1.0$ VO(acac)$_2$ per albumin molecule.

**TABLE II**

| Conc (mM) | Glucose transport (pmol/min/well) |
|----------|-----------------------------------|
| KRBH BSA |                                   |
| Basal    | 1.95                              |
| 100 nM insulin | 42.16                        |
| 0.5 mM VO(acac)$_2$ | 17.96                        |
| 0.5 mM VO(malto)$_3$ | 10.46                        |
| 0.5 mM VO(Hhida)$_3$ | 2.11                         |
| 1.0 mM VOSO$_4$ | ND$^a$                       |

$^a$ [N-(2-Hydroxyethyl)iminodiacetato]oxovanadium(IV).

Not determined because the chemical state of the VO$^{2+}$ ion is undefined in KRBH.
that both inorganic salts and organic chelates of VO$_2^{+}$ in vitro have not been fully clarified. It has been demonstrated that both inorganic salts and organic chelates of VO$_2^{+}$ exert their insulin-mimetic effect, at least in part, by stimulating lipogenesis in adipocytes via membrane-associated phosphotyrosine phosphatases and a cytosolic, non-insulin receptor protein tyrosine kinase (7). Other enzyme systems may also be involved. Since enhancement of lipogenesis by VO(acac)$_2$ has been shown to be far greater than that by VOSO$_4$ in the studies by Shechter and co-workers (7), the structure and affinity of the organic ligand for VO$_2^{+}$ are likely to be critical in the expression of insulin-mimetic activity.

It has been previously postulated on the basis of EPR spectra of VO(acac)$_2$ that isomerizations and displacement of equatorial solvent molecules hydrogen bonded to equatorial or axial oxygens of the VO$_2^{+}$ chelate. Of particular interest, therefore, is the observation through Figs. 5 and 6 that VO(acac)$_2$ formed a specific adduct with BSA of 1:1 stoichiometry. Protein-chelate adduct formation was correlated with enhanced insulin-mimetic activity of VO(acac)$_2$ over that with other organic chelates of VO$_2^{+}$, with the exception of VO(malto)$_2$, the influence of added BSA was observed also for VO(malto)$_4$, the addition of which to the insulin-mimetic effect of VO(acac)$_2$ that isomerizations and displacement of equatorial or axial oxygen ligands may be important in controlling drug potency and distribution of organic chelates of VO$_2^{+}$ to insulin-sensitive tissues, namely binding of the organic chelate to serum transport proteins. This factor has not been considered heretofore.

Differential enhancement of the insulin-mimetic action of VO$_2^{+}$ chelates by BSA reported in Table II may be due to variation in the specific activity of the protein-chelate adduct, a different binding affinity of the chelates for BSA, or the stripping out of the VO$_2^{+}$ ion from its chelate environment. It is also possible that other serum transport proteins such as transferrin and transthyretin may form specific adducts with organic chelates of VO$_2^{+}$ with differential enhancement of their insulin-mimetic action. Our results thus point to a variety of protein-chelate interactions that may be associated with enhancement of their insulin-mimetic action. Of particular interest is that they may lead to multiple routes for development of pharmacologic agents for treatment of type II diabetes.

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