Binding of vanadium compounds perturbs conformation and aggregation state of insulin*

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Abstract The interactions between zinc-free insulin and vanadium compounds, $NaVO_3$, $VO(acac)_2$ and $VO(ma)_2$, have been investigated by fluorescence spectroscopy, circular dichroism (CD) and Fourier-transformed infrared (FT-IR) spectroscopy. The results showed that binding of vanadium compounds produced a static quenching of the intrinsic fluorescence of insulin. The apparent association constants were determined to be $(0.17 \pm 0.01) \times 10^4\ L \cdot mol^{-1}$ for $NaVO_3$, $(2.8 \pm 0.2) \times 10^4\ L \cdot mol^{-1}$ for $VO(acac)_2$, and $(4.0 \pm 0.1) \times 10^4\ L \cdot mol^{-1}$ for $VO(ma)_2$, respectively. The light scattering intensity of insulin decreased upon incubation with the vanadium compounds, suggesting the disaggregation of insulin. The attenuation of the band at 273 nm of insulin CD spectra also supported the disaggregation of insulin observed above. A new band at $1650 \sim 1653\ cm^{-1}$ appeared in the FT-IR spectra of insulin upon incubation with the vanadium compounds, indicating the formation of an α-helix structure at B (9-19) *motif*. This α-helix structure suggests a structural change of insulin from an extended conformation (T state) to a helical conformation (R state), which is essential for binding of insulin to its receptor. In conclusion, binding of vanadium compounds results in conformational changes and disaggregation of insulin. These changes might account for the enhancement of binding affinity for insulin to its receptor in the presence of vanadium compounds.

Keywords: insulin, vanadium compounds, conformation.

In recent years, the researches on the vanadium compounds attract more and more interest. Almost all the vanadium compounds, whether the simple vanadium salts, the peroxovanadium (V) compounds or the vanadium (IV) chelates, exhibit hypoglycemic effects when taken orally[1~5]. It was reported that there was no obvious correlation between the intestinal absorption and their lipophilicity, and the difference in hypoglycemic effect among a variety of vanadium compounds could not totally contribute to the intestinal absorbability since the difference remained even the gastrointestinal tract was bypassed by i. v. injection[6], indicating different mechanisms of glucose lowering might exist for different vanadium compounds. Although the working mechanism of *in vivo* antidiabetic action of vanadium is poorly understood, *in vitro* and *in vivo* data showed that vanadium does affect various aspects of the insulin signaling pathway[4]. Kwong et al.[7], based on the influence of vanadium compounds on the insulin binding with its receptor, suggested that peroxovanadate acted as a modulator by forming a ternary complex of modulator-receptor-insulin. But it was still unclear whether the vanadium compounds acted on insulin receptor or on insulin itself or both. Since specific binding between insulin and its receptor is the primary event for insulin to exert biological function and only the monomeric insulin can bind with its receptor, the interaction between zinc-free insulin and vanadium compounds would be one of the key problems to be studied in order to clarify the antidiabetic mechanism of vanadium compounds.

1 Materials and methods

1.1 Reagents and instruments

Instruments: Shimadzu 540 fluorescence spectrophotometer, JASCO J-720 spectropolarimeter, Bio-Rad FTS-65A FT-IR spectrometer, and pHS-2 pH meter.

Reagents: Native zinc human insulin (26 IU/mg) and N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid (HEPES) were purchased from Sigma. Vanadium compounds were gifts from Professor Crans (Colorado State University, USA). All other chemicals were of analytical grade. Stock solutions of Na-

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VO_3, bis (acetylacetonato) oxovanadium (IV) ($VO(acac)_2$) and bis(maltolato)-oxovanadium (IV) ($VO(ma)_2$) were freshly prepared in HEPES-buffered isotonic saline (NaCl 135 mmol·L^{-1}, HEPES 15 mmol·L^{-1}, pH 7.4). All solutions were prepared by double distilled de-ionized water.

Preparation of Zn-free insulin: A 10 mg·mL^{-1} insulin solution was prepared by dissolving zinc human insulin in aqueous HCl solution (pH 3.0). Zinc ions and other trace metal ions were removed by extensive dialysis against 1 mmol/L HCl for 48 h at 4 ℃ using a dialysis bag with the exclusive limit of 3000 Dalton. The concentration of insulin monomer was determined by measuring the absorbance at 280 nm using a value of $\varepsilon_{280} = 5700$ L·mol^{-1}·cm^{-1}[8]. The zinc content of the samples was determined by atomic absorption spectrometry.

1.2 Effects of vanadium compounds on intrinsic fluorescence of insulin

The reaction solutions were prepared by adding varied amount of the solutions of each vanadium compound to insulin solutions to a final concentration of zinc-free insulin of 4.0×10^{-5} mol/L and the total volume of 3 mL. The solutions were allowed to react for 1 h at (20 ± 0.5) ℃ and (37 ± 0.5) ℃ respectively. The fluorescence spectra were recorded on a Shimadzu 540 fluorescence spectrophotometer. Intrinsic emission fluorescence of insulin was scanned in the range of 200 ~ 400 nm with $\lambda_{ex} = 280$ nm. The changes in fluorescence intensity at $\lambda_{em} = 310$ nm and 280 nm were measured in the presence and absence of vanadium compounds.

1.3 Circular dichroism (CD) spectroscopy of insulin

All samples were prepared in HEPES isotonic buffer solutions (NaCl 135 mmol·L^{-1}, HEPES 15 mmol·L^{-1}; pH 7.4) with the final concentration of zinc-free human insulin and vanadium compounds to be 4.0×10^{-5} mol·L^{-1} and 5.0×10^{-4} mol·L^{-1}, respectively. The mixture of vanadium compounds and insulin were allowed to react at (37 ± 0.5) ℃ for 1 h. CD spectra were then recorded on a Jasco J-720 spectropolarimeter using a quartz cell with a path length of 0.1 cm; other parameters used were bandwidth of 2.0 nm, scan rate of 50 nm·min^{-1}, time constant of 4 s, and sensitivity of 10 mdeg·cm^{-1}.

1.4 Fourier transformed infrared spectroscopy (FT-IR) of insulin

The mixtures of insulin (4.0×10^{-5} mol·L^{-1}) and the various vanadium compounds (5.0×10^{-4} mol·L^{-1}) in solutions (NaCl 135 mmol·L^{-1}, HEPES 15 mmol·L^{-1}; pH 7.4) were incubated at 37 ℃ for 1 h. Then, the samples were lyophilized and dissolved in D_2O and incubated at 4 ℃ for 24 h, and then lyophilized again. The samples were re-dissolved in D_2O to a final concentration of insulin of 0.2 mg·mL^{-1}. FT-IR spectra were recorded on a Bio-Rad FTS-65A FT-IR spectrometer. The spectra of insulin were obtained by subtracting the spectrum of water vapor recorded under identical conditions. The deconvoluted spectra between 1700 cm^{-1} and 1600 cm^{-1} were obtained using a Win-IR Curvefit program with Gaussian band profiles[9].

2 Results and discussions

2.1 Preparation of zinc-free insulin

The zinc-free insulin was prepared by the method described previously[10]. The zinc content was determined by atomic absorption spectrometry to be less than 2×10^{-4} mole per mole of insulin monomer.

2.2 Effects of vanadium compounds on the intrinsic fluorescence of insulin

The results in Fig. 1 show that all the three vanadium compounds can quench the intrinsic fluorescence of insulin. When excited at 280 nm, insulin monomer gives a fluorescent peak at 310 nm, which originates from four Tyr residues, A14, A19, B16 and B26[11], and a scattering peak at 280 nm. The intensity of the latter reflects the aggregation states of insulin in solution[12]. The monomeric insulin is the dominating species in the HEPES buffer solution (NaCl 135 mmol·L^{-1}, HEPES 15 mmol·L^{-1}; pH 7.4) at the concentration mentioned above, in spite of the presence of a trace amount of various aggregates[13,14]. When vanadium compounds were added, the intensity of the characteristic fluorescence peak of Tyr at 310 nm decreased and that originating from light scattering at 280 nm decreased correspondingly, which indicated the tendency towards disaggregation of insulin aggregates by vanadium compounds.

In general, fluorescence quenching falls into two categories: dynamic quenching and static quench-

ing[15]. It can be distinguished by their different temperature dependency and the nature of quenching can be determined on the basis of the Stern-Volmer equation:

$$F_0/F = 1 + K[Q],$$

where F_0 and F are the fluorescence intensities in the absence and presence of various concentrations of a fluorescence quencher. In the case of dynamic quenching, K is the Stern-Volmer dynamic quenching constant K_D, while in the case of static quenching, it is the static association constant K_S.

As shown in Fig. 2, the quenching ability of three vanadium compounds at 37 ℃ was lower than that at 20 ℃, especially for $VO(acac)_2$ and $NaVO_3$. This suggested that it is mainly a static quenching and implies the formation of complexes of vanadium compounds-insulin, since the increase in temperature will destabilize the complexes and thus lead to lower values of the static quenching constants. At lower concentration of vanadium compounds, the Stern-Volmer plots at both temperatures were linear and their apparent formation constants were obtained by the slope as shown in Table 1.

Table 1. The apparent association constants of vanadium compounds and insulin

T(℃)	$K_S(10^4\ mol^{-1}\cdot L)$		
	$NaVO_3$	$VO(acac)_2$	$VO(ma)_2$
20	0.17±0.01	2.8±0.2	4.0±0.1
37	0.11±0.01	1.2±0.3	3.7±0.3

Table 1 shows the apparent association constants calculated from the slope of the Stern-Volmer plots at the linear range of the concentrations. It is shown that $VO(ma)_2$ and $VO(acac)_2$ exhibit a much higher tendency to bind to insulin than $NaVO_3$. The order of the affinity is $VO(ma)_2 > VO(acac)_2 \gg NaVO_3$; this difference in binding affinity indicated that the ways of binding of the three vanadium compounds to insulin may be different. But at higher concentrations of vanadium compounds, the Stern-Volmer plots display upward curvature, indicating that vanadium compounds quench the intrinsic fluorescence of insulin by collisional encounters in addition to complex formation (Fig. 2).

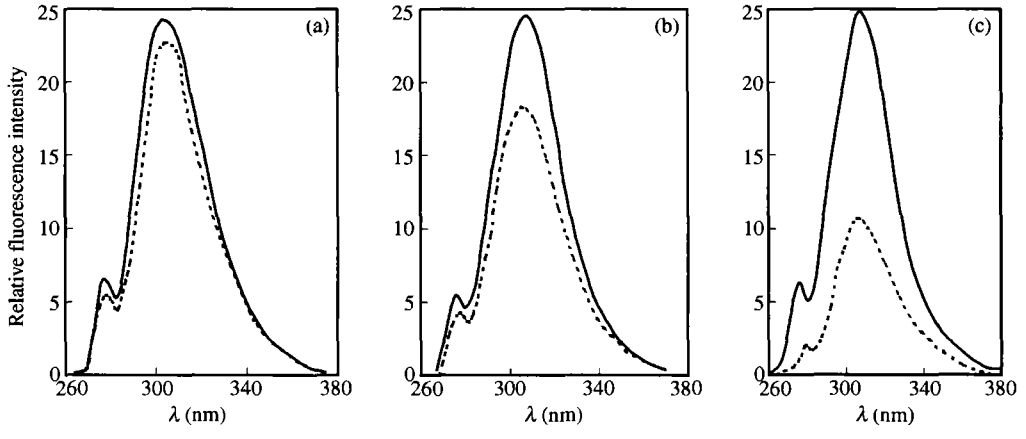


Fig. 1. Fluorescence spectra of native human insulin (4×10^{-5} mol·L^{-1}) in the presence of various concentrations of different vanadium compounds (4×10^{-5} mol·L^{-1}). Solid line, insulin; dashed line, insulin incubated with $NaVO_3$(a), $VO(acac)_2$(b), and $VO(ma)_2$(c).

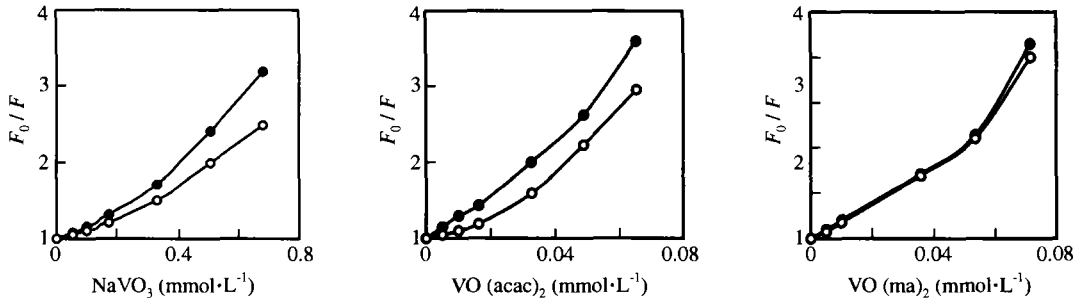


Fig. 2. Stern-Volmer plots of quenching of insulin fluorescence by different vanadium compounds at different temperatures (● 20 ℃; ○ 37 ℃).

Of the three vanadium compounds, the quenching action of $NaVO_3$ was extremely weak, while that of $VO(ma)_2$ and $VO(acac)_2$ are rather strong, with $VO(ma)_2 > VO(acac)_2$. The difference indicated that their binding mode might be different and the difference was evidently related to the species. Anyway they were common in inducing the disaggregation of insulin.

2.3 Effects of vanadium compounds on CD spectra of insulin

Fig. 3 shows the CD spectra of insulin upon incubation with different vanadium compounds. Three characteristic negative minima at 208, 222, and 273 nm in 200～300 nm region of the spectrum can be seen. The CD bands at 273 nm are assigned to disulfide bridge, Tyr-B26 and Phe-B24 or Phe-B25[16].

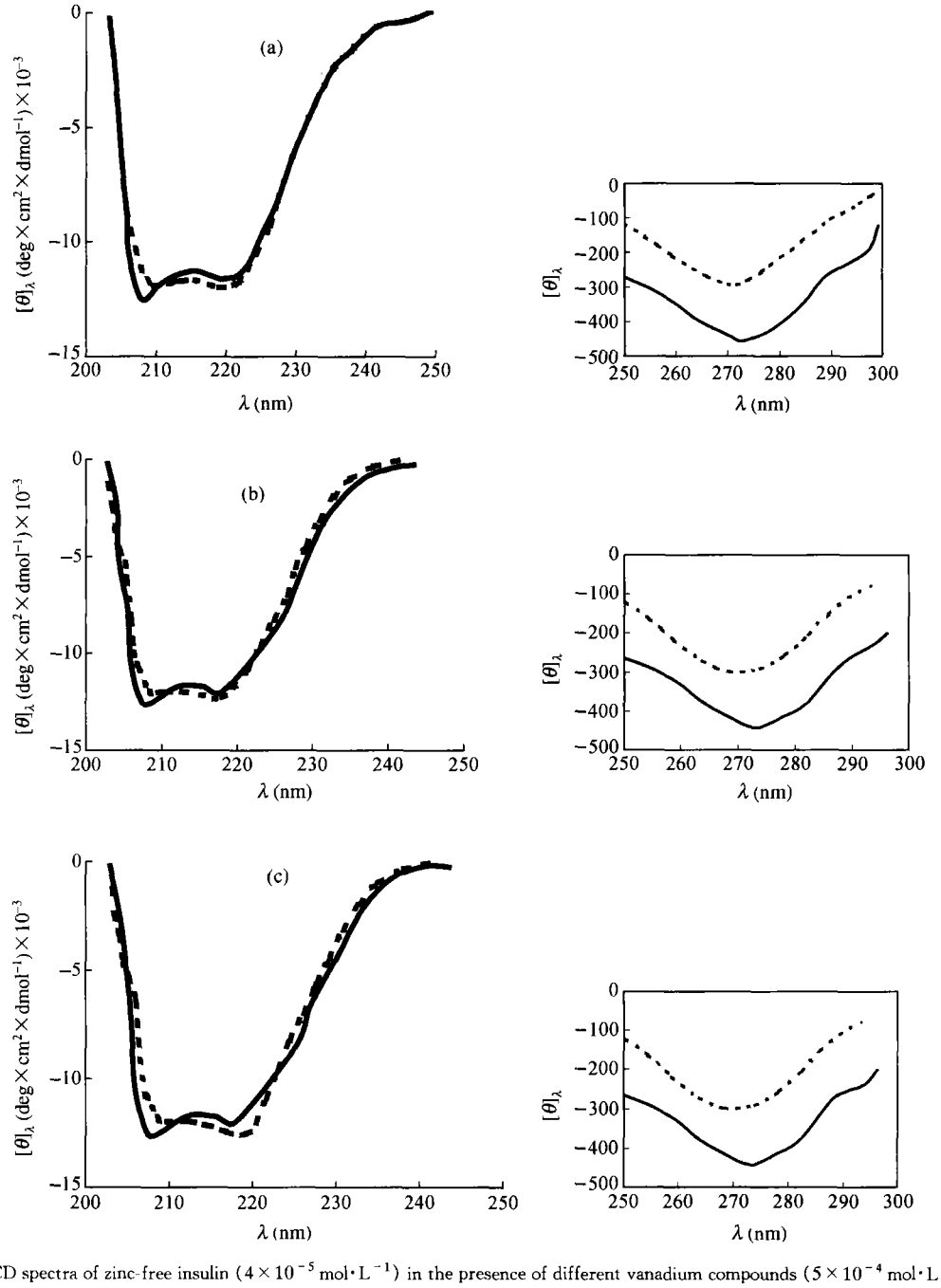


Fig. 3. CD spectra of zinc-free insulin (4×10^{-5} mol·L^{-1}) in the presence of different vanadium compounds (5×10^{-4} mol·L^{-1}). Solid line, insulin; dashed line, insulin incubated with $NaVO_3$(a), $VO(acac)_2$(b), and $VO(ma)_2$(c).

Attenuation of this band is correlated with conditions causing disaggregation; while strengthening of the band is associated with those enhancing aggregation[16]. A number of the metal ions that strengthen the band at 273 nm have been shown to be able to replace zinc ions in the formation of crystalline insulin[16]. The band at 222 nm can be assigned to β structure and the band at 208 nm may be attributed mainly to α-helix[17].

As shown in Fig. 3, the interaction of three vanadium compounds with zinc-free insulin resulted in the changes of all three bands, i.e. slight decrease in α-helix and increase in β structure. It is remarkable that the band at 273 nm is decreased, as described above, the attenuation of this band is associated with the disaggregation of insulin. Therefore it is conceivable that binding of the three vanadium compounds to insulin causes disaggregation of the insulin. Meanwhile Tyr-B26, Phe-B24 or Phe-B25 are located at the binding domain of insulin to its receptor, thus the vanadium compounds may change the conformation of the binding domain of the insulin and may perturb the binding of the insulin to its receptor.

2.4 Effects of vanadium compounds on FT-IR spectra of insulin

The influence of vanadium compounds on the components of secondary structure of insulin was studied by means of FT-IR spectroscopy. Fig. 4 presents FT-IR spectra of the deconvoluted and fitted individual component bands of amide I region of zinc-free insulin. The individual bands were assigned according to Ref. [18] and the percentage of each band was calculated (Table 2).

Table 2. Quantitative estimation of secondary structures of Zn^{2+}-free insulin (4×10^{-5} mol·L^{-1} of Zn^{2+}-free insulin and 5×10^{-4} mol·L^{-1} of vanadium compounds)

Structure	Position (cm^{-1})	Content (%)				Peptide segments
		Control	$NaVO_3$	$VO(acac)_2$	$VO(ma)_2$	
β-turn	1687	0.22	0.57			B(20~22)
	1681	2.70	4.25	3.77	4.99	B(7~8), A(18~20)
β-sheet	1671~1672	8.47	8.35	8.26	7.92	
	1631~1635	16.00	16.61	6.35	22.28	A(1~9)
	1620~1621		2.06	2.29	6.94	
	1628			12.86		
3_{10}-helix	1657~1659	37.79	18.11	32.23	11.99	A(12~17)
Extended chain	1611~1612		0.18	0.28	1.48	
	1665		12.60		9.49	B(1~6)
α-helix	1650~1653		16.80	12.60	11.27	B(9~19)
Random	1641~1642	33.24	20.47	21.36	11.27	A(10~11,21), B(27~30), B(1~3)

It is observed that under the action of $VO(acac)_2$, a new peak at 1628 cm^{-1} appeared; while the reaction with $NaVO_3$ and $VO(ma)_2$ caused the appearance of a new peak at 1665 cm^{-1}. The new peak appeared at 1650~1653 cm^{-1} is noteworthy, because it reflects the increase in α-helix content in B (9-19) region and indicates a change from an extended conformation (T state) to a helical conformation (R state)[19,20]. It is known that the change from T to R state is critical for insulin-receptor interaction[21]. The sequence of the influence exerted by vanadium compounds was as follows: $NaVO_3 > VO(ma)_2 > VO(acac)_2$. In the T→R transition, Tyr (B26) is likely more exposed to the solvent[21], which would result in the quenching of intrinsic fluorescence of insulin.

3 Discussion

As shown by the present results, the reaction of vanadium compounds with zinc-free insulin may induce the conformation change of insulin from T to R state. For the vanadium compounds we tested, $NaVO_3$ exerted stronger effect than $VO(ma)_2$ and $VO(acac)_2$. On the other hand, the apparent association constants obtained from the quenching constants of vanadium compounds to the intrinsic fluorescence of insulin showed that the binding of $NaVO_3$ to insulin was weaker than $VO(ma)_2$ and $VO(acac)_2$. The reason for this difference might be that the apparent association constants obtained reflect only the overall conformation change due to the interaction between the vanadium compounds and proteins, whereas the T→R conformation transition reflects the specific local

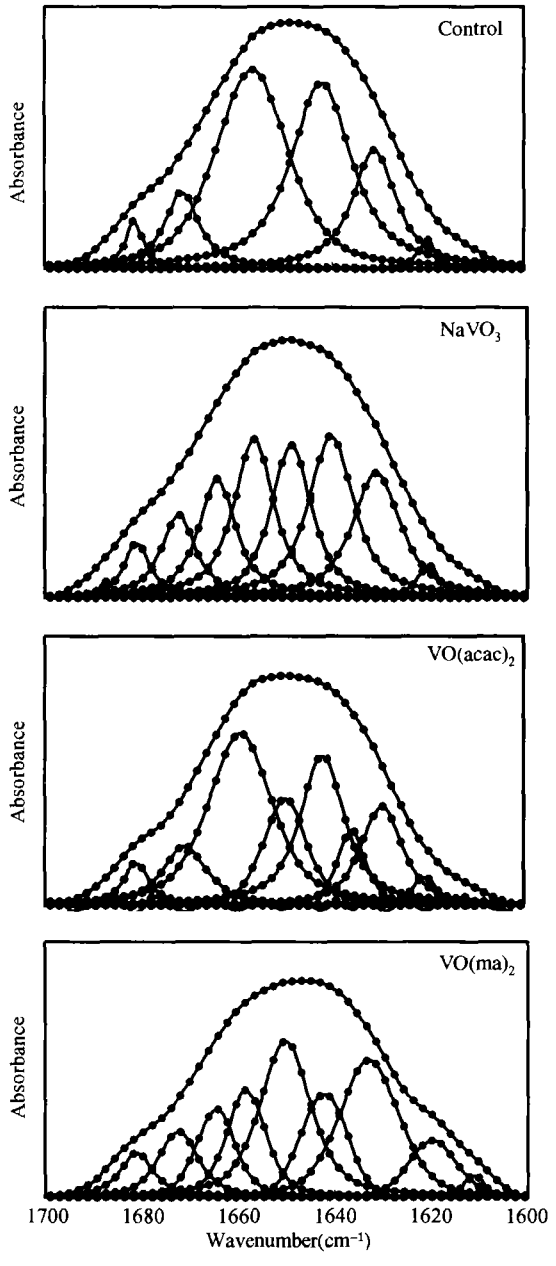


Fig. 4. FT-IR spectra of amide I region of zinc-free insulin (4×10^{-5} mol·L^{-1}) in the presence or absence of vanadium compounds (5×10^{-4} mol·L^{-1}) and the fitted individual component bands.

action on the secondary structure. It also showed that the binding mode of $NaVO_3$ may be different from that of $VO(ma)_2$ and $VO(acac)_2$.

Considering that these two vanadyl complexes induced the disaggregation of zinc-free-insulin, they were unlikely to bind with insulin as VO^{2+} and even more not to the Zn^{2+} binding sites, because it is known that Zn^{2+} binding will enhance the insulin aggregation[16]. However, the binding of the oxidation products of vanadium compounds could not be excluded. As we know, under the experimental conditions, $NaVO_3$ mainly exists in the form of $H_2VO_4^-$ including a small quantity of HVO_4^{2-} [22].

$VO(ma)_2$ can be oxidized slowly to $[VO_2(ma)_2]^-$[23]. $VO(acac)_2$ has higher redox stability in solution and its major species of hydrolysis is a positive-charged 1:1 complex[24](Fig. 5). and $NaVO_3$ is formed by oxidation[24]. It was reported that the anionic species formed from the oxidition of $VO(ma)_2$ and $VO(acac)_2$ could enter the human erythrocytes *via* anion channel, and furthermore, $VO(ma)_2$ is more sensitive to oxidation than $VO(acac)_2$ [25]. In addition, human insulin, as an allosteric protein, can interact with two classes of ligands: cyclic organic molecules (such as resorcinol, phenol, cyclohexanol, etc.)[26,27] and monovalent anions (such as SCN^-, N_3^-, I^- and Cl^-)[28,29]. Among the three vanadium complexes studied, $VO(ma)_2$ and $VO(acac)_2$ are of cyclic structures (Fig. 6), whereas $H_2VO_4^-$, $NaVO_3$ and $[VO_2(ma)_2]^-$ are single-charged anions. These two different features might account for the different influence of vanadium compounds on the conformation of insulin and their different binding modes. As to $VO(ma)_2$, it is not only of cyclic structure but its oxidation products are single-charged anions. Thus it exhibits stronger binding affinities and conformation perturbation to insulin.

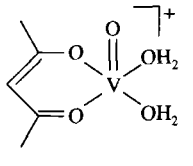
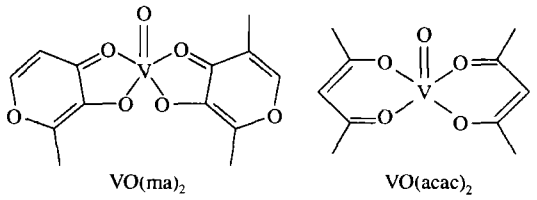


Fig. 5. 1:1 complex formed in solution of $VO(acac)_2$ [24].



$VO(ma)_2$ $VO(acac)_2$

Fig. 6. Structures of $VO(ma)_2$ and $VO(acac)_2$.

The binding site of insulin to its receptor is considered to be a large hydrophobic domain involving Gly(A1), Glu(A5), Tyr(A19), Asn(A21) of A chains and Val(B12), Tyr(B16), Phe(B24), Phe(B25), Tyr(B26) of B chains[30]. The vanadium binding induced static quenching of the intrinsic fluorescence of insulin revealed the reduction of intensity

of scattering peak at 280 nm, which reflects the aggregation states of insulin in solution[12] along with that of the peak at 310 nm. These changes indicate that under the action of vanadium compounds, the aggregates of insulin tend to disaggregate. In the CD spectra, the attenuation of the band at 273 nm of insulin is also related to disaggregation, since this band can be assigned to disulfide bridge, Tyr-B26 and Phe-B24 or Phe-B25[16]. Meanwhile, Tyr-B26 and Phe-B24 or Phe-B25 is just located at the binding domain of insulin to its receptor. Thus the binding of these vanadium compounds may perturb the conformation of receptor-binding domain of insulin. In the FT-IR spectra of insulin, after the addition of vanadium compounds a new band at 1650 ~ 1653 cm^{-1} appeared, which indicates the formation of an α-helix structure at B (9-19) motif, which revealed the perturbation to the binding site and a transition from extended conformation (T state) to a helical conformation (R state)[19,20], and this transition might be essential for insulin binding to its receptor[21].

In conclusion, binding of vanadium compounds results in disaggregation and conformational changes of insulin, which might account for the vanadium-enhanced binding affinity for insulin to its receptor, and this may be related to the glucose-lowering effect of vanadium compounds.

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References

1 Shechter, Y. et al. Insulin-like stimulation of glucose oxidation in rat adipocytes by vanadyl (IV) ions. Nature, 1980, 284(5756): 556.
2 Shechter, Y. et al. Vanadium salts and the future treatment of diabetes. Endeavour, 1993, 17(1): 27.
3 Posner, B. I. et al. Peroxovanadium compounds. A new class of potent phosphotyrosine phosphatase inhibitors which are insulin mimetics. J Biol Chem, 1994, 269(6): 4596.
4 Fantus, I. G. et al. Multifunctional actions of vanadium compounds on insulin signaling pathways: evidence for preferential enhancement of metabolic versus mitogenic effects. Mol Cell Biochem, 1998, 182(1-2): 109.
5 Thompson, K. H. et al. Vanadium compounds as insulin mimics. Chem Rev, 1999, 99(9): 2561.
6 Reul, B. A. et al. Effects of vanadium complexes with organic ligands on glucose metabolism: a comparison study in diabetic rats. Br J Pharmacol, 1999, 126(2): 467.
7 Kwong, D. W. et al. Modulatory effects of peroxovanadates on insulin receptor binding. J Inorg Biochem, 1996, 64(3): 163.
8 Frank, B. H. et al. Physical studies on proinsulin-association behavior and conformation in solution. Biochem Biophys Res Commun, 1968, 32(2): 155.
9 Pullen, R. A. et al. Receptor-binding region of insulin. Nature, 1976, 259(5542): 369.
10 Palmieri, R. et al. ^1H Fourier transform NMR studies of insulin: coordination of Ca^{2+} to the Glu(B13) site drives hexamer assembly and induces a conformation change. Biochemistry, 1988, 27(9): 3387.
11 Holladay, L. A. et al. Conformational stability and self-association of zinc-free bovine insulin at neutral pH. Biochim Biophys Acta, 1977, 494(1): 245.
12 Brand, J. G. et al. Fluorescence characteristics of native and denatured monellin. Biochim Biophys Acta, 1977, 493(1): 178.
13 Hogue, C. W. et al. Comparison of terbium (III) luminescence enhancement in mutants of EF hand calcium binding proteins. J Biol Chem, 1992, 267(19): 13340.
14 Kadima, W. et al. The influence of ionic strength and pH on the aggregation properties of zinc-free insulin studied by static and dynamic laser light scattering. Biopolymers, 1993, 33(11): 1643.
15 Eftink, M. R. et al. Exposure of tryptophanyl residues in proteins. Quantitative determination by fluorescence quenching studies. Biochemistry, 1976, 15(3): 672.
16 Goldman, J. et al. Zinc binding, circular dichroism, and equilibrium sedimentation studies on insulin (bovine) and several of its derivatives. Biochemistry, 1974, 13(22): 4566.
17 Ettinger, M. J. et al. Optical activity of insulin. I. On the nature of the circular dichroism bands. Biochemistry, 1971, 10(5): 824.
18 Wei, J. A. et al. FT-IR studies of secondary structures of bovine insulin and its derivatives. Biochim Biophys Acta, 1991, 1080(1): 29.
19 Smith, G. D. et al. Structure of a rhombohedral R6 insulin/phenol complex. Proteins, 1992, 14(3): 401.
20 Smith, G. D. et al. The structure of a complex of hexameric insulin and 4′-hydroxyacetanilide. Proc Natl Acad Sci U S A, 1994, 91(19): 8851.
21 Pittman, I. T. et al. A spectroscopic investigation of the conformational dynamics of insulin in solution. Biochemistry, 1995, 34 (33): 10578.
22 Crans, D. C. et al. Vanadium chemistry and biochemistry of relevance for use of vanadium compounds as antidiabetic agents. Mol Cell Biochem, 1995, 153(1-2): 17.
23 Sun, Y. et al. Oxidation kinetics of the potent insulin mimetic agent bis(maltolato)oxovanadium(IV) (BMOV) in water and in methanol. Inorg Chem, 1996, 35(6): 1667.
24 Crans, D. C. Chemistry and insulin-like properties of vanadium (IV) and vanadium(V) compounds. J Inorg Biochem, 2000, 80 (1-2): 123.
25 Yang, X. G. et al. in Abstracts of Papers Presented at the Third International Symposium on Chemistry and Biological Chemistry of Vanadium, Nov., 2001, Osaka, Japan, I-04.
26 Kim, Y. et al. pH dependent conformational changes in the T- and R-states of insulin in solution: circular dichroic studies in the pH range of 6 to 10. Biochem Biophys Res Commun, 1992, 186(2): 1115.
27 Ciszak, E. et al. Crystallographic evidence for dual coordination around zinc in the T_3R_3 human insulin hexamer. Biochemistry, 1994, 33(6): 1512.
28 Brzovic, P. S. et al. Structural asymmetry and half-site reactivity in the T to R allosteric transition of the insulin hexamer. Biochemistry, 1994, 33(44): 13057.
29 Choi, W. E. et al. The allosteric transition of the insulin hexamer is modulated by homotropic and heterotropic interactions. Biochemistry, 1993, 32(43): 11638.
30 Gammeltoft, S. Insulin receptors: binding kinetics and structure-function relationship of insulin. Physiol Rev, 1984, 64(4): 1321.