Specific Binding of Vanadyl Ion (VO^{2+}) with Thiolate of the Cysteine-34 Residue in Serum Albumin, Demonstrated by CD Spectroscopy and Kinetic Property

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Binding feature of the vanadyl ion (VO^{2+}) with bovine serum albumin (BSA) was studied with CD spectrometry based on comparisons with that of Cu^{2+}. The strong positive CD band at 590 nm in the VO^{2+}–albumin system was assigned to the binding of VO^{2+} and thiolate of Cys-34 in BSA.

Increasing worldwide interest in the insulin mimetic action of vanadium ions and their complexes[1] has led to further investigation of their chemical features in living systems to better understand their pharmacological activities. Recently, oral administration of vanadyl sulfate ($VOSO_4$) in human type 2 (non-insulin-dependent) diabetic subjects has revealed that the improvement of diabetes mellitus in terms of both blood glucose and hemoglobin A_{1c} levels positively depends on the total vanadium concentrations in the blood.[2] We have analyzed the metallokinetics of vanadyl species (VO^{2+}) in the blood of live rats given $VOSO_4$[3] and vanadyl complexes[4] intravenously by the blood-circulation monitoring-electron paramagnetic resonance (BCM-EPR) method. During the study, we proposed that vanadyl ion bound with albumin because the EPR signal of $VOSO_4$ in bovine serum albumin (BSA) was fairly similar to that in flesh blood of rats.[3] In contrast, on the basis of EPR and chemical speciation studies vanadyl ion was proposed to bind transferrin rather than albumin.[5,6] However, the binding nature of VO^{2+} in the blood has not yet been precisely determined.[6] Although EPR is known to be useful for analysis of paramagnetic VO^{2+} in living systems,[7] no detailed binding features with biomolecules such as proteins and enzymes have been revealed. We therefore have fundamentally studied the binding nature of $VOSO_4$ with serum albumins, which consist 50–60% of the serum proteins, by means of other useful spectroscopy than EPR. Using the CD spectral method, we report herein that VO^{2+} exclusively binds with cysteine-34 (Cys-34) in albumins, but not with the N-terminal histidine (His)-containing tripeptide moiety.

The purity of $VOSO_4$ and $CuCl_2$ (Nacalai Tesque, Kyoto, Japan) was determined by complexometry with EDTA. Bovine (BSA) and porcine (PSA) serum albumins (both fraction V, Sigma, St. Luise, MO, USA) were defatilized as reported.[8] The SH group of Cys-34 in BSA was modified with iodoacetamide (Wako Pure Chemicals, Osaka, Japan), and the SH-masked protein was purified by dialysis as reported,[9] in which the SH content was determined with 5,5-dithiobis-2-nitrobenzoic acid (Wako Pure Chemicals).[10] Gly-Gly-His was the product of Peptide Institute Inc. (Osaka, Japan). CD and UV–vis absorption spectra were measured with JASCO J-720 (Tokyo, Japan) and Shimadzu Multispec-1500 (Kyoto, Japan) spectrometers.

When 0.5 mM BSA was titrated with 0–1.5 mM VO^{2+} in 0.1 M $NaClO_4$ adjusted to pH 7.4 at 37 °C, a positive CD spectral maximum at 590 nm developed (Figure 1a), the spectra at pH 4.0–10.0 being stable. The Vis spectral maxima of 0.5 mM VO^{2+}–BSA were observed at 580 and 750 nm. In contrast, titration of BSA with Cu^{2+} gave completely different CD spectra under the same conditions (Figure 1b). In the Cu^{2+}–BSA system, monitoring of positive and negative CD spectral intensities at 474 and 570 nm, respectively, exhibited 1:1 complex formation in Cu^{2+}:BSA, indicating that Cu^{2+} binds with the N-terminal Asp-Thr-His moiety of BSA, as indicated previously.[11] A spectral intensity at 690 nm developed when Cu^{2+} was added above equimole of BSA, suggesting the presence of a second binding site of Cu^{2+} in BSA, as previously proposed by CD spectra.[12] The Vis spectral maxima of 0.5 mM Cu^{2+}–BSA was observed at 510 and 670 nm.

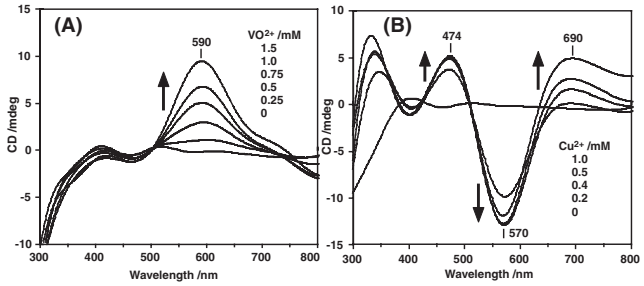


Figure 1. CD spectral titrations of 0.5 mM BSA with $VOSO_4$ (A) and $CuCl_2$ (B) in 0.1 M $NaClO_4$ at pH 7.4 and 37 °C. Final concentrations of added each metal ion are indicated in the figures.

To analyze the VO^{2+} binding site in BSA, the VO^{2+}–BSA complex was titrated with Cu^{2+}. Increasing Cu^{2+} concentrations induced the spectral pattern of Cu^{2+}–BSA with positive and negative CD spectral maxima from the original VO^{2+}-binding form (Figure 2), however, no spectral maximum at 690 nm due to the second binding site of Cu^{2+} in BSA was observed, suggesting the stronger binding of VO^{2+} than Cu^{2+} at the second Cu^{2+}

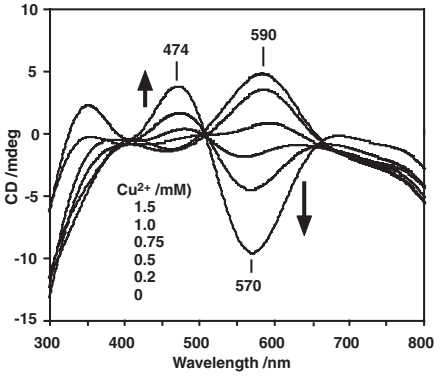


Figure 2. CD spectral titrations of 0.5 mM BSA-0.75 mM VO^{2+} system with $CuCl_2$ in 0.1 M $NaClO_4$ at pH 7.4 and 37 °C. Final concentrations of added Cu^{2+} are indicated in the figure.

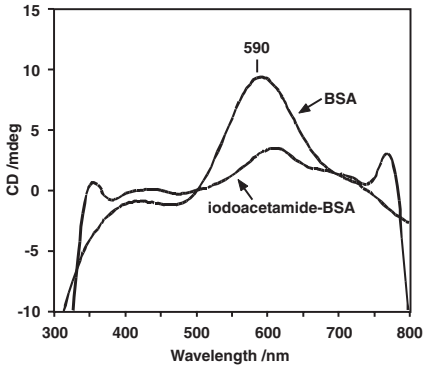


Figure 3. CD spectra of 0.5 mM BSA or 0.5 mM iodoacetamide-BSA by addition of 1.5 mM $VOSO_4$ in 0.1 M $NaClO_4$ at pH 7.4 and 37 °C.

binding site.

To determine the VO^{2+} binding site in BSA, we used PSA, which lacks His at the N-terminal. Titration of PSA with VO^{2+} led to development of a positive band at 590 nm similar to that of the VO^{2+}–BSA system, indicating that VO^{2+} does not bind with the N-terminal moiety. In fact, no CD spectral change due to complex formation of VO^{2+} with Gly-Gly-His was observed. Titration of PSA with Cu^{2+} led to development of a positive absorption band at 690 nm, as observed in the Cu^{2+}–BSA system, suggesting the presence of a second Cu^{2+} binding site in PSA. In addition, we measured the CD spectrum of a system containing VO^{2+} and iodoacetamide-modified BSA, which is a partially thiolate-masked form, and found that the absorption intensity at 590 nm due to VO^{2+}–BSA binding was clearly lowered (Figure 3). The thiol contents of VO^{2+}–, Cu^{2+}–, and iodoacetamide–BSA were 63%, 85%, and 9% of the intact BSA when the metal ions or iodoacetamide were added at equimolar concentrations to BSA.

Based on the results, we calculated the VO^{2+} and Cu^{2+} binding constants (K) and maximal binding numbers (n) in BSA, the theoretical equation where the concentration of metal–BSA complex was derived from the binding equilibrium being fitted to the positive CD spectral intensities at 590 nm due to VO^{2+}–BSA and those at 474 and 690 nm due to Cu^{2+}–BSA.[12,13] $LogK_1$ and n_1 of Cu^{2+}–BSA were determined to be 6.32 and 1.0, respectively, as previously reported ($\log K_1 = 6.20$, $n_1 = 1.0$),[12] and $\log K_2$ and n_2 were to be 4.44 and 2.0, respectively. While, $\log K$ and n of VO^{2+}–BSA were estimated to be 5.15 and 3.0,

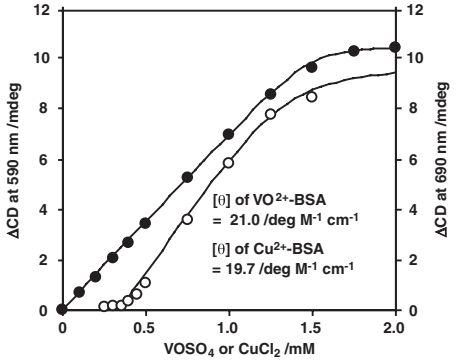
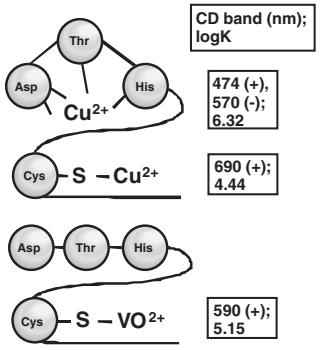


Figure 4. CD spectral titration cuves of BSA (0.5 mM)-VO^{2+} system (●) and BSA (0.5 mM)-Cu^{2+} system (○) in 0.1 M $NaClO_4$ at pH 7.4 and 37 °C. The lines represent the theoretical curves fitted to the data. The values of molar ellipticity ([θ]) for each complex are indicated in the figure.



Scheme 1. Possible reactions of BSA with Cu^{2+} and VO^{2+}, assigned CD spectral bands, and estimated binding constants.

respectively (Figure 4). These results indicated that VO^{2+} binds stronger than Cu^{2+} to the second Cu^{2+} binding site in BSA, as depicted in Scheme 1.

The binding of VO^{2+}–thiolate has previously been observed in simple complexes such as bis(methylcysteinato)-, bis(dithiocarbamato)-, and bis(1-oxypyridine-2-thiolato)-vanadyl.[15] The present result regarding the VO^{2+}–thiolate complex formation in BSA is the first finding in a biochemical system.[16] These new results will be important for the analysis of not only the antidiabetic action of vanadyl complexes that exhibit blood glucose-lowering effects in streptozotocin-induced type 1 diabetic rats,[1] but also the structure of vanadyl states in the blood of live rats, as observed in the BCM-EPR.[3,4]

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13 Binding parameters were obtained as follows. Equilibrium binding model [(M-BSA) = K·(M)·(BSA)] was fitted to each profile of the CD spectral intensities using nonlinear least squares regression program, MULTI,[14] where (M-BSA), (M), and (BSA) are the concentrations of metal ion–BSA complex, free metal ion, and free BSA, respectively. The (M) and (BSA) were defined by the following equations: $(M) = 0.5\cdot[-(1/K + n\cdot(BSA)_0 - (M)_0) + \{(1/K + n\cdot(BSA)_0 - (M)_0)^2 + 4/K\cdot(M)_0\}^{1/2}]$, $(BSA) = 0.5/n\cdot[-(1/K - n\cdot(BSA)_0 + (M)_0) + \{(1/K - n\cdot(BSA)_0 + (M)_0)^2 + 4\cdot n/K\cdot(BSA)_0\}^{1/2}]$, and $(M)_0$ and $(BSA)_0$ are the initial concentrations of metal ion and BSA, respectively.
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