^{51}V NMR as a Probe of Metal Ion Binding in Metalloproteins

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Vanadium is widely recognized as a biologically important element.[1] The recent discovery of the first two naturally occurring vanadium-proteins, a V(V)-bromoperoxidase[2] and a V-nitrogenase,[3] has focused our need to learn more about vanadium-protein interactions, particularly involving pentavalent vanadium. An attractive method to characterize metal binding sites of metalloproteins is NMR spectroscopy of the bound metal ion which can serve as a highly selective probe, giving information about site symmetries, the nature of the coordinated ligands, exchange kinetics, and the motional properties of the protein.[4-6] Unlike most other biologically important isotopes, the NMR receptivity of the ^{51}V nucleus is exceptionally high due to a large magnetic moment, high natural abundance, (99.76%), and rapid quadrupolar relaxation in solution. The ^{51}V NMR chemical shifts are very sensitive to changes in the nature of the ligands,[7-10] thereby providing an excellent diagnostic tool for detailed investigations of vanadium(V) coordination environments. In the present paper, we report the first ^{51}V NMR study of a V(V)-protein complex: $V(V)_2$-human transferrin. Human transferrin is a glycoprotein that functions as an iron transport protein, although it may also be involved in the transport of other metal ions, including vanadium.[11] The protein is a single polypeptide chain with two homologous regions, each of which binds one metal atom.[12] The degree of similarity between the two metal binding sites is not known and is a topic of much current interest.[6,13-16] While the sequence homology of both halves of transferrin suggests that the binding ligands could be identical, the pH dependence difference for iron binding[16] and the ESR spectra of Fe- and VO^{2+} derivatives[17,18] as well as the ^{205}Tl NMR spectrum of Tl_2-transferrin,[6] suggest that the two sites are not equivalent.

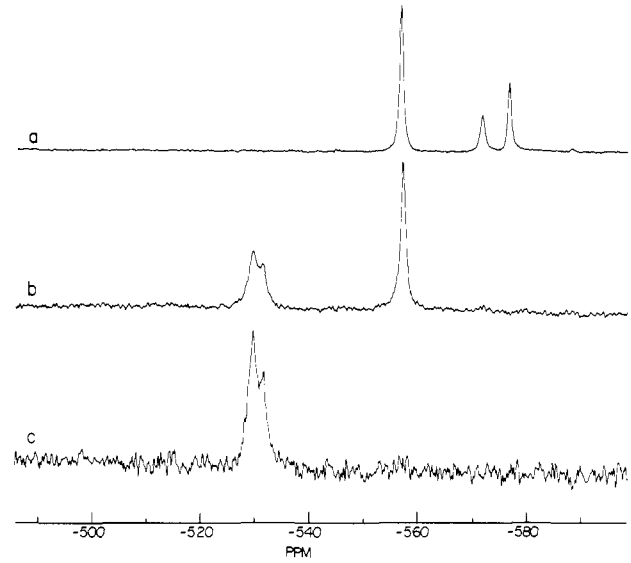

Figure 1. ^{51}V NMR evidence of binding of vanadate to apo-transferrin: (a) 0.826 mM NH_4VO_3 dissolved in 0.1 M HEPES buffer, pH 7.5; 26 342 scans. (b) 2.3:1 M/M mixture of V(V) and apo-transferrin at 0.826 mM in 0.1 M HEPES buffer, pH 7.5; 205 159 scans. (c) Equimolar mixture of (V) and apo-transferrin at 0.340 mM in 0.1 M HEPES buffer, pH 7.5; 200 010 scans.

Figure 1 shows the ^{51}V NMR spectrum of a solution of NH_4VO_3 in HEPES buffer in the presence and absence of transferrin.[19] The spectrum of an aqueous vanadate solution (Figure 1a) agrees with the previously reported spectra.[9,10] Upon addition of 1 equiv of transferrin, two new overlapping peaks at -529.5 and -531.5 ppm are observed, with a total line width of 420 Hz (Figure 1c). The signal areas of these resonances increase linearly with incremental addition of vanadate up to a vanadate/protein molar ratio of 2:1 at which point an additional peak at -557 ppm appears, indicating free vanadate. The areas of the protein-bound vanadate peaks also decrease linearly with incremental addition of iron. Furthermore, the addition of Ga^{3+} leads to rapid displacement of bound vanadium, Line widths and chemical shifts are independent of concentration (range 10^{-4} to 10^{-3} M), pH (5-9), nature of the buffer solution, and presence of excess free vanadate (Figure 1b). On this basis we assign these resonances to protein-bound vanadium which is in the limit of slow metal ion exchange on the NMR time scale (2.5×10^{-4} s).[20]

The absolute intensity of the vanadium-transferrin peak pattern observed in our experiments is about 15% of that observed for an equimolar solution of free vanadate, This indicates that the protein-bound vanadium is in the slow-motion limit ($\omega\tau_c >> 1$). It is well-known that spin-spin relaxation of half-integer quadrupolar nuclei is multiexponential, giving rise to strong deviations from Lorentzian line shapes in the slow-motion regime.[21] For ^{51}V ($I = 7/2$) a sum of four exponentials is predicted,[22] but in the limit of very long correlation times only a single component (corresponding to the $+^1/_2 \rightarrow -^1/_2$ transition) may be observable.

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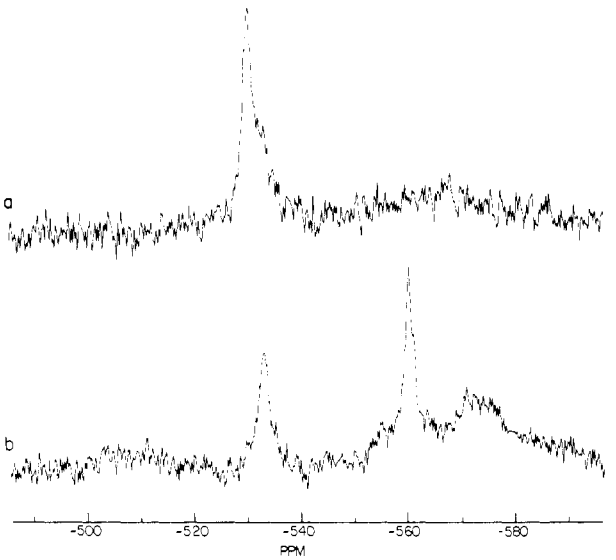

Figure 2. ^{51}V NMR evidence of selective binding of vanadium and iron: (a) Equimolar mixture of V(V) and apo-transferrin at 0.54 mM in 0.1 M HEPES buffer, pH 5.8; 87 285 scans. (b) Equimolar mixture of V(V) and Fe_C-transferrin at 0.5 mM in 0.1 M HEPES buffer, pH 5.8; 228, 590 scans. Fe_C-transferrin was prepared and identified (urea–polyacrylamide gel electrophoresis) according to published procedures.[24]

Thus we interpret the experimentally observed intensity loss as being due to extremely rapid relaxation of the other components which broadens their signal contribution beyond detectability. The remaining intensity of 15% is close to the theoretically predicted value (19%).

While the observation of two peaks with approximately equal intensity for the bound vanadate agrees well with the qualitative result obtained by UV–vis spectroscopy,[23a] the ^{51}V NMR technique is capable of distinguishing these sites according to their different chemical shifts, hence demonstrating their chemical inequivalence. Titrations carried out at pH 7.5 in 0.1 M HEPES buffer for vanadate/protein ratios between 0.1 and 2.3 confirm the large complex formation constants published previously[23] and indicate indiscriminate binding to the C- and N-terminal sites. However, Figure 2a shows that if vanadate solution is added to a HEPES buffer solution at pH 5.8 of transferrin loaded with ferric iron in the C-terminal site, no site scrambling occurs and only a single peak at −532 ppm is observed (in addition to absorptions due to free vanadate). Likewise, we have selectively observed the −529.5 ppm peak when adding vanadate solution to a HEPES buffer solution at pH 7.5 of transferrin loaded with ferric iron in the N-terminal site.[25] Thus we assign the peaks at −529.5 and −531.5 ppm to V(V) bound to the C- and N-terminal sites, respectively. In conclusion we have shown that ^{51}V NMR is a highly sensitive tool to monitor binding of vanadate to apo-human transferrin and could be a widely applicable technique to characterize V(V) binding to metalloproteins in general. Such studies as well as investigations of small molecular complexes that model protein binding sites are currently in progress.

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Registry No. Vanadium, 7440-62-2; vanadate, 14333-18-7.

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Synthesis of Chiral Molecular Clefts. New Armatures for Biomimetic Systems

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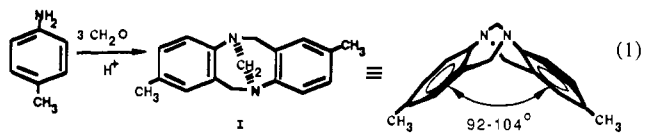
Natural receptors are often described as clefts, grooves, or depressions on protein, membrane, or polynucleotide surfaces. The essential characteristic of a small molecule receptor is *concavity*.[1] The great majority of synthetic receptors have used macrocyclic rings to enforce the formation of concave surfaces.[2,3] We report here the first syntheses of nonmacrocyclic chiral molecules which contain a deep cleft or groove large enough to bind small organic molecules. The rigid chiral grooves are maintained by conformational constraints intrinsic to the architecture of these synthetic receptors. The concave surfaces in these new molecules are produced without recourse to macrocyclic structural elements.

Analogues of Tröger's base [2,8-dimethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocine, I] are useful chiral structural elements for biomimetic systems.[4] Such molecules are readily prepared from aniline derivatives and formaldehyde (eq 1) and



(1)

have a folded structure wherein the three methylene carbons and two nitrogen atoms form a hingelike bridge between the two aryl units. Tröger's base analogues have been incorporated into a macrocyclic ring to provide chiral, water-soluble cyclophanes which act as receptors for benzenoid substrates.[5b] All analogues of Tröger's base examined have been found to be sharply folded. The angle formed by the least-squares planes containing the two aryl rings varies depending on the ring substituents and ranges from 92° to 104°.[5] We reasoned that two cup-shaped molecules joined in this manner would form a substantial concave surface and define an interesting new binding site.

To test this idea the well-known adduct **1**, available in quantitative yield from anthracene and dimethyl acetylenedicarboxylate, was treated with nitric acid in CH_3NO_2 to afford the mononitrate **2** in 76% yield.[6,7] This nitro compound was quantitatively reduced

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