MAGNETIC RESONANCE IN CHEMISTRY
Magn. Reson. Chem. 2004; **42**: 745–749
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mrc.1419

MRC

A vanadium-51 NMR study of the binding of vanadate and peroxovanadate to proteins†

Dieter Rehder,[1]* Marian Časný[1] and Reiner Grosse[2]

[1] Institut für Anorganische und Angewandte Chemie, Universität Hamburg, 20146 Hamburg, Germany
[2] Institut für Physikalische Chemie II der Universität Dortmund, 44227 Dortmund, Germany

Received 10 February 2004; Revised 17 March 2004; Accepted 19 March 2004

^{51}V quadrupolar central transition NMR spectra of buffered (pH 7.6–8.0) solutions of bovine apo-transferrin (Tf) and bovine prostatic acid phosphatase (Pp) treated with vanadate show normal features (chemical shifts between −515 and −542 ppm) corresponding to the complexation of VO_2^+ to the Tf binding site and the Pp active centre, respectively. Addition of H_2O_2 leads to the temporary formation of complexed $VO(O_2)^+$ ($\delta \approx -595$). Vanadate-dependent bromoperoxidase from the alga *Ascophyllum nodosum* exhibits an unusually high shielding both for the native ($\delta = -931$) and the peroxo form ($\delta = -1135$) of the enzyme. A resonance at −471 ppm is traced back to an inactive form with oxovanadium(V) in a trigonal-bipyramidal array.

KEYWORDS: ^{51}V NMR; vanadate-dependent peroxidase; transferrin; acid phosphatase

INTRODUCTION

Vanadate has been recognized as the active centre of peroxidases in marine algae such as *Corallina officinalis*[1] and *Ascophyllum nodosum*[2] and the fungus *Curvularia inaequalis*.[3] These enzymes catalyse the oxidation of halide to hypohalous acid and of (prochiral) sulfides to (chiral) sulfoxides by peroxide [Eqns (1) and (2)].[4,5] Vanadate is covalently attached to the protein via a histidine and a tight hydrogen bonding network involving water and amino acid side-chains.

$$Hal^- + H_2O_2 + H^+ \longrightarrow HalOH + H_2O \quad (1)$$

$$RSR' + H_2O_2 \longrightarrow RS(O)R' + H_2O \quad (2)$$

In the native form of the enzyme, vanadium is in a trigonal-bipyramidal array. The coordination geometry changes to tetragonal-pyramidal in the peroxo form[6] (Fig. 1, **1** and **2**). In a previous paper,[7] we reported on unusually high ^{51}V shielding in the native form of the *A. nodosum* bromoperoxidase, obtained on a Varian DP60 wide-line instrument. In the present study, carried out with aqueous solutions of the peroxidase on a Bruker MSL 400 wide-line spectrometer under static conditions, these features were confirmed, and the investigations were extended to studies of the enzyme treated with H_2O_2.

†Dedicated to Professor Wolfgang von Philipsborn on the occasion of his 75th birthday.

*Correspondence to: Dieter Rehder, Institut für Anorganische und Angewandte Chemie, Universität Hamburg, 20146 Hamburg, Germany. E-mail: dieter.rehder@chemie.uni-hamburg.de

Contract/grant sponsor: Deutsche Forschungsgemeinschaft;
Contract/grant numbers: RE 431/13-4; RE 431/18-1.
Contract/grant sponsor: COST; Contract/grant number: D12-0027-99.

More recently, striking similarities of tertiary structure motifs of vanadate-dependent peroxidases and vanadate-inhibited phosphatases have been noted.[9] These similarities extend to close homologies between the active centres of the peroxidases and vanadate-inhibited phosphatases, structural features at the vanadate sites (Fig. 1, **3**) and even to the functions of these proteins.[8] We therefore incorporated a specific acid phosphatase in our investigations. In addition, vanadate–transferrin interactions are included. Transferrin is an effective transport protein for vanadate, which is bound to the metal binding sites as the VO_2^+ cation,[10] and ^{51}V NMR characteristics of the peroxide-free forms have been reported previously.[11,12] A few other ^{51}V NMR investigations on vanadate–protein interactions are available, such as vanadate binding to ribonucleases[13] and copper–zinc–superoxidedismutase,[14] and will be considered in discussing our data.

The ^{51}V nucleus (nuclear spin = 7/2) is a sensitive NMR probe (receptivity relative to ^{1}H = 0.38) with a suitably small quadrupole moment (-0.05×10^{-28} m^2). In the present systems, where vanadium is bound to the proteins, the situation is far from extreme narrowing conditions, but within the motional narrowing limit, i.e. only the central transition with its favourable relaxation time in the millisecond regime is observed.[11,12]

EXPERIMENTAL

Analytical-grade KVO_3 and buffers (HEPES, Tris) were obtained from Fluka and bovine prostatic acid phosphatase (Pp, 10 units per g solid) and bovine apo-transferin (Tf) from Sigma. The proteins were dissolved in aqueous HEPES buffer, pH 7.6, treated with vanadate solution and incubated for 12 h at room temperature before measurement in order

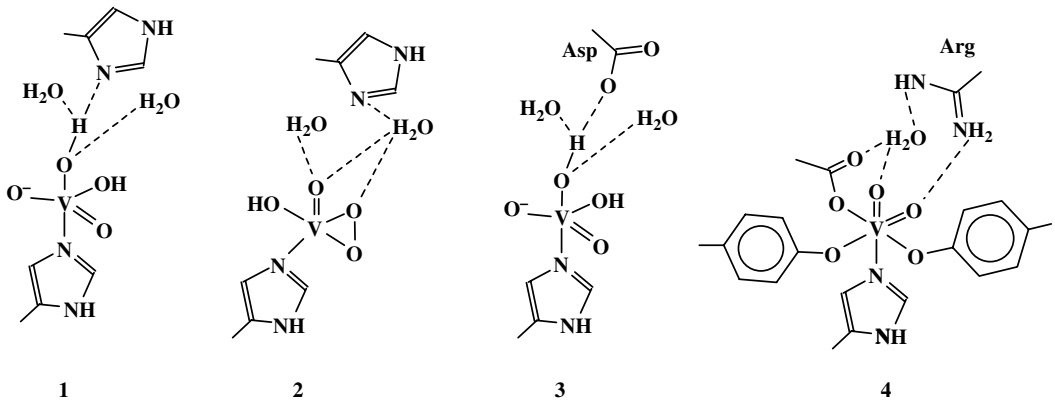

Figure 1. Active centres of the native (**1**) and the peroxo form (**2**) of vanadate-dependent peroxidases and vanadate-inhibited prostatic acid phosphatase (**3**) and the proposed VO_2^+ binding sites in transferrins (**4**). Only part of the hydrogen bonding interactions are shown. The structures **1**,[2] **2**[6] and **3**[8] are based on single-crystal x-ray diffraction results.

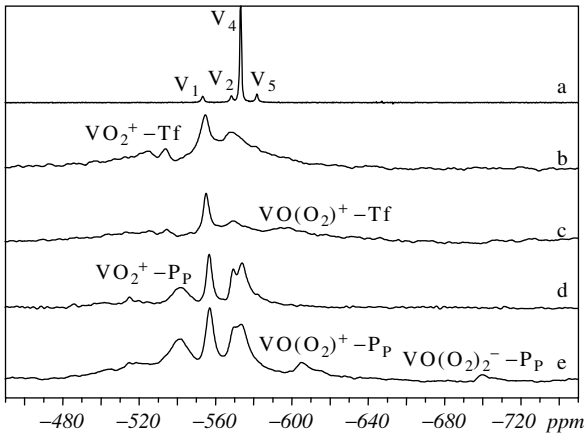

Figure 2. ^{51}V NMR spectra: (a) 5 mM KVO_3; (b) 5 mM vanadate + 1.7 mM transferrin; (c) the ternary system vanadate–transferrin–H_2O_2; (d) 5 mM vanadate + 3.3 mM phosphatase; (e) the ternary system vanadate–phosphatase–H_2O_2. All spectra were obtained in HEPES buffer, pH 7.6, and $c(H_2O_2) = 4.4$ mM after ca 12 h of incubation.

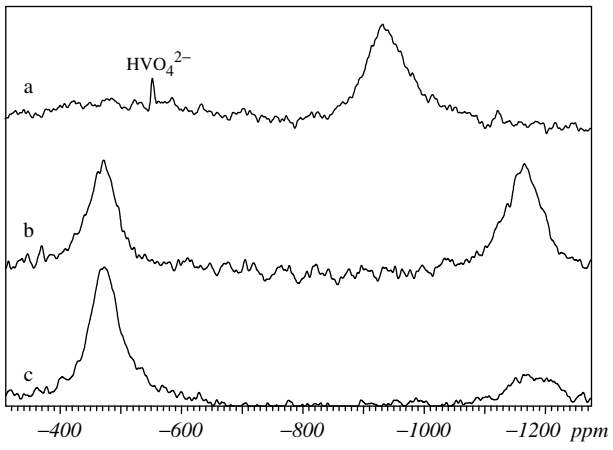

Figure 3. ^{51}V NMR spectra of the bromoperoxidase from *A. nodosum*, 0.15 mM in Tris buffer, pH 8.0. (a) A freshly prepared solution; (b) 24 h after addition of H_2O_2 (30 mM); (c) 72 h after addition of H_2O_2.

to ascertain equilibration. A second portion of these samples was further treated with 2 µl of 3% H_2O_2. Final concentrations were c(vanadate) = 5 mM, c(Tf) = 1.7 mM (based on M = 81 kDa), c(Pp) = 3.3 mM (based on M = 43 kD) and $c(H_2O_2) = 4.4$ mM. Partial reduction of vanadate to vanadyl by the proteins was excluded by EPR.

Highly purified apo-bromoperoxidase from *Ascophyllum nodosum* was isolated from algae collected in the Bretagne, following literature procedures.[15] The purified enzyme was reconstituted by dialysis against vanadate and concentrated by ultracentrifugation to 0.15 mM (based on M = 80 kDa for half of the homodimer). The specific activity was 800 U/mg. H_2O_2 was added in the form of 15 M H_2O_2 to a final concentration of 50 mM (in this experiment, we have used H_2O_2 enriched in ^{17}O by ca 45%; for preliminary results on ^{17}O NMR of the bromoperoxidase–system H_2O_2, see Ref. 16).

NMR spectra (at 297 K) were obtained at a transmitter frequency of 105.182 MHz on a Bruker MSL 400 instrument using a high-power Solenoid (5 mm) probe under static conditions, applying a single-pulse quadrature cycle sequence. The samples were sealed in tubes of 30 mm length and 5 mm width. Typical parameter settings were sweep width 83 or 125 kHz, line broadening factor 200 Hz (500 Hz in the case of the peroxidase), 90° pulse 3 µs and recycling delay time 1 s. All $\delta(^{51}V)$ values are quoted relative to external $VOCl_3$.

RESULTS AND DISCUSSION

Data from this work are given in Table 1 together with data from the literature for comparison. A 5 mM solution of potassium vanadate (KVO_3) in HEPES buffer, pH 7.6, shows the usual vanadate pattern, i.e. signals corresponding to mono-, di-, tetra- and pentavanadate: $H_xVO_4^{(3-x)-}$ (x = 1, 2; pK_a for $H_2VO_4^-$ = 8.1[17]) at −553 ppm, $H_xV_2O_7^{(4-x)-}$ (x = 1, 2) at −568 ppm, $V_4O_{12}^{4-}$ at −573 ppm (dominant signal) and $V_5O_{15}^{5-}$ at −582 ppm [Fig. 2(a)].

As this solution is mixed with a solution of apo-transferrin and incubated for 12 h (the time necessary for complete equilibration), the signals broaden, and mono- and divanadate become the dominant species. The broadening mainly reflects the high viscosity of the solution. In addition,

Table 1. ^{51}V NMR data for the systems investigated in this work and data from the literature

System	V1[a]	V2[a]	V4/V5[a]	$[VO_2L]^{+b}$	$[VO(O_2)L]^{+b}$	Fig. Ref.
KVO_3 20 mM, HEPES, pH 7.6	−553	−568	−573/−582			2(a)
KVO_3 + Tf in HEPES, pH 7.6	−555	−568	Shoulder	−525, −532 sh, −534[230]		2(b)
Na_3VO_4 + Tf in HEPES, pH 7.5				−533		11
Vanadate + Tf in HEPES, pH 7.4				−529.5, −531.5		12
KVO_3 + Tf + H_2O_2 in HEPES, pH 7.6	−555	−570	Shoulder	−525, −535	−594	2(c)
KVO_3 + Pp in HEPES, pH 7.6	−557	−569	−574	(−487), (−515), −539 sh, −542[600]		2(d)
KVO_3 + Pp + H_2O_2 in HEPES, pH 7.6	−557	−569	−574	(−505), (−515), −542	−605[470], −700	2(e)
Peroxidase, Tris buffer, pH 8.0	−555			−930[5100]		3(a)
Peroxidase + H_2O_2 in Tris, pH 8.0				−472[3600]	−1155[3900]	3(b)
Vanadate + RNase-T_1 in Tris, pH 7.2				−514		13b
NH_4VO_3 + uridine + RNase-A in NaOAc				−506		13a
KVO_3 + RNase-T_1 + inosine in Tris, pH 7.2				−525		13b
NH_4VO_3 + SOD in HEPES, pH 7.4	−558	[c]	[c]	[c]		14
Vanadate + Ala–His, pH 7.6				−518		22a
Vanadate + Ala–His + H_2O_2, pH 7.6	−624/−725[d]				−627 to −683	22a
Vanadate + peptide + H_2O_2 in HEPES, pH 7					−743 to −757	24

[a] The V*n* stand for mono-, di-, tetra- and pentavanadate; see text. Tf = apo-serotransferrin, Pp = bovine prostatic acid phoaphatase, RNase = ribonuclease.

[b] Refers to the vanadium–protein complex; minor signals in parentheses; sh = shoulder; $W_{1/2}$ (Hz) in square brackets.

[c] Broad signal centred at −576 ppm.

[d] The signals reflect the peroxovanadates $HVO_3(O_2)^{2-}$ (−624 ppm) and $H_xVO_2(O_2)_2^{(3-x)-}$ (−725 ppm at pH 7.6).[22a]

two new low-intensity but distinct signals arise at −525 and −534 ppm (including a shoulder at −532 ppm) [Fig. 2(b)], which can be assigned to VO_2^+ complexes in an environment of a donor set dominated by *O*-functions.[18] The two binding sites in transferrin contain two tyrosines, a histidine and an aspartate (Fig. 1, **4**), thus providing the appropriate donor set. Two alternative coordination patterns have been proposed, viz. (i) coordination of all of the four amino acid side-chain functions[12,19] and (ii) coordination of two Tyr and Asp and hydrogen-bonding of the His to one of the oxo groups of the VO_2^+ moiety.[20] The binding constants reported for the vanadate–transferrin complex are $pK_1 = 13.2$ and $pK_2 = 12.2$.[21] Within the limits of error, the resonances at −534 and −532 ppm corresponds to what has been reported previously;[11,12] the two binding sites giving rise to two distinct resonances (Table 1), separated by 2 ppm.[12] The additional −525 ppm signal observed in our preparation therefore should not represent vanadium at a binding site, a view which is corroborated by the fact that this signal is observed only with an excess of vanadate [*c*(V):*c*(Tf) >2]. A possible explanation for this resonance is unspecific binding of vanadate to the protein. However, in such a case, exchange with free vanadate would be expected, as observed for the unspecific binding of vanadate to Cu–Zn–superoxide dismutase, leading to considerable broadening of the vanadate resonances, but not to substantial shifts with respect to the undisturbed system.[14] The assignment of the −525 ppm signal therefore remains uncertain.

Addition of H_2O_2 (final concentration 4.4 mM) produces a new resonance at −594 ppm [Fig. 2(c)], which corresponds to a peroxo complex with the $VO(O_2)^+$ moiety. This signal decays within ca 24 h, restoring the original transferrin–vanadate spectrum: peroxovanadium complexes are generally unstable,[22] and vanadate catalyses the decomposition of hydrogen peroxide.[23] Several studies on the systems vanadate–peptide–H_2O_2 have shown that oxo–monoperoxovanadium complexes with small peptides (such as Ala–His) provide ^{51}V NMR resonances between −627 and −683 ppm,[22] and oxo–diperoxovanadium complexes give rise to resonances around −750 ppm.[22,24] $\delta(^{51}V)$ values between −570 and −625 ppm are typical for oxo–monoperoxovanadium complexes with three- and four-dentate *ON*-ligands.[25] The resonance at −594 ppm observed in the vanadate–transferrin–H_2O_2 system, clearly distinct from those of free peroxovanadate at pH 7.6 (Table 1), can therefore unambiguously be ascribed to a $VO(O_2)^+$–Tf complex.

Addition of vanadate to acid phosphatase yields a strong signal at −541 ppm (including a shoulder at −539 ppm) along with two minor component at −487 and −515 ppm. The signals for free vanadate are still present, with a reduction in intensity for the resonance corresponding to tetravanadate and a predominance of the monovanadate resonance [Fig. 2(d)], as expected as the overall concentration of free vanadate decreases. A trigonal-bipyramidal environment for the vanadium centre, the expected geometric arrangement at the active site of phosphatase (cf. Fig. 1, **3**), should give rise to deshielding with respect to a tetragonal arrangement (see also below),[26] which is not observed here for the main signal, a fact which may hint at strong distortion towards a square

pyramid. The complexes formed between ribonucleases and vanadate provide resonances between −505 and −525 ppm (Table 1). For these systems, a trigonal-bipyramidal ('transition state analogous') arrangement has been proposed.[13] In the light of the deshielding observed for authentic trigonal-bipyramidal complexes (δ values of −470 ppm and less), again substantial distortions from this geometry should prevail.

H_2O_2 generates two new signals, at −605 and −700 ppm, in the peroxovanadium domain [Fig. 2(e)]. The −605 ppm signal, corresponding to a monoperoxovanadium complex, is at about the same position as in the case of vanadate–transferrin–H_2O_2. The second signal, at −700 ppm, represents a bis(peroxo)vanadium species. Free bis(peroxo)vanadate, $HVO_2(O_2)_2^{2-} + H^+ \rightleftharpoons H_2VO_2(O_2)_2^-$ at pH 7.6, resonates at −725 ppm. We therefore assign the −700 ppm signal to $\{VO(O_2)_2^-\}$ complexed to the phosphatase. Again, the signals for the peroxo species vanish after about 24 h.

The spectral patterns observed so far may be considered 'normal' in the sense that the chemical shifts correspond to those observed for solutions of defined vanadium(V) coordination compounds. In contrast, the bromoperoxidase from *A. nodosum* (Fig. 1, **1**), exhibits unusual features (Table 1 and Fig. 3). A 0.15 mM solution of the enzyme in Tris buffer of pH 8.0, containing intrinsically bound vanadate only, shows a strong and very broad (half-width $W_{1/2} = 5100$ Hz compared with 230–600 Hz for the Tf and Pp systems) resonance at −930 ppm along with a trace at −555 ppm for monovanadate [Fig. 3(a)]. Such an extreme high-field shielding is completely unexpected for a V^V compound; the largest shielding values have so far been reported for $VO(NO_3)_3$(−745 ppm),[27] peroxovanadates [$HVO_2(O_2)_2^{2-}$ at pH 9: −764 ppm[22]] and oxovanadium compounds containing highly electronegative ligands such as fluoride {VOF_3/THF, −757 ppm,[28] VOF_3/CH_3CN_6 − 793 ppm [The $\delta(^{51}V)$ of VOF_3 in CH_3CN noted by Hibbert *et al.*[27] corresponds to $[VOF_4]^-$]}. The active centre of the peroxidase provides a histidine donor, and a variety of hydrogen bonds for the vanadate moiety, including water molecules and a distal histidine (Fig. 1, **1**). Hence a shielding situation comparable to that of the transferrin and phosphatase complexes should be expected. In a theoretical DFT-based study on vanadate complexes containing the dipeptide glycylglycine, Bühl points out that microsolvation by water and protonation of oxo sites lead to a drastic increase in shielding.[29] Three water molecules are actually present in the immediate neighbourhood of vanadate,[2,3] and protons may be shuttled via hydrogen bonds from aspartate, serine and protonated histidine at the active site.

Addition of H_2O_2 (final concentration 30 mM) gives rise to two new signals at −471 ppm (this signal does not reflect a vanadate–Tris complex; for the vanadate–Tris systems, signals are observed at, *inter alia*, −500 and −534 ppm[30]) and −1165 ppm [Fig. 3(b)]. Whereas the latter is in accord with the increase in shielding on going from an oxo to an oxo–peroxo complex (Fig. 1, **2**), the former is at a position expected for a peroxide-free complex $\{VO_4N\}$ in a trigonal-bipyramidal array.[26] This is the genuine coordination environment of vanadium in the haloperoxidases. Addition of H_2O_2 to the native enzyme (represented by the −930 ppm signal) thus leads to the peroxo form (−1155 ppm) and, concomitantly, possibly to 'denaturation' of the native enzyme, i.e. (following the path of reasoning mentioned above) removal of protons and water in the microsolvation sphere, but leaving intact the intrinsic coordination geometry. Support for denaturation comes from activity measurements; after the NMR experiment, the activity of the enzyme reduces to a few percent of the original activity. The signal for the rebuilt enzyme, −471 ppm, remains the only one after longer periods of time, during which the −1165 ppm signal slowly decays [Fig. 3(c)].

CONCLUSION

Vanadate in HEPES buffer, pH 7.6, added to aqueous bovine apo-serotransferrin (Tf) (absolute vanadate concentration 5 mM, molar ratio V:Tf = 5:1.7) or bovine prostatic acid phosphatase (Pp) (V:Pp = 5:3.3) gives rise to ^{51}V NMR resonances mainly between −515 and −542 ppm, belonging to VO_2^+ complexes to the Tf binding sites and the Pp active centre, respectively. The shielding corresponds to oxovanadium centres in a tetragonal-pyramidal or octahedral environment dominated by *O*-functional ligands. The coordinating ligands are two Tyr, Asp, His and the two oxo groups in the case of $\{VO_2^+$–Tf$\}$, and His, two oxo groups and OH/water in the case of $\{VO_2^+$–Pp$\}$. The results for the Tf complexes are essentially in accord with those reported previously by Butler and Eckert,[12] and by Saponja and Vogel.[11] H_2O_2 generates comparatively short-lived (ca 24 h) peroxo complexes of composition $\{VO(O_2)^+$–Tf$\}$, [$\delta(^{51}V) = -594$ ppm], $\{VO(O_2)^+$–Pp$\}$(−605 ppm) and $\{VO(O_2)_2^-$–Pp$\}$(−700 ppm). Vanadate–dependent bromoperoxidase from the marine brown alga *A. nodosum* provides an unusually high-field ^{51}V NMR signal at −931 ppm. This extreme shielding, which we have observed previously,[7] is tentatively traced back to a microsolvation sphere and extensive hydrogen bonding at vanadate in the active centre. This proposal follows suggestions for enhanced shielding in vanadate–GlyGly complexes based on DFT calculations carried out by Bühl.[29] The −930 ppm signal shifts to −1155 ppm on addition of excess H_2O_2, reflecting the formation of the peroxo form of the enzyme. Simultaneously, the enzyme appears to be transformed to an inactive form (including fragmentation as an option for the deactivation), with a signal at −471 ppm, typical of oxovanadium in a trigonal-bipyramidal array, $\{VO_4N\}$,[26] the intrinsic arrangement of vanadium also in the native form.[2]

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (grants RE 431/13-4 and RE 431/18-1) and the COST project D12-0027-99. We thank Dr Hans Vilter, Trier, for supplying the algal raw material and guiding the isolation and purification of the enzyme.

REFERENCES

1. Isupov MI, Dalby AR, Brindley AA, Izumi Y, Tanabe T, Murshudov GN, Littlechild JA. *J. Mol. Biol.* 2000; **299**: 1035.

2. Weyand M, Hecht HJ, Kiess M, Liaud M, Vilter H, Schomburg D. *J. Mol. Biol.* 1999; **293**: 595.
3. Messerschmidt A, Wever R. *Proc. Natl Acad. Sci. USA* 1996; **93**: 392.
4. (a) Rehder D. *Inorg. Chem. Commun.* 2003; **6**: 604; (b) Butler A, Baldwin AH. *Struct. Bonding* 1997; **89**: 109.
5. (a) ten Brink HB, Tuynman A, Dekker HL, Hemrika W, Izumi Y, Oshiro T, Schoemaker HE, Wever R. *Inorg. Chem.* 1998; **37**: 6780; (b) ten Brink HB, Holland HL, Schoemaker HE, van Lingen H, Wever R. *Tetrahedron: Asymmetry* 1999; **10**: 4563.
6. Messerschmidt A, Prade L, Wever R. *Biol. Chem.* 1997; **378**: 309.
7. Vilter H, Rehder D. *Inorg. Chim Acta* 1987; **136**: L7.
8. (a) Tanaka N, Dumay V, Liao Q, Lange AJ, Wever R. *Eur. J. Biochem.* 2002; **269**: 2162; (b) Renirie R, Hemrika W, Wever R. *J. Biol. Chem.* 2000; **275**: 11 650.
9. Hemrika W, Renirie R, Dekker HL, Barnett P, Wever R. *Proc. Natl. Acad. Sci USA* 1997; **94**: 2145.
10. Harris WR, Carrano CJ. *J. Inorg. Biochem.* 1984; **22**: 20.
11. Saponja JA, Vogel HJ. *J. Inorg. Biochem.* 1996; **62**: 253.
12. Butler A, Eckert H. *J Am. Chem. Soc.* 1989; **111**: 2802.
13. (a) Borah B, Chen CW, Egan, W, Millar M, Wlodawer A, Cohen JS. *Biochemistry* 1985; **24**: 2058; (b) Rehder D, Holst H, Quaas R, Hinrichs W, Hahn U, Saenger W. *J. Inorg. Biochem.* 1989; **37**: 141.
14. Wittenkeller L, Abraha A, Ramasamy R, Mota de Freitas D, Theisen LA, Crans DC. *J. Am. Chem. Soc.* 1991; **113**: 7872.
15. (a) Vilter H. *Methods Enzymol.* 1994; **282**: 665; (b) Časný M. Dissertation, Hamburg, 2003.
16. Časný M, Rehder D, Schmidt H, Vilter H, Conte V. *J. Inorg. Biochem.* 2000; **80**: 157.
17. Elvingson K, Gonzáles-Baró A, Pettersson L. *Inorg. Chem.* 1996; **35**: 3388.
18. Rehder D, Weidemann C, Duch A, Priebsch W. *Inorg. Chem.* 1988; **27**: 584.
19. Smith CA, Ainscough EW, Brodie AM. *J. Chem. Soc., Dalton Trans.* 1995; 1121.
20. Butler A, Carrano CJ, *Coord. Chem. Rev.* 1991; **109**: 61.
21. Kiss T. Abstracts of the 6th European Conference on Bioinorganic Chemistry, Lund and Copenhagen, 2002; p. 48.
22. (a) Andersson I, Angus-Dunne S, Howarth O, Pettersson L. *J. Inorg. Biochem.* 2000; **80**: 51; (b) Schmidt H, Andersson I, Rehder D, Pettersson L. *Chem. Eur. J.* 2001; **7**: 251; (c) Gorzsás A, Andersson I, Pettersson L. *Dalton Trans.* 2003; 2503.
23. Conte V, Di Furia F, Moro S. *Inorg. Chim. Acta* 1998; **272**: 62.
24. Tracey AS, Jaswal JS. *J. Am. Chem. Soc.* 1992; **114**: 3835.
25. Časný M, Rehder D. *Dalton Trans.* 2004; 839.
26. Santoni G, Licini G, Rehder D. *Chem. Eur. J.* 2003; **9**: 4700.
27. Hibbert RC, Logan N, Howarth OW. *J. Chem. Soc., Dalton Trans.* 1986; 369.
28. Priebsch W, Rehder D. *Inorg. Chem.* 1985; **24**: 3058.
29. Bühl M. *J. Comput. Chem.* 1999; **20**: 1254.
30. Tracey AS, Gresser MJ. *Inorg. Chem.* 1988; **27**: 1269.