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## Nitrite reduction in aqueous solution mediated by amavadin homologues: N<sub>2</sub>O formation and water oxidation

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Abstract: Reactions of two vanadium(IV) complex anions homologues of amavadin,  $[V(HIDPA)_2]^{2-}$  and  $[V(HIDA)_2]^{2-}$  (HIDPA = N-oxyiminodiproprionate, HIDA = N-oxyiminodiacetate) with nitriteion (NO2-) in aqueous solution were investigated by experimental (absorption spectroscopy in the visible range, by measurements of the dioxygen formed into solution from water oxidation and identification of nitrogen oxide species of gaseous atmosphere from nitrite reduction by an infrared analyser) and theoretical methods. Two reactions, mediated by the V complexes, with environmental and biological significance were observed in this system, i.e. reduction of nitrite to N<sub>2</sub>O and water oxidation to molecular oxygen. The reduction of nitrite, studied by DFT calculations, occurs via formation of NO ( $\Delta G^{\neq}$  = 14.3 kcal/mol), which is strongly pH dependent and slightly endergonic, and then it is easily converted into N<sub>2</sub>O with the overall activation barrier  $\Delta G^*$  of 11.8 kcal/mol. This process includes dimerization of NO assisted by one molecule of the V-complex, protonation and oxidation of the formed ONNO- ligand by another amavadin molecule or by nitrite and the NO bond cleavage/proton transfer in the ONNOH- ligand. The results indicate that amavadin exhibits an unusual nitrite reductase type activity that could be involved in the nitrogen metabolism of Amanita muscaria and other fungi bearing this V complex.

#### Introduction

Amavadin is a vanadium metallobiomolecule, isolated in 1972 by Bayer and Kneifel from the fungus *Amanita muscaria*.<sup>[1]</sup> Later, it was also found in two other fungi of this genus that has more than 600 species.<sup>[2]</sup>.Natural amavadin is a vanadium(IV) species bearing the  $[V(S,S-HIDPA)_2]^{2-}$  complex anion (S,S-HIDPA = (S,S)-N-oxyimino-(2,2')-diproprionate) with two ligands derived from *S*, *S'*-*N*-hydroxyiminodiproprionic acid (Figure 1 and Chart 1). Differently of the majority of vanadium(IV) complexes, it does not bear the oxovanadium (V=O) group. Each ligand in amavadin is tetradentate<sup>[3]</sup> and the coordination number of vanadium is eight, which is rare for the first series of transition metals.



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Supporting information for this article is given via a link at the end of the document.



Chart 1.

Amavadin may play an important biological role exhibiting catalase- and peroxidase- type activities.<sup>[2,4,5]</sup> Its homologues  $[V(HIDPA)_2]^{2-}$  (including diastereomers of the natural compound) and  $[V(HIDA)_2]^{2-}$  (HIDA = *N*-hydroxyiminodiacetate, Chart 1) are also known to mediate or catalyse a number of reactions, in particular, various oxidation processes<sup>[2]</sup> such as hydroxylation, oxygenation and peroxidative halogenation of alkanes and benzene,<sup>[6]</sup> peroxidative oxygenation of benzene and mesitylene,<sup>[7]</sup> carboxylation of linear and cycloalkanes,<sup>[8-13]</sup> oxidation of alcohols<sup>[14]</sup> as well as the cyanide addition to aldehydes.<sup>[14]</sup>

On the other hand, mechanistic details of reactions,<sup>[11,13,15]</sup> NMR<sup>[16]</sup> and EPR<sup>[17]</sup> spectroscopic information, involving amavadin and its homologues, have been obtained from DFT calculations.

Recently, it was reported by some of us that amavadin in acidic medium is an efficient mediator of water oxidation in the presence of a strong oxidant (Ce<sup>4+</sup>).<sup>[15]</sup> This was the first example of such a type of activity exhibited by a V complex under nonphotochemical conditions.<sup>[18]</sup> Experimental data and DFT calculations suggested for this reaction a mononuclear Water Nucleophilic Attack (WNA) mechanism (Scheme 1) which includes (i) initial protonation of amavadin and its oxidation by Ce4+ to the corresponding V(V) complex II, (ii) decoordination of the protonated carboxylic group and ligation of a water molecule, (iii) deprotonation of the highly acidic coordinated H<sub>2</sub>O molecule, (iv) the ligand centred oxidation of the formed complex V by  $Ce^{4+}$ and (v) nucleophilic addition of another water molecule at the OH- ligand in VI with simultaneous proton transfer to give the hydrogen peroxide complex VII. The hydrogen peroxide is further oxidized to O2 in a well-known metal-mediated process.

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Scheme 1. Proposed mechanism of water oxidation by Ce(IV) mediated by the amavadin analogue.  $^{\rm [15]}$ 

Stimulated by these exciting findings, we decided to explore other oxidants milder than Ce<sup>4+</sup> that mediated by amavadin homologues could also oxidize water. We pointed our attention to nitrite (NO<sub>2</sub><sup>-</sup>) due to its great significance from the environmental and biological viewpoints. To our knowledge, the homogeneous chemical oxidation of water by nitrites was not yet reported. Nitrite still exhibits a rather strong oxidizing activity in acidic medium that is noticeably weaker than of Ce<sup>4+</sup> (E°(Ce<sup>4+</sup>/Ce<sup>3+</sup>) = 1.62 V, E°(HNO<sub>2</sub> + H<sup>+</sup>/NO + H<sub>2</sub>O) = 1.00 V, E°(2HNO<sub>2</sub> + 4H<sup>+</sup>/N<sub>2</sub>O + 3H<sub>2</sub>O) = 1.30 V].

Nitrite is a pollutant in drinking and waste water. Its reduction to nitrogen oxides NO and N<sub>2</sub>O – which should be captured – or preferentially to environmentally benign molecular nitrogen underlies the water denitrification process and has a tremendous ecological significance.<sup>[19]</sup>

Furthermore, the nitrite reduction is an important biochemical process associated with *e.g.* neurotransmission, immune response and apoptosis in various organisms.<sup>[20-26]</sup> Soil bacteria metabolize nitrite to NO via nitrite reductases – enzymes of the EC 1.7 group<sup>[27]</sup> with the iron,<sup>[28]</sup> copper<sup>[29]</sup> or molybdenum<sup>[26]</sup> based active centres. Both NO and N<sub>2</sub>O – another product of the biological nitrite reduction – are greenhouse gases that also participate in the destruction of the ozone layer. Besides the anthropogenic way, the soil bacteria are a principal cause of the NO emission. Various fungi including *A. muscaria* also contribute to the nitrogen cycle with nitrite as possible metabolic substrates.<sup>[30-32]</sup> Therefore, the understanding of mechanistic details of the nitrite reduction is important in environmental chemistry.<sup>[21]</sup>

Despite such a great importance, the chemical homogeneous reduction of nitrites by inorganic transition and internal transition metal compounds was investigated rather scarcely. Most of the works deal iron<sup>[33-45]</sup> or copper<sup>[46-52]</sup> complexes which mimic the nitrite reductases. However, the NO<sub>2</sub><sup>-</sup> reduction with Ru,<sup>[53]</sup> Mo,<sup>[54-56]</sup> Ti, Cr,<sup>[56]</sup> Co,<sup>[57-59]</sup> and U<sup>[60]</sup> was also reported. Vanadium species are unconventional for this reaction, and there are only few publications about the nitrite reduction by the V(III) species from an analytical approach<sup>[56,61]</sup> and there is no one describing this reaction with the V(IV) complexes. Additionally, rational design based on metallobiomolecules<sup>[62-64]</sup> has been employed for similar purpose. Herein, complexes related with a vanadiumbiomolecule with metal in the oxidation state four are used for nitrite reduction, suggesting a possible natural process which could occur in A. muscaria and other fungi bearing this vanadium species. In the first part of the article, experimental results of the reaction between the homologues of amavadin and nitrite in aqueous solution are presented. In the second part, a detailed theoretical study of the reaction mechanism is discussed.

#### **Results and Discussion**

#### Reaction features and visible spectra of solutions

Addition of the oxidant NaNO<sub>2</sub> in the aqueous solutions of complexes  $[V(HIDA)_2]^{2-}$  or  $[V(HIDPA)_2]^{2-}$  (0.01 M) results in an immediate colour change from the initial blue to red due to oxidation of the vanadium(IV) complexes into the corresponding vanadium(V) species. This behaviour is similar for samples covered with aluminium foil and, hence, protected from the light radiation and for the unprotected ones. Then, the colour of all solutions returns to blue, although with different shades, within several hours or days depending on the complex and conditions (see below). Such a colour change was previously observed with  $H_2O_2^{[4]}$  or Ce(IV)<sup>[15]</sup> used as oxidants, and it was attributed to the reduction of the V(V) complexes back to V(IV) as a result of the water oxidation process. Formation of bubbles of gas was detected visually in the solutions for both  $[V(HIDA)_2]^{2-}$  and  $[V(HIDPA)_2]^{2-}$ .

The absorption spectra of  $[V(HIDA)_2]^{2-}$  and  $[V(HIDPA)_2]^{2-}$ , with nitrite 0.005 M, are characterized by band with maxima at 570 nm and 565 nm, respectively (Figure 2 and 3). In the case of  $[V(HIDA)_2]^{2-}$ , these spectra are similar for the light-protected and unprotected samples. Colour stabilization is reached within *ca.* 24 hours and a partial degradation of the complex occurs in both cases, see Figure 2.







**Figure 3.** Absorption spectra of the aqueous solution of  $[V(HIDPA)_2]^{2-}$  (0.01 M) before  $(\cdot \cdot \cdot)$  and after addition of NaNO<sub>2</sub> (0.005 M) and after the reaction completion for the light protected  $(\cdot \cdot \cdot)$  and unprotected  $(- \cdot)$  samples.

associated with the V-mediated water oxidation – a process whose mechanism was proposed and investigated previously.<sup>[15]</sup> An eightfold increase of the dissolved oxygen (from 0.46 mg/L) was detected after 20 h. The amount of the released oxygen is half of the initial amount of nitrite in solution (0.11 mmol/L vs. 0.22 mmol/L, respectively). This is in agreement with the general reaction scheme (Scheme 2) involving the formation of N<sub>2</sub>O but not NO as the final product of the nitrite reduction (in the latter case, the amount of the oxygen released should be four times lower than the amount of the nitrite consumed).

 $N_2O + 3H_2O$ 



 $2NO_2^- + 6H^+$ 

Scheme 2. Overall reaction of water oxidation with NO<sub>2</sub><sup>--</sup> mediated by amavadin or related complexes. {V<sup>IV</sup>} is amavadin or its homologues, {V<sup>V</sup>} is the corresponding oxidized V(V) complex.

#### Theoretical mechanistic study of nitrite reduction

With aim to understand the mechanism of the nitrite reduction with amavadin, theoretical calculations at the DFT level of theory were undertaken. Taking into account the acidic character of the reaction mixture, the monoprotonated form of the amavadin model [V(HIDA)(HIDAH)] (1) was considered for these calculations. The experimental observations suggest that the nitrite reduction with amavadin consists of two main steps, *i.e.* (*i*) reduction of NO<sub>2</sub><sup>-</sup> to NO and (*ii*) conversion of NO into N<sub>2</sub>O. Additionally, the possibility of the direct reduction of nitrite to N<sub>2</sub>O is also considered. Each of these processes is discussed in the following sections (and in Supporting Information).

Formation of NO. The reaction starts with the liberation of one coordination place in 1 due to the cleavage of the V–O(H) bond to give 2 which is slightly endergonic (by 2.9 kcal/mol). The nitrite reduction strongly depends on the pH value of the reaction mixture. Therefore, three main possible pathways were considered, *i.e.* without further participation of a proton and with additional involvement of one (for these two cases, see Supporting Information for details) or two protons in the reaction (Scheme 3).

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The reaction of nitrite with  $[V(HIDPA)_2]^{2-}$  depends on exposure to the light radiation in contrast with  $[V(HIDA)_2]^{2-}$ . For the light-protected samples the former complex returns to its original blue colour after *ca*. 10 days, much slower than for  $[V(HIDA)_2]^{2-}$ , and there is no sign of the complex degradation. A typical example of  $[V(HIDPA)_2]^{2-}$  photodegradation at ambient light conditions is shown in Figure 3. Upon irradiation (*ca*. 2h with the xenon lamp) the return to blue colour of the solution occurs much faster, exhibiting the complex degradation (see Experimental Section).

The oxidation of  $[V(HIDA)_2]^{2-}$  or  $[V(HIDPA)_2]^{2-}$  by nitrite strongly depends on pH of the reaction mixture. In a strongly acidic solution (pH = 1.72), the colour change from initial blue to red occurs immediately upon addition of the oxidant. The decrease of the reaction mixture acidity by addition of NaOH to pH > 2.5 prevents the oxidation of the V complexes by NO<sub>2</sub><sup>-</sup>, and no colour change is observed.

#### **Gaseous reaction products**

Gaseous species derived from the nitrite reduction for three solutions, [V(HIDPA)2]2- (protected and unprotected from light) and [V(HIDA)<sub>2</sub>]<sup>2-</sup> (protected from light), were qualitatively identified by analysis of the gas phase above the solution using the infrared analyser (see Supporting Information for details). In all samples,  $N_2O$  is the only detected reduction product in the gaseous atmosphere, despite its high (comparing with other gases) solubility in aqueous solution. The presence of the other nitrogen oxides (NO and/or NO2) was not detected. The results for  $[V(HIDPA)_2]^2$  protected from light are shown in Figure 4. Meanwhile, analysis of the literature on this topic<sup>[33,44,47,57,59,65]</sup> suggests that the reaction may involve the formation of NO (and/or hyponitrite) as an intermediate which is rapidly converted into N2O mediated by complex. Theoretical DFT calculations of the reaction mechanism confirmed this hypothesis (see below).





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Scheme 3. Mechanisms of the nitrite reduction to NO upon reaction with the amavadin model **1** (relative Gibbs free energies are indicated in parentheses in kcal/mol).

Reaction with additional involvement of two protons. This mechanism can operate in a sufficiently strong acidic medium corresponding to the experimental conditions. The OH<sup>-</sup> anion liberating as a result of the N–O bond cleavage in **2**•••HNO<sub>2</sub> is captured by a proton (H<sub>3</sub>O<sup>+</sup>) that should be much more efficient than the grab by a metal centre as in the pathway with additional involvement of one proton (see Supporting Information). For such a process, transition state **TS3** was found. As a result of the N–O bond cleavage and simultaneous proton transfer, NO associated with a V(V) complex ([V(HIDA)(HIDAH)](NO) **2**<sup>0</sup>•••NO) and two water molecules are formed. No minimum corresponding to the inner sphere V(V) or V(IV) complex with coordinated NO or NO<sup>+</sup> ligand was found. Thus, this process is truly outer sphere including the electron transfer (ET) from V(IV) to the NO moiety.

The HNO<sub>2</sub> reduction step is barrierless with the negative Gibbs free energy of activation -0.7 kcal/mol. The negative  $\Delta G^{\pm}$  value may be explained by the fact that the total energy rather than the Gibbs free energy was minimized during the geometry optimization. Indeed, in terms of enthalpy at 0 K, the activation barrier is positive (2.5 kcal/mol). The overall activation barrier for this mechanism relative to the initial complex **1** is 14.3 kcal/mol. The formation of NO within this mechanism is only slightly endergonic by 3.2 kcal/mol relative to **1**. Thus, the computational data demonstrate a significant effect of the medium acidity on the nitrite reduction that is in agreement with the experimental data.

**Formation of N<sub>2</sub>O.** The further conversion of NO into N<sub>2</sub>O – the principal nitrogen containing product of the nitrite reduction detected experimentally – should include two main processes, *i.e.* the NO reduction and the preceding or following N–N bond formation.

1. Initial reduction of NO followed by the N–N bond formation. There are two reducing agents in the reaction mixture which, in principle, could reduce NO to NO<sup>-</sup>, *i.e.* the amavadin homologue and the NO<sub>2</sub><sup>-</sup> anion (or HNO<sub>2</sub> acid), see also Supporting Information.

Reduction of NO by the V(IV) complex **2**. The reduction of NO by V(IV) requires the coordination of nitric oxide to the metal centre (inner sphere ET) or, at least, a close interaction between NO and V which could permit an outer sphere ET. Equilibrium structures for one inner sphere V(IV) complex  $^{3}[V^{IV}(NO^{*})(HIDA)(HIDAH)]^{-}(^{3}7)$  and an outer sphere complex

<sup>3</sup>[V<sup>IV</sup>(HIDA)(HIDAH)]('NO)<sup>−</sup> (<sup>3</sup>2•••NO) were obtained (Scheme 4). In <sup>3</sup>7, NO is coordinated via the N atom forming a weak coordination V–NO bond [I(V–NO) = 2.468 Å]. In <sup>3</sup>2•••NO, the V–ON distance is 3.145 Å and, therefore, NO is in the second coordination sphere. No equilibrium structure corresponding to the inner sphere complex with NO coordinated by the O atom <sup>3</sup>[V<sup>IV</sup>(<u>O</u>N<sup>•</sup>)(HIDA)(HIDAH)]<sup>−</sup> was found. All attempts of its optimization led to the adduct <sup>3</sup>2•NO. The outer sphere complex <sup>3</sup>2•••NO is by 6.7 kcal/mol more stable than the inner-sphere complex <sup>3</sup>7. The low spin antiferromagnetic biradical structures of 2•••NO and 7 were also optimized but they are slightly less stable (by 1.5 kcal/mol) than the corresponding high spin triplet structures.



Scheme 4. Initial steps of the NO-to-N<sub>2</sub>O conversion mechanism based on the initial NO reduction (relative Gibbs free energies are indicated in parentheses in kcal/mol).

The reduction of NO by V(IV) in <sup>3</sup>2•NO results in the formation of the closed shell V(V) complex <sup>1</sup>[V<sup>V</sup>(<u>O</u>N)(HIDA)(HIDAH)]<sup>-</sup> (<sup>1</sup>8) where NO is coordinated to vanadium by the oxygen atom (the V–ON bond length is 2.275 Å). However, the energy of such ET is 32.2 kcal/mol, *i.e.* too high to permit the realization of this process. In contrast, the intramolecular reduction of NO in <sup>3</sup>7 leads to the closed shell V(V) complex <sup>1</sup>[V<sup>V</sup>(<u>N</u>O)(HIDA)(HIDAH)]<sup>-</sup> (<sup>1</sup>7) and requires only 8.5 kcal/mol.

2. Initial N–N bond formation followed by reduction of NO. In this mechanism of the NO-to-N<sub>2</sub>O conversion, the N–N bond is formed between two unreduced NO molecules. The N–N bond formation may occur without an involvement of the metal complex (see Supporting Information), or with participation of one or two molecules of the vanadium species.

(i) Mononuclear mechanism involving the V(IV) complex 2. Dimerization of NO may be assisted by one molecule of the V(IV) complex 2 (Scheme 5). Adduct <sup>3</sup>2•NO reacts with another NO molecule aivina the doublet complex <sup>2</sup>[V<sup>V</sup>(<u>O</u>NNO)'(HIDA)(HIDAH)]<sup>-</sup> (11). Spin density in 11 is delocalized among the ONNO ligand and it is virtually zero at the V atom (Figure 5). Therefore, 11 should be described as the V(V) complex bearing the ONNO<sup>-</sup> anion-radical ligand. Thus, dimerization of NO in the presence of 2 is accompanied by the single electron reduction of the ONNO dimer by the V(IV) atom. This process requires only 10.1 kcal/mol.



Scheme 5. Mononuclear mechanisms of the NO-to-N<sub>2</sub>O conversion based on the initial N–N bond formation. Gibbs free energies are indicated relative to 2<sup>0</sup>•••NO in kcal/mol. <sup>*a*</sup> For NO<sub>2</sub><sup>-</sup> as oxidant. <sup>*b*</sup> For 2 as oxidant. <sup>*c*</sup> For the oxidation of 11 by HNO<sub>2</sub>. <sup>*d*</sup> For the oxidation of 14 by NO<sub>2</sub><sup>-</sup>. <sup>*e*</sup> For the oxidation of 14 by NO<sub>2</sub><sup>-</sup>.



Figure 5. Spin density distribution in complex 11.

Complex 11 may then undergo the second single electron reduction either by NO2-/HNO2 or by V(IV) in 2 to give [V<sup>V</sup>(ONNO)(HIDA)(HIDAH)]<sup>2-</sup> (12)or [V<sup>V</sup>(ONNOH)(HIDA)(HIDAH)]<sup>-</sup> (13) depending on acidity of the medium. Such a reduction is almost energetically neutral for  $NO_2^-$  ( $\Delta G = 0.5$  kcal/mol) or clearly exergonic for HNO<sub>2</sub> and 2  $(\Delta G = -8.4 \text{ and } -3.6 \text{ kcal/mol})$ . Nitrous oxide N<sub>2</sub>O may be directly formed from 12 or 13 upon monomolecular O-N bond cleavage via TS5 or TS6, respectively, with the activation barriers of 0.3 and 8.8 kcal/mol, correspondingly. In the case of 13, the N<sub>2</sub>O formation could also occur via TS7 with the assistance of one water molecule which plays the role of a proton shuttle. However, the water assisted step is 13.2 kcal/mol less favourable than the monomolecular pathway due to the entropic factor.

Alternatively, the ONNO<sup>-</sup> ligand in **11** may be initially protonated. The protonation is slightly exergonic by -3.7

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kcal/mol and results in the decoordination of the ONNOH' ligand to give the outer sphere complex [V<sup>V</sup>(HIDA)(HIDAH)](HONNO)' (14) (see Supporting Information for computational details). The obtained HONNO' radical in complex 14 is reduced by NO<sub>2</sub><sup>-</sup> or 2 to give [V<sup>V</sup>(<u>O</u>NNOH)(HIDA)(HIDAH)]<sup>-</sup> 13 with the  $\Delta$ G values of – 11.6 or –15.6 kcal/mol, respectively. Complex 13 is transformed to N<sub>2</sub>O and **6** (see Scheme 7).

The mechanisms based on the protonation of **11** to **14** or on the oxidation of **11** to **12** by **2** are the most favourable one with the overall activation barrier of 11.8 kcal/mol relative to the initial level  $2^{0}$ ···NO. Meanwhile other mechanisms based on the direct oxidation of **11** by NO<sub>2</sub><sup>-</sup> or HNO<sub>2</sub> have only slightly higher overall activation barrier 12.3–12.6 kcal/mol and, therefore, may be realized concurrently.

(ii) Mechanism involving two molecules of the V(IV) complex. In this mechanism, complex <sup>3</sup>2···NO may dimerize, giving **15** (Scheme 6). Both *trans*- and *cis*-isomers of **15** in both triplet and closed shell singlet spin states have been optimized. The calculations indicate that the singlet *trans*-isomer *trans*-**15** is the most stable among these four structures (see for the energies Table S1 in Supporting Information). The  $\Delta G$  energy of dimerization of <sup>3</sup>2···NO is 12.3 kcal/mol. Since *trans*-**15** is a closed shell structure, both V atoms have the oxidation state +V. Hence, dimerization of <sup>3</sup>2···NO is accompanied by the two electron reduction of the ONNO ligand by the V(IV) centres.



Scheme 6. Binuclear mechanisms of the NO-to-N<sub>2</sub>O conversion based on the initial N–N bond formation (relative Gibbs free energies are indicated in parentheses in kcal/mol).

Complex trans-15 may undergo direct decomposition to the V(V) species  $[V^{V}(=O)(HIDA)(HIDAH)]^{2-}$  (5),  $[V^{V}(HIDA)(HIDAH)]$  (16) and N<sub>2</sub>O via **TS8**. However, the activation energy of this step is high (21.7 kcal/mol) allowing its exclusion from consideration.

Alternatively, *trans*-**15** may be doubly protonated to give **17**. The estimated energy of the protonation is -11.0 kcal/mol (see Supporting Information for details). The hyponitrous acid HONNOH may be liberated from **17** ( $\Delta G = -18.8$  kcal/mol) and then easily decomposed to N<sub>2</sub>O and water. The overall activation barrier for this mechanism is 15.8 kcal/mol relative to **2**<sup>0</sup>···NO and it appears to be slightly less favourable than the mechanism based on one molecule of the V(IV) complex.

#### Conclusions

FULL PAPER

Investigations of the past decades showed that amavadin and homologues compounds exhibit interesting chemical properties being efficient catalysts or mediators of a number of redox reaction. In this work, we demonstrate that two chemical processes with environmental and biological significance occur in a simple chemical system bearing amavadin (or its homologues), nitrite and water.

First, these vanadium species mediate the homogeneous water oxidation to molecular oxygen. This process usually requires very strong oxidants such as Ce<sup>4+</sup>, HSO<sub>5</sub><sup>-</sup>, CIO<sup>-</sup> or IO<sub>4</sub><sup>-</sup>.<sup>[66]</sup> However, in the presence of amavadin or its homologues, even significantly weaker oxidants such as nitrite become active towards water oxidation. Vanadium atom in amavadin and its homologues can experience only the single electron oxidation to V(V), the highest oxidation state for this metal. However, in accord with DFT calculations, the *N*-oxyiminodicarboxylate ligands in these complexes are non-innocent and may also undergo a relatively facile single electron oxidation due to the presence of the electron rich NO group. Such ligand centred oxidation allows the accumulation of an additional oxidative strength which is sufficient for the oxidation of water<sup>[16]</sup> and is likely associated with an unusual <sup>51</sup>V NMR spectrum.<sup>[16]</sup>

process The second occurring the amavadin(homologue)/nitrite/H2O system is the reduction of nitrite with the V(IV) species. Experimental observations and results of theoretical DFT calculations indicate that such a reduction occurs via formation of NO which is then converted to the final N<sub>2</sub>O product. The most plausible mechanism is shown in Scheme 7. The formation of NO is strongly pH dependent and efficiently occurs with involvement of three protons, two of them protonating the amavadin model molecule and the NO2- ion and the third proton being directly involved in the N-O bond cleavage capturing the releasing OH<sup>-</sup> anion. The transformation of nitric oxide into N<sub>2</sub>O includes (i) dimerization of NO which is assisted by the V(IV) centre in the amavadin model molecule and accompanied by the single electron reduction of ONNO by V(IV), (*ii*) protonation of the ONNO. ligand in  $^2[V^{V}(\underline{O}NNO)^{*}(HIDA)(HIDAH)]^{-}$  11 (iii) reduction of HONNO\* in  $[V^{V}(HIDA)(HIDAH)](HONNO)^{*}$  14 by a V(IV) complex or  $NO_2^{-}$ N-Ô cleavage/proton and (iv)bond transfer  $[V^{\vee}(ONNOH)(HIDA)(HIDAH)]^{-}$  13 to give N<sub>2</sub>O and the hydroxo complex [V<sup>∨</sup>(OH)(HIDA)(HIDAH)]<sup>-</sup> 6.



**Scheme 7.** The most plausible mechanism of the nitrite reduction with amavadin model **1** (relative Gibbs free energies are indicated in parentheses in kcal/mol). <sup>*a*</sup> For NO<sub>2</sub><sup>-</sup> a oxidant. <sup>*b*</sup> For **2** as oxidant.

The overall activation energies of the NO formation and the NO-to- $N_2O$  conversion are 14.3 and 11.8 kcal/mol,

respectively. The endergonic character of the NO formation by 3.15 kcal/mol and the low activation barrier for the NO transformation to  $N_2O$  do not allow an efficient accumulation of NO in the reaction mixture explaining why nitric oxide was not detected experimentally.

Some transition metal compounds are able to reduce nitrite to N<sub>2</sub>O, including some minerals,<sup>[43-45]</sup> and herein is revealed that amavadin (a metallobiomolecule) mediates a similar reaction, in contrast with other metallobiomolecules (nitrite reductases) concerning final product. However, the formation of N<sub>2</sub>O by living organisms is known with other substrates.<sup>[67]</sup>

The biological function of amavadin in organisms is still unknown. Meanwhile, results of this work suggest that this complex exhibits the nitrite reductase type activity and, therefore, may be involved in the nitrogen metabolism of Amanita muscaria and other fungi bearing amavadin. Moreover, it can be associated with the molecular oxygen production under suitable biological conditions or biological thiols (see Supporting Information). The nitrite reductase, peroxidase and catalase type activities of amavadin uncovered in this and previous works<sup>[4,68-</sup> <sup>70]</sup> suggest that this vanadium metallobiomolecule may play a multiple role in organisms depending on the cellular environment, including a different protein moiety. However, due to the high coordination number of metal centre and being an anion, amavadin favours interactions with cationic side-chains of the proteins, as observed for its homologue [V(HIDA)2]<sup>2-</sup> by ammonium ion.<sup>[71]</sup> Additionally, if amavadin participates in the metabolic reduction of nitrite, the reaction should occur at higher pH than the tested in this work, since the metabolic pH is less acid, with plausible cooperation of suitable amino acid residues.

These results suggest that homologues of amavadin could be employed to reduce nitrite contamination in aqueous environments, e.g., a bell jar, opaque to the light radiation, with the complex immobilized into an acidic moiety in the interior side and a cylinder to store N<sub>2</sub>O (which has technological applications and it is not easy to prepare)<sup>[41]</sup> arising from the nitrite reduction could be used for that purpose.

Finally, studies with other oxidants using amavadin and its homologues as mediators should be a profitable field concerning new approaches on chemical synthesis and catalysis.

#### **Experimental Section**

The proligands, H<sub>3</sub>HIDPA, and H<sub>3</sub>HIDA (Chart 1), were synthesized according to Bayer and Kneiffel.<sup>[72]</sup> The solutions of the complexes, usually 0.01 M, were prepared *in situ* with deoxygenated distilled water (to be sure that the presence of molecular oxygen results only from the oxidation of water), oxovanadium(IV) sulfate pentahydrate and H<sub>3</sub>HIDPA or H<sub>3</sub>HIDA. The initial pH of these solutions was *ca.* 1.72. All reactions and operations were carried out at room temperature. Some experiments were carried out in absence of light radiation by covering the samples with an aluminium foil or using some coloured filters (see Supporting Information).

Absorption spectra were recorded with Perkin-Elmer L35 and Jasco V-560 spectrophotometers in the wavelength range 380-850 nm using quartz cells (b = 1 cm). The pH measurements were performed with a Metrohm 827 pH lab. Water oxidation was detected and quantified by the oxygen meter with SG9-SevenGo proTm equipment calibrated with a saturated solution of distilled water in air (see Supporting Information). Preliminary tests for the identification of the reduction product of nitrite were carried out (see Supporting Information). For the final identification of the gaseous products from nitrite reduction, three experiments were carried out with complexes [V(HIDPA)2]2- and [V(HIDA)2]2- fully covered with an aluminium foil, and another similar experiment was carried out just for [V(HIDPA)2]2- but uncovered and it was irradiated in continuous mode using a 450 W xenon lamp in a Fluorolog (Horiba Jobin Yvon spectrofluorometer) with incident light bandpass of 515.0±14.7 nm (this range is based on tests with coloured filters, see Supporting Information). The identification of the gaseous

species on the samples was carried out using an ABB EL 3020 infrared analyser (for details see Supporting Information).

#### **Computational Details**

The full geometry optimization of all structures and transition state has been carried out at the DFT/HF hybrid level of theory using the B3LYP\* functional  $^{[73-76]}$  with the Gaussian-09 program package.  $^{[77]}$  It was shown that while the popular pure functionals, such as BP86, underestimate the relative stability of high spin states and many hybrid functionals with a high contribution of the Hartree-Fock term overestimate the relative stability of high spin states, the modified B3LYP functional (B3LYP\*) correctly describes the relative energies of the low and high spin states in a number of transition metal complexes.<sup>[75,76]</sup> Since in the proposed mechanisms the relative stability of the high and low spin states is a crucial issue, especially the B3LYP\* functional was selected for the calculations. This functional was previously employed by some of us with success for the theoretical treatment of water oxidation mediated by amavadin.<sup>[15]</sup> The B3LYP\* functional was constructed using the keywords IOp(3/76=1000001500), blyp, IOp(3/77=0720008500) and IOp(3/78=0810010000) in Gaussian.

The relativistic Stuttgart pseudopotential that described 10 core electrons (MDF10) and the appropriate contracted basis set[78] were employed for the V atoms, whereas the standard basis set 6-31G\* was applied for all other atoms. Single point calculations with the 6-311+G\*\* basis set for all non-metal atoms were then performed. The MP4(SDQ)/6-31G\* level was used for the estimates of the NO dimerization energy.

To test the effect of the functional on the rate limiting activation barrier, the calculations of the key species with the M06 functional<sup>[79]</sup> has also been performed. The activation barriers for the NO formation calculated with the B3LYP\* and M06 functionals differ by less than 2 kcal/mol (14.3 and 16.2 kcal/mol, respectively).

No symmetry operations have been applied for any of the calculated structures. Restricted approximations for the structures with closed electron shells and unrestricted methods for the structures with open electron shells have been employed. The geometry optimization of all structures was carried out for water solution using the SMD model with default parameters.[80]

The Hessian matrix was calculated analytically for the optimized structures in order to prove the location of correct minima (no imaginary frequencies) or saddle points (only one imaginary frequency) and to estimate the thermodynamic parameters, the latter being calculated at 25°C. The nature of all transition states was investigated by the analysis of vectors associated with the imaginary frequency and by the calculations of the intrinsic reaction coordinates (IRC) using the Gonzalez-Schlegel method.<sup>[81–83]</sup>

All energies discussed in the text are Gibbs free energies if not stated otherwise. The computational details for the reaction steps with participation of a proton are discussed in Supporting Information.

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