Metalloporphyrins

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Iron Catalysis for In Situ Regeneration of Oxidized Cofactors by Activation and Reduction of Molecular Oxygen: A Synthetic Metalloporphyrin as a Biomimetic NAD(P)H Oxidase**

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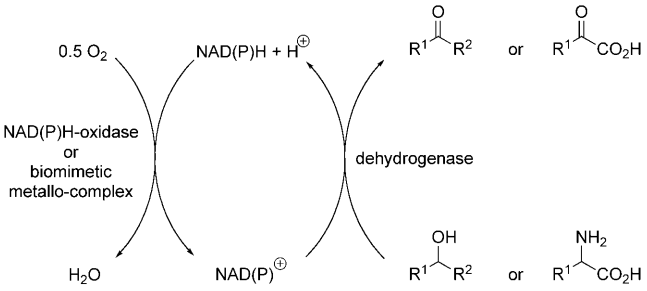
A major challenge in biomimetic catalysis is the development of synthetic low-molecular-weight compounds that are able to mimic the catalytic function of enzymes.[1a] Thus, biomimetic redox enzymes should, on the one hand, be able to function as a catalyst in water and on the other hand accept cofactors, in particular NADH and NADPH (NAD(P) = nicotinamide adenine dinucleotide (phosphate)) and their oxidized forms NAD^+ and $NADP^+$, respectively, as co-substrates. The in situ recycling of the expensive cofactors, a process carried out mostly by means of biotransformations, is considered a key technique for conducting enzymatic redox reactions in an attractive fashion.[1b] For the reduction mode of the cofactor regeneration (to regenerate the reduced forms NADH and NADPH) Steckhan et al. developed a "biomimetic formate dehydrogenase" for the regeneration of NAD(P)H by oxidation of formic acid into carbon dioxide by using a suitable rhodium complex.[2] For the oxidation mode of the cofactor regeneration, NAD(P)H oxidases[3] as natural catalysts have been applied as well as chemoenzymatic,[4] electrochemical,[5] and biomimetic[6] catalyst systems. However, the biomimetic catalysts developed so far produce undesired hydrogen peroxide as a by-product instead of (preferably) water.[6] To the best of our knowledge no biomimetic catalyst for the regeneration of $NAD(P)^+$ from NAD(P)H by activation and reduction of molecular oxygen into water, similar to the mode of action of a NAD(P)H oxidase, is known. The mode of action of such a water-producing NAD(P)H oxidase is depicted in Scheme 1. In addition only a few synthetically suitable NAD(P)H oxidases, which serve as key tools for the "oxidative cofactor regeneration", are known. However, in part these enzymes show a lack of stability under process conditions, different preferences for the two cofactors NADH and NADPH, and the production of unwanted hydrogen peroxide (instead of water) as a by-product.[1b,3] Additionally, from this perspective the availability of an "artificial" biomimetic, water-producing NAD(P)H oxidase would be desirable and a valuable alternative to NAD(P)H-oxidase-type enzymes in preparative syntheses.



Scheme 1. Concept of the NAD(P)H-oxidase-catalyzed or biomimetic in situ cofactor regeneration of $NAD(P)^+$.

Herein we report the application of a synthetic, water-soluble iron(III) porphyrin as an artificial, biomimetic water-producing NAD(P)H oxidase. In analogy to enzymes, the metalloporphyrin is suitable for the in situ regeneration of both cofactors NAD^+ and $NADP^+$ by activation and reduction of molecular oxygen, and is also compatible with different preparative enzymatic oxidative reactions. Furthermore, to the best of our knowledge, this represents the first application of a synthetic metalloporphyrin as a catalyst for the activation and reduction of molecular oxygen into water by means of a natural cofactor in aqueous solution. Additionally a novel alternative is presented for carrying out enzymatic oxidation reactions under in situ regeneration of the oxidized cofactor $NAD(P)^+$ by means of a non-enzymatic, synthetic catalyst serving as an "artificial enzyme mimic".

At the beginning of our work we searched for a low-molecular-weight and water-soluble metal complex that accepts the natural cofactors NAD(P)H as a hydride donor for the activation of molecular oxygen, and would thereby be able to reduce oxygen into water while simultaneously being recycled as a catalyst. As the Fe^{III} porphyrin subunit, located in the active site of monooxygenases, exhibits comparable characteristics in the initial steps of monohydroxylation (though here a one-electron transfer involving a further cofactor takes place),[7] we focused our preliminary screening on low-molecular-weight Fe^{III} complexes having a water-

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soluble porphyrin ligand.[8,9] A set of highly water-soluble porphyrin ligands was recently developed by Jux et al.[10] The Fe^{III} complexes **1–3**, which are based on such porphyrin derivatives having a fourfold and eightfold positively charged or eightfold negatively charged substitution pattern, were used in a spectrophotometric study to evaluate their suitability as catalysts for the oxidation of the cofactor NADH in the presence of molecular oxygen with formation of the oxidized form NAD^+ (water or hydrogen peroxide as by-product). However, none of the water-soluble metalloporphyrin complexes **1–3** were suitable for the oxidative transformation of the cofactor NADH into NAD^+ in aqueous medium (Figure 1). A potential reason for this result could arise from hindered access of the charged cofactor to the iron center because of the sterically demanding (charged) substituents in the iron porphyrin complexes **1–3**.

As an alternative we tested the structurally simplified iron(III) complex **4**,[11] containing the water-soluble, fourfold meso-tetrakis(4-sulfonatophenyl)porphyrin (TSPP) as a ligand. At first we used spectrophotometry to study the suitability of the iron TSPP complex **4** as a catalyst for the oxidation of the cofactor NADH in the presence of molecular oxygen with formation of its oxidized form NAD^+ (and water or hydrogen peroxide as a by-product). Here, the cofactor NADH was successfully accepted as a substrate in aqueous reaction medium and transformed into NAD^+ by oxidation (Figure 1). The activity with respect to the amount of catalyst **4** (in mg) was determined to be 6.3 $U\,mg^{-1}$. Thus, this "biomimetic specific activity" is in an interesting activity range for synthetic applications. Furthermore, **4** is also suitable for the regeneration of the cofactor $NADP^+$ through oxidation of NADPH. For this transformation a lower, but still synthetically attractive specific activity of 3.7 $U\,mg^{-1}$ (59% compared to the activity when using NADH) was obtained. Converting these values into a turn over frequency (TOF), which is more common for chemocatalysts, gave 0.11 s^{-1} for the oxidation of NADH and 0.06 s^{-1} for the oxidation of NADPH. To the best of our knowledge this is the first example demonstrating the suitability of a metal-containing porphyrin complex as a catalyst for the oxidation of the cofactors NADH as well as NADPH with molecular oxygen in an aqueous reaction medium.[12]

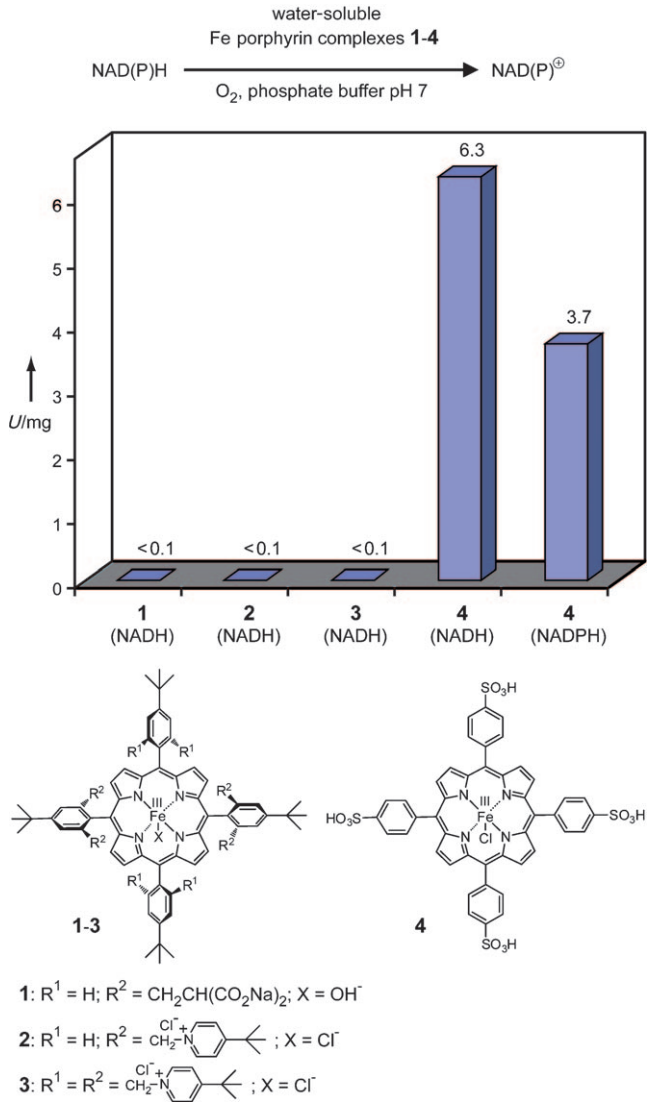
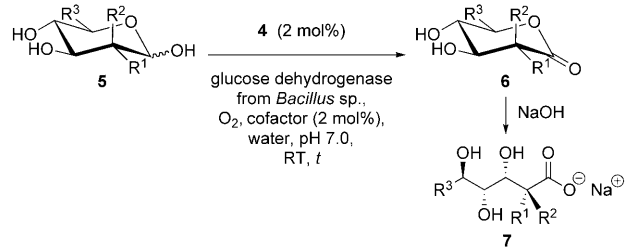


Figure 1. Activities of the iron(III) porphyrins **1–4** for the oxidation of NAD(P)H; the activities of 6.3 $U\,mg^{-1}$ and 3.7 $U\,mg^{-1}$ obtained for catalyst **4** correspond to TOFs of 0.11 s^{-1} and 0.06 s^{-1}, respectively.

Given the encouraging results of the activity studies on the iron TSPP complex **4**, a catalytic biomimetic cofactor oxidation for the in situ regeneration of the cofactor, in combination with a biotransformation in aqueous reaction medium was performed. Accordingly, the cofactor was used in catalytic amounts, thus fulfilling the prerequisite for a "biomimetic NAD(P)H oxidase" (see Scheme 1). For the coupled biotransformation reaction the enzymatic oxidation of D-glucose (**5a**) into D-gluconolactone (**6a**) in the presence of a glucose dehydrogenase was chosen. In this reaction the oxidized form of the cofactor $NAD(P)^+$ is required and transformed into its reduced form. Subsequently, the in situ formed D-gluconolactone (**6a**) is hydrolyzed into D-gluconic acid, which is neutralized by the addition of a solution of sodium hydroxide with the formation of the sodium salt of D-gluconic acid[13] (**7a**), thereby maintaining the pH value at pH 7.0. This synthetic principle based on the use of metalloporphyrin **4** as a "biomimetic NAD(P)H oxidase" is depicted in Table 1.

In the presence of 2 mol% of **4** and 2 mol% of the cofactor NADH, the cofactor regeneration proceeded successfully and by using a glucose dehydrogenase from *Bacillus* sp. the desired sodium salt of D-gluconic acid (**7a**) was obtained with a (product-related) conversion of greater than 95% (Table 1, entry 1). Using the oxidized form of this cofactor, NAD^+, also led to a conversion of greater than 95% as well as when using the cofactor $NADP^+$ (entries 2 and 3). The corresponding turn over numbers (TONs) for the reactions described in entries 1–3 are each in the range of 48–50. Subsequently, additional biotransformations were carried out using other monosaccharides to evaluate the developed method for the in situ cofactor regeneration of $NAD(P)^+$ for these reactions. We were pleased to find that analogously D-mannonic acid (**7b**) and D-xylonic acid (**7c**) were obtained with good to excellent (product-related)

Table 1: Iron(III) porphyrin catalyzed in situ cofactor regeneration for the enzymatic oxidation of monosaccharides **5**.



Entry[a]	Substrate	R^1	R^2	R^3	Cofactor	t [h]	Conv. [%][b]
1	D-glucose (**5a**)	OH	H	CH_2OH	NADH	24	>95
2	D-glucose (**5a**)	OH	H	CH_2OH	NAD^+	24	>95
3	D-glucose (**5a**)	OH	H	CH_2OH	$NADP^+$	24	>95
4	D-mannose (**5b**)	H	OH	CH_2OH	$NADP^+$	113	73
5	D-xylose (**5c**)	OH	H	H	$NADP^+$	66	>95

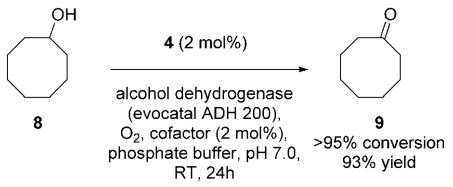
[a] For experimental protocols, see the Supporting Information. [b] The conversion corresponds to the formation of **7** (product-related conversion); the formation of side products in the enzymatic oxidation of **5** was not observed and the hydrolysis of **6** into **7** proceeds quantitatively.

conversions of 73% and greater than 95%, respectively, and TONs of 37 and 48–50, respectively (entries 4 and 5).

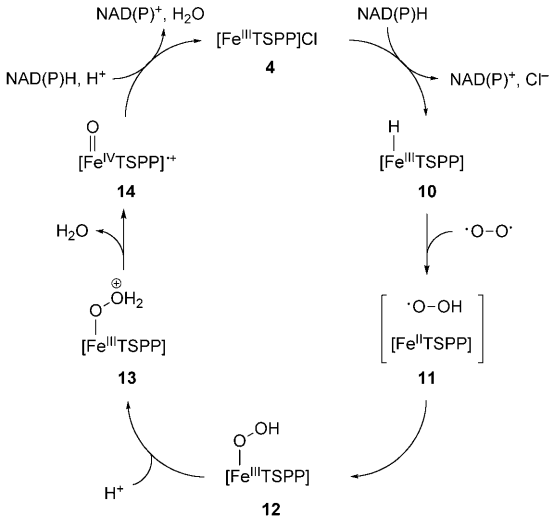
The need for the presence of all involved components (molecular oxygen, glucose dehydrogenase, and **4**) for the catalytic process was additionally confirmed by experiments in which one component had been omitted; none of these experiments showed any activity.[14] An interesting question concerns the type of by-product formed in the reduction of molecular oxygen. In the previous biomimetic synthesis and also in part when using NAD(P)H oxidases, the disadvantageous by-product hydrogen peroxide is formed, which causes deactivation of the involved enzymes and cofactors. Accordingly such reactions proceed successfully only in the presence of a catalase, which is used to decompose the hydrogen peroxide formed in situ.[6] The formation of hydrogen peroxide as a by-product would be also generally conceivable in our reactions. With test reactions being negative with respect to the presence of hydrogen peroxide (see the Supporting Information), and successful biotransformations proceeding without the addition of a catalase, however, a four-electron transfer on molecular oxygen yielding the attractive co-product water can be assumed for our reaction.

Furthermore, the metalloporphyrin **4**, which was successfully identified as a biomimetic NADH oxidase, turned out to be highly suitable for the cofactor regeneration of other oxidative reactions. This is exemplified for the alcohol dehydrogenase catalyzed oxidation of cyclooctanol (**8**) into the corresponding ketone[15] (Scheme 2). Thus, in the presence of an alcohol dehydrogenase, 2 mol% of the catalyst **4**, and cofactor NAD^+ the oxidation to cyclooctanone (**9**) proceeded with an excellent conversion of greater than 95%, and after simple isolation by extraction the desired product **9** was obtained in 93% yield.

The catalytic mechanism of the reaction involving the iron(III) porphyrin **4** is proposed as follows (Scheme 3):



Scheme 2. Iron(III) porphyrin catalyzed in situ cofactor regeneration for the enzymatic oxidation of cyclooctanol (**8**).



Scheme 3. Postulated reaction mechanism of the iron(III) porphyrin catalyzed in situ cofactor regeneration of $NAD(P)^+$.

Starting from the metalloporphyrin **4** and the reduced form of the cofactor, an NAD(P)H iron(III) hydride complex (**10**) is formed[16] and subsequently transformed into the iron hydroperoxo complex **12** by coordination and reduction of molecular oxygen. This step might proceed by homolytic cleavage of the Fe–H bond in the formation of the complex **11**, which consists of an Fe^{II} species and a hydroperoxyl radical. After protonation and elimination of water (analogous to the mechanism of P450-monooxygenases) the formed iron peroxo complex **12** is transformed into a Fe^{IV} oxo species of type **14**.[17] The final step consists of the regeneration of the iron(III) porphyrin **4** and simultaneous formation of water as a by-product.

In conclusion, we have reported the first application of a synthetic metalloporphyrin, namely the complex **4**, as a catalyst for the activation and reduction of molecular oxygen into water by means of a natural cofactor NAD(P)H in aqueous solution. Furthermore the synthetic and water-soluble iron(III) porphyrin **4** proved to be an artificial biomimetic NAD(P)H oxidase, which is compatible with different types of preparative enzymatic oxidations. Thus, a novel non-enzymatic, synthetic catalyst-based technology has been found for the in situ regeneration of the oxidized cofactor $NAD(P)^+$ in synthetic enzymatic oxidations. As a result of the catalytic efficiency and ready availability of the metalloporphyrin **4**, this biomimetic recycling of the cofactors

NAD^+ and $NADP^+$ in water can be regarded as an alternative to enzymatic cofactor regenerations with NAD(P)H oxidases.

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