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Aqueous oxidation of alcohols catalyzed by artificial metalloenzymes based on the biotin–avidin technology

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Abstract

Based on the incorporation of biotinylated organometallic catalyst precursors within (strept)avidin, we have developed artificial metalloenzymes for the oxidation of secondary alcohols using *tert*-butylhydroperoxide as oxidizing agent. In the presence of avidin as host protein, the biotinylated aminosulfonamide ruthenium piano stool complex **1** (0.4 mol%) catalyzes the oxidation of *sec*-phenethyl alcohol at room temperature within 90 h in over 90% yield. Gel electrophoretic analysis of the reaction mixture suggests that the host protein is not oxidatively degraded during catalysis.

Keywords: Artificial metalloenzymes; Ruthenium; Oxidation; Piano stool complex; Biotin–avidin technology

Homogeneous and enzymatic catalysis are in many respects complementary [1–4]. By incorporating an organometallic complex into a host protein, we aim at creating artificial metalloenzymes with properties reminiscent both of enzymes and of homogeneous catalysts. With this goal in mind, we have recently described the incorporation of achiral biotinylated rhodium–diphosphine complexes into (strept)avidin ((strept)avidin refers to either avidin or streptavidin, used as a host protein) [5]. These artificial metalloenzymes were used as catalysts for the enantioselective hydrogenation of *N*-protected dehydroaminoacids (ee up to 96%). The perspective of improving the performance of such artificial metalloenzymes using combined chemical- and genetic optimization strategies (i.e., chemogenetic) provides the opportunity to focus on more challenging reactions. For example, it may be interesting to test the power of such methodology for the oxidation of alcohols. Along similar lines, several groups have recently reported the creation of artificial metalloenzymes for the enantioselective oxidation of sulfides to sulfoxides [6–8].

The oxidation of alcohols to the corresponding aldehydes and ketones is one of the most important functional group transformations in organic synthesis. While alcohol dehydrogenases often perform this task very efficiently [3,4], mild homogeneous catalysts are scarce. Traditionally, these reactions have been performed with stoichiometric chromium reagents. However, today's environmental restrictions render most of the stoichiometric metal oxidants obsolete. For this purpose, the use of *tert*-butylhydroperoxide (TBHP) as an oxidizing agent has been investigated in conjunction with several homogeneous transition-metal catalysts, including chromium, ruthenium, rhodium, and copper [9–13].

The present Communication outlines our efforts to create active artificial metalloenzymes for the oxidation of alcohols using biotinylated transition metal complexes in the presence of (strept)avidin as host protein.

In contrast to transition-metal catalyzed hydrogenation reactions (where nearly any rhodium–diphosphine moiety is an active catalyst), the number of versatile

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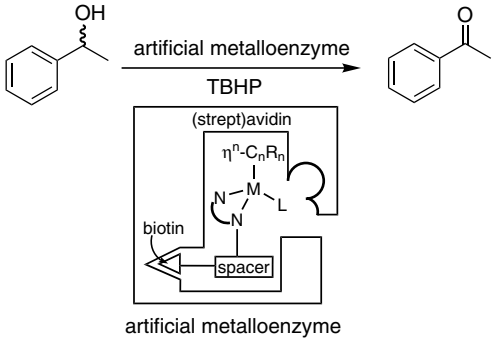


Scheme 1. Artificial metalloenzymes for the oxidation of *sec*-phenethyl alcohol based on biotinylated d^6 piano stool complexes.

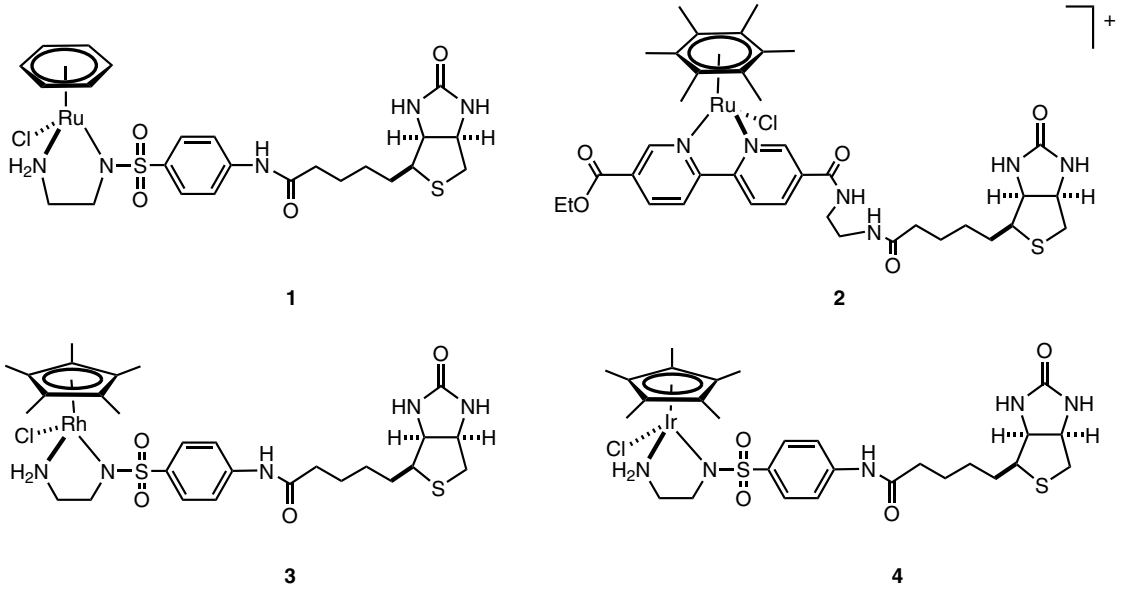
organometallic catalysts for the mild oxidation of secondary alcohols in water is limited [14–16]. With no homogeneous system to build upon, we decided to focus on d^6 piano stool complexes as catalyst precursors as illustrated in Scheme 1. Biotinylation of the metal was introduced by means of a chelating ligand, thus ensuring stability of the coenzyme as well as its quantitative incorporation within (strept)avidin [5].

Initial oxidation experiments using *sec*-phenethyl alcohol as substrate were performed with biotinylated amino-sulfonamide Ru(II) catalyst **1** (Scheme 2). A detailed experimental procedure is provided in the Supporting Information. Using streptavidin as a host protein, we found that, in the presence of **1**, the oxidation proceeded efficiently using *tert*-butylhydroperoxide (TBHP) as terminal oxidant. When *sec*-phenethyl alcohol (250.0 equiv., 62.5 μmol) was treated with aqueous TBHP (300.0 equiv., 75.0 μmol) in the presence of the ruthenium coenzyme **1** (1.0 equiv., 0.25 μmol) dissolved in DMF (31 μl), streptavidin (0.08 μmol of the tetrameric protein) in a mixture of water (500 μl) and acetone (150 μl), acetophenone was obtained with 81% conversion after 90 h at room temperature (Table 1, entry 1).

Extraction of the reaction mixture with pentane allows to isolate the product devoid of any traces of the hybrid catalyst which remains exclusively in the aqueous phase. Following this treatment, the aqueous orange-red solution was subjected to non-denaturing gel-electrophoresis. Inspection of the gel suggests that, despite the presence of a strong oxidizing agent, the host protein remains tetrameric (Fig. 1). There is no evidence for protein degradation by oxidative cleavage or denaturation (to afford monomeric streptavidin). Performing the reaction in the absence of host protein under the same conditions, the reaction is nearly quantitative, but affords a black residue, suggesting the formation of RuO_2, which is supposed to result from the degradation of an arene-ruthenium complex by *tert*-butylhydroperoxide [17].

As control experiments, the catalytic reaction was carried out in the absence of either the ruthenium catalyst or TBHP. The presence of both compounds was found to be essential. No oxidized product was detected in the absence of TBHP (thus excluding an Oppenauer-type oxidation with acetone acting as a hydrogen acceptor [15]), and very low conversions were obtained in the absence of the ruthenium complex (<1% based on *sec*-phenethyl alcohol). Carrying out the reaction in the presence of air revealed no difference, while substitution of TBHP with H_2O_2 afforded no trace of oxidized product.

While *sec*-phenethyl alcohol was oxidized to afford exclusively acetophenone, oxidation of benzyl alcohol (entry 2) afforded a mixture of benzaldehyde and



Scheme 2. Biotinylated organometallic coenzymes tested for the oxidation of alcohols.

Table 1
Oxidation of alcohols catalyzed by different artificial metalloenzymes[a]

Entry	Coenzyme	Substrate	Host protein	Conversion (%)[b]
1	**1**	Phenethyl alcohol	Streptavidin	81
2	**1**	Benzyl alcohol	Streptavidin	68
3[c]	**1**	Cyclohexanol	Streptavidin	80
4[d]	**1**	Phenethyl alcohol	Streptavidin	43
5	**2**	Phenethyl alcohol	Streptavidin	69
6[c]	**3**	Phenethyl alcohol	Streptavidin	3
7[c]	**4**	Phenethyl alcohol	Streptavidin	12
8	**1**	Phenethyl alcohol	Ser112Gly	75
9	**1**	Phenethyl alcohol	Pro64Gly	70
10	**1**	Phenethyl alcohol	Avidin	92

[a] Unless otherwise stated, the reaction was carried out under nitrogen at room temperature for 90 h with alcohol (62.5 μmol), aqueous TBHP (75.0 μmol), coenzyme (0.25 μmol) dissolved in DMF (31 μl), protein (0.08 μmol) dissolved in a mixture of water (500 μl) and acetone (100 μl).
[b] Determined by GC integration.
[c] The reaction was performed for 140 h.
[d] The coenzyme was dissolved in DMSO (31 μl).

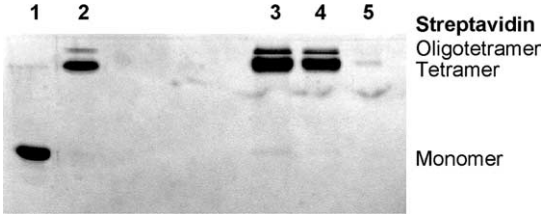


Fig. 1. Non-denaturing gel-electrophoresis demonstrating the integrity of the host protein following a catalytic run. (Lane 1: denatured pure streptavidin, revealing monomeric protein; Lane 2: non-denatured pure streptavidin, revealing tetrameric and oligotetrameric protein; Lane 3: aqueous reaction mixture resulting from a catalytic run, identical to Lane 2; Lanes 4 and 5: supernatant and precipitate, respectively, resulting from centrifugation at 16000*g* of a catalytic run).

benzoic acid in a ratio of 4:1 (68% total conversion (Table 1, Entry 2)).

With cyclic alcohols such as cyclohexanol (Table 1, Entry 3), the corresponding ketone was obtained without the formation of ring-opened or lactone products (80% conversion).

Using DMSO instead of DMF to solubilize the catalyst precursor, led to a decrease in conversion (Table 1, Entry 4). Dimethyl sulfoxide can act as a reductant in certain aerobic oxidation reactions catalyzed by late transition metals and dimethyl sulfone is produced during the reaction [18]. In contrast, DMSO is also a stoichiometric oxidant in a variety of chemical [19,20] and biological [21] oxidation reactions, yielding dimethyl sulfide as a byproduct. However, in our case there is no evidence for presence of either dimethyl sulfone or dimethyl sulphide (GC analysis). Presumably DMSO lowers the oxidation rate by coordinating to the Ru(II) center.

To optimize the catalytic activity of such metalloenzymes, two complementary approaches can be pursued: chemical modification of the first coordination sphere of the biotinylated catalyst or genetic modification of the protein.

Our first attempts focused on the use of a biotinylated bipyridine Ru(II) catalyst **2** (Table 1, Entry 5). With this coenzyme, a slight decrease in catalytic activity was observed (69% conversion, compared to 80% conversion for the sulfonamide Ru(II) complex **1**).

We also examined the oxidation of *sec*-phenethyl alcohol in the presence of [Rh(η^5-C_5Me_5)(Biot-Ligand)] **3** and [Ir(η^5-C_5Me_5)(Biot-Ligand)] **4**. The results displayed in Table 1 suggest that the iridium catalyst **4** was more active that its rhodium analog **3**. However, both systems are much less active than the corrresponding ruthenium-based catalysts **1** and **2**: acetophenone was obtained in 3% yield and 12% yield after 140 h with catalyst precursors **3** and **4**, respectively (Table 1, Entries 6–7).

Having identified the most promising organometallic fragment (Table 1, Entry 1), we subjected the host protein to site-directed mutagenesis. Two streptavidin mutants were tested with the most promising organometallic catalyst precursor **1**. Neither Ser112Gly nor Pro64Gly streptavidin mutants displayed enhanced activity (Table 1, Entries 8–9). Using avidin as the host protein, we found that, in the presence of **1**, the oxidation of *sec*-phenethyl alcohol proceeds nearly to completion (Table 1, Entry 10).

In summary, we have developed *active* artificial metalloenzymes for the oxidation of alcohols in water based on the non-covalent incorporation of biotinylated d^6 piano stool complexes in (strept)avidin. Having established that the protein does not suffer from oxidative damage despite the harsh reaction conditions, our next goal is to develop *selective* artificial metallo-alcohol dehydrogenases for the kinetic resolution of secondary alcohols.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2005.02.001.

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