ChemComm

Cite this: *Chem. Commun.*, 2012, **48**, 5745–5747

www.rsc.org/chemcomm

COMMUNICATION

Ferritin-supported palladium nanoclusters: selective catalysts for aerobic oxidations in water†

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Received 24th February 2012, Accepted 17th April 2012
DOI: 10.1039/c2cc31401k

Confinement of nanometallic Pd within the core of a hyperthermophilic ferritin cage (from *Pyrococcus furiosus*) is reported. The resulting nanostructured hybrid catalysts can be used for highly specific aerobic oxidation of alcohols in water.

Alcohol oxidation is one of the most fundamental and central reactions in organic chemistry, also on an industrial scale.[1] It is quite astounding that today 'traditional' oxidants such as chromates and hypochlorites still prevail on the preparative scale.[1] Next to environmental issues, selectivity is often not satisfactory. Hence, the quest for more sustainable and selective oxidation systems is ongoing. As stoichiometric oxidants, molecular oxygen and hydrogen peroxide appear appealing due to their high thermodynamic driving force and the unproblematic side products formed. To activate O_2 or H_2O_2, a broad variety of transition metal and enzyme catalysts have been investigated.[2]

Water is an attractive solvent for O_2-driven oxidations as the formation of explosive organic hydroperoxides is not an issue here. Previously, we and others have reported on highly active, water soluble Pd complexes for the selective oxidation of primary and secondary alcohols.[3]

For the use of metal catalysts in water, Pd-nanoclusters are emerging as key-players, following the pioneering work of Moiseev and co-workers in the 1980's.[4] However, controlling the size and morphology of these nanoclusters is a major challenge.[5] Polymeric and biopolymeric matrices have been shown to be especially effective in attaining narrow size distributions of Au, Pd or Pt nanoclusters within chitosan,[6] polyvinyl-2-pyrrolidone (PVP)[7] and polystyrene matrices.[8]

In that respect confining Pd-nanoclusters within a protein core as *e.g.* supplied by ferritins may be an attractive method.[9] Indeed, metallic nanoparticles of Pd,[10a] Pt,[10b] Au,[10c,d] Ag,[10c,d] Cu[10e] and Ni[10f] have been reported. However, so far only ferritins from mesophilic host organisms have been reported, which limits the application of this nanoparticle@ferritin concept to chemical transformations proceeding well at mild temperature. Furthermore, even then the robustness of these composite particles is limited.

Here, we report on the application of a highly stable ferritin from the hyperthermophile *Pyrococcus furiosus* (*Pf*Ferritin)[11] to overcome the aforementioned limitation, which we demonstrate using the example of nano-Pd-catalysed oxidation of alcohols at elevated temperatures.

*Pf*Ferritin was produced by recombinant expression in *Escherichia coli* followed by simple heat denaturation and precipitation of the mesophilic host proteins.[11] As a result, essentially pure *Pf*Ferritin (as judged by SDS-gel electrophoresis) was obtained. To obtain the Pd-loaded *Pf*Ferritin apo-*Pf*Ferritin was incubated with Pd^{2+}-salts followed by size exclusion chromatography purification of the Pd-loaded *Pf*Ferritin and H_2-driven reduction (ESI†). Overall, the desired nano-Pd@*Pf*Ferritin composite material was obtained in high purity through a very simple procedure.

Microscopic and spectroscopic characterisation of this material revealed that indeed Pd-nanoparticles of uniform size (5 ± 1 nm) were formed within the *Pf*Ferritin core (ESI†). The maximal capacity of *Pf*Ferritin to accommodate Pd atoms was 200, which is in good accordance with previous reports.[12] Interestingly, the composite material, examined with dynamic light scattering, appeared to be somewhat smaller than apo-*Pf*Ferritin (Fig. 1). It remains to be clarified whether this is a spectroscopic artefact or the result of a true shrinkage, possibly due to attractive Pd-nanoparticle–*Pf*Ferritin interactions.

Next, we investigated the catalytic properties of nano-Pd@*Pf*Ferritin for the oxidation of primary and secondary alcohols (Table 1). Pleasingly, nano-Pd@*Pf*Ferritin catalysed the aerobic oxidation of a range of benzylic primary alcohols

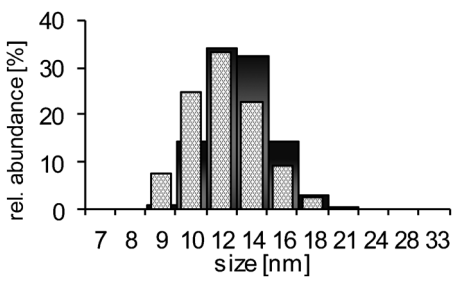

Fig. 1 Size distribution of apo-*Pf*Ferritin (■) and nano-Pd@*Pf*Ferritin (▒) determined by DLS.

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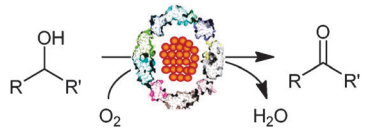
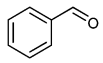
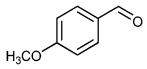
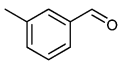
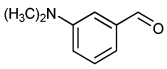
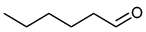
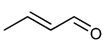
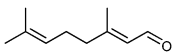
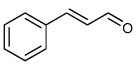
† Electronic supplementary information (ESI) available: Detailed experimental procedures and supporting experimental data. See DOI: 10.1039/c2cc31401k

Table 1 Substrate scope of nano-Pd@*Pf*Ferritin in the aerobic oxidation of various alcohols



Product[a]	Yield [%]	TON[b]
	16	37
	61	138
	21	48
	8	19
	4	12
	0	0
	12	36
	0	0
	67	197
	1	3
	1	3
	44	101

Conditions: [Substrate] = 40 mM, [nano-Pd@*Pf*Ferritin] = 1.3 μM in water (unbuffered), T = 80 °C, p = 30 bar (of a O_2/N_2 8 : 92 mixture), t = 24 h.[a] No other products were detectable. [b] Turnover number calculated on the basis of Pd.

and certain allylic alcohols. In all cases, the oxidation proceeded selectively to the aldehyde stage and no overoxidation products or any other side products were detectable *via* GC- and HPLC-analysis. Non-activated and sterically demanding primary alcohols as well as secondary alcohols were somewhat sluggish substrates. The stability of benzaldehydes can be rationalized by the fact that Pd is a poor aldehyde oxidation catalyst (in contrast to Au catalysts[13]). In addition autoxidation of benzaldehyde is rather slow, especially in the presence of (traces of) benzyl alcohol.[14]

In order to gain more insight into the oxidation mechanism, we investigated the initial rate of oxidation of a range of *m*- and *p*-substituted benzylalcohols. Generally, electron-donating substituents accelerated the oxidation rate. From the corresponding Hammett plot (Fig. 2) a ρ-value of −1.65 was calculated for

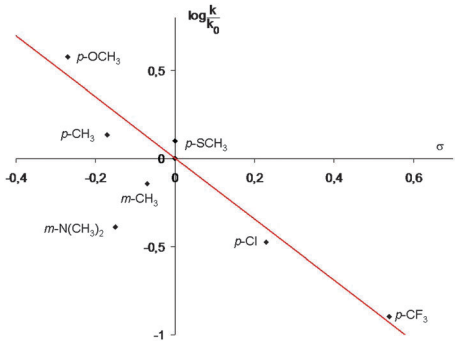


Fig. 2 Hammett plot for the aerobic oxidation of substitute benzyl alcohols by nano-Pd@*Pf*Ferritin. Conditions: [benzyl alcohol] = 40 mM, [nano-Pd@*Pf*Ferritin] = 1.3 μM in water (unbuffered)/DMSO (80/20), p = 30 bar (of a O_2/N_2 8 : 92 mixture), t = 15 h.

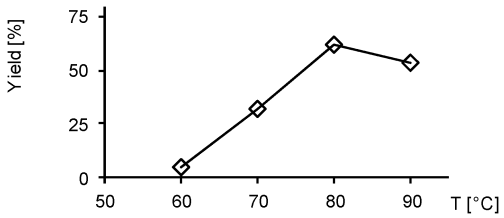


Fig. 3 Time course of the nano-Pd@*Pf*Ferritin-catalysed oxidation of 4-methoxybenzyl alcohol. Conditions: [4-methoxybenzyl alcohol] = 40 mM, [nano-Pd@*Pf*Ferritin] = 1.3 μM in water (unbuffered), p = 30 bar (of a O_2/N_2 8 : 92 mixture), t = 15 h.

the linear free energy relationship indicating the buildup of a positive charge in the transition stage, possibly by hydride abstraction from the alcohol-C-atom. This value is considerably more negative than that for the previously reported water-soluble Pd–phenanthroline catalyst (ρ-value of −0.58).[15]

The temperature-dependence of the nano-Pd@*Pf*Ferritin-catalysed oxidation was somewhat unexpected as the reaction rate increased only linearly with temperature (Fig. 3). This apparent deviation from exponentiality might be an indication of diffusion being the overall rate-limiting step of the oxidation sequence.[16] However, further experiments will be necessary to clarify this. The apparent optimal reaction temperature around 80 °C most likely is the result of increasing reaction rate and decreasing protein stability under the given reaction conditions. Nevertheless, the catalyst could be reused at least twice without significant loss in activity (ESI†).

The typical progression curve of nano-Pd@*Pf*Ferritin-catalysed oxidations exhibited an interesting behavior. Following a high initial reaction rate, the activity of nano-Pd@*Pf*Ferritin always decreased by almost one order of magnitude over time (Fig. 4, ■). The catalyst however remained active. After 2.5 days 80% conversion of 2-methoxy benzyl alcohol was observed.

We suspected a sort of product inhibition to account for this decrease in the reaction rate, which was validated in a control experiment after preincubation of nano-Pd@*Pf*Ferritin with benzaldehyde. As a result, a linear time course was observed (Fig. 4, ◊). Though further experiments will be necessary to clarify the nature of this aldehyde-induced inhibition, it may be speculated that reversible Schiff base formation (*e.g.* with Lys145 and/or Lys156) reduces the channel size and therewith increases the diffusion limitation.[17]

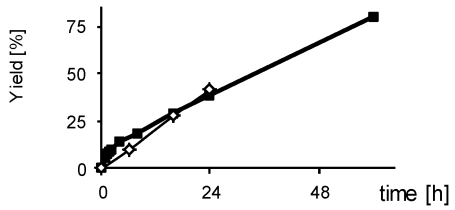


Fig. 4 Time course of the nano-Pd@*Pf*Ferritin-catalysed oxidation of 4-methoxybenzyl alcohol. Conditions: [4-methoxybenzyl alcohol] = 40 mM, [nano-Pd@*Pf*Ferritin] = 1.3 μM in water (unbuffered), p = 30 bar (of a O_2/N_2 8 : 92 mixture), (■) time course of a representative reaction, (◊) time course in the presence of 40 mM benzaldehyde.

In conclusion, we have prepared a well-defined novel metal–protein hybrid catalyst using the ferritin cage from *P. furiosus*. The high thermal stability of *Pf*Ferritin makes it an attractive alternative to the commonly used mesophilic analogs due to (1) facile preparation in high purity and (2) high robustness, also under demanding reaction conditions. This robustness was demonstrated using the example of aerobic oxidation of alcohols at elevated temperatures.

Further experiments elucidating the scope of *Pf*Ferritin as a host for nano-particulate catalysts are currently ongoing in our laboratory.

This project was financially supported by the Dutch Ministry of Economic Affairs and the B-Basic partner organizations (www.b-basic.nl), through B-Basic, a public–private NWO-ACTS programme. Dr Ing. V. C .L. Butselaar and Dr H.-P. de Hoog are acknowledged for performing the electron microscopy measurements.

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