Merging homogeneous catalysis with biocatalysis; papain as hydrogenation catalyst†

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Papain, modified at Cys-25 with a monodentate phosphite ligand and complexed with $Rh(COD)_2BF_4$, is an active catalyst in the hydrogenation of methyl 2-acetamidoacrylate.

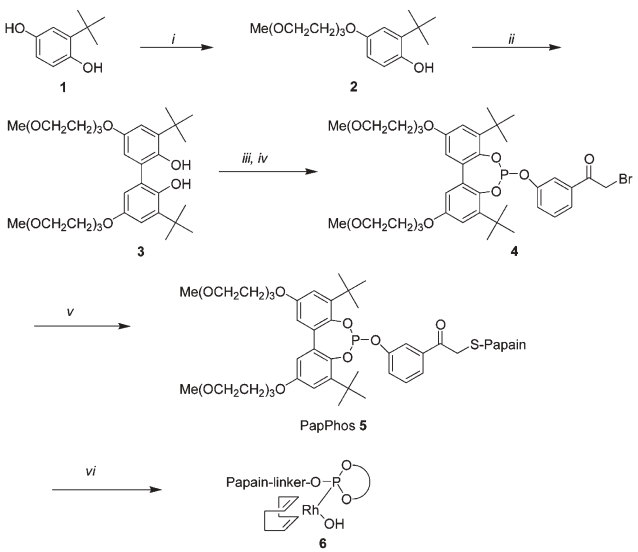
Although asymmetric catalysis has reached an advanced stage in the laboratory, the method of choice for the preparation of enantiopure intermediates, in the production of pharmaceuticals, is resolution by crystallization of diastereomeric salts.[1] One of the main reasons for this is the strong time-to-market pressure related to their production. Fortunately, high throughput experimentation can be used increasingly for the rapid identification of a chiral catalyst.[2] The availability of large libraries of chiral catalysts and, in particular, of the corresponding ligands can be, however, a serious bottleneck.[3] This problem is aggravated by the cumbersome and lengthy syntheses that are associated with bidentate phosphorus-based ligands. For this reason, we and others have developed simple monodentate ligands that can be synthesised in few simple synthetic steps.[4] These ligands can be synthesised in an automated manner, opening the doors to large libraries.[5]

In view of the tremendous advance in the methods for preparation of large libraries of proteins[6,7] we were wondering if proteins could be used as a convenient source of chirality to obtain chiral transition metal catalysts. Few examples have been reported in the area of semi-synthetic enzymes.[8] Up until now, the growing efforts of the scientific community toward the creation of artificial metallo-enzymes have been concentrated on either covalent incorporation of co-factors or supramolecular approaches.

Remarkable examples of the non-covalent approach are provided by the studies of Whitesides[9] and Ward[10] based on the attachment of a rhodium catalyst to biotin, allowing its complexation to avidin resulting in an enantioselective hydrogenation catalyst.[11] Keinan and Mahy, instead, reported antibodies containing metal porphyrin complexes. In the work of Watanabe, apo-myoglobin was reconstituted with Cr^{III} Schiff base complexes, whilst Gross adopted iron and manganese corrole complexes conjugated with albumin. The metallo-enzymes obtained were all tested in asymmetric sulfoxidation reactions.[12]

In the covalent approach instead, following the pioneering work of Kaiser,[13] work has been done on the chemical modification of reactive residues in proteins.[8] Davies and Distefano, for example, introduced a Cu^{II} 1,10-phenanthroline complex in adipocyte lipid binding protein, whilst Janda proposed an aldolase antibody derivatized with a Cu-binding bis-imidazolyl co-factor; both systems showed hydrolytic activity.[14] Moreover, Reetz reported the introduction in papain of maleimide based manganese–salen and Rh–dipyridin complexes as epoxidation and hydrogenation catalysts.[15] Another example of covalent attachment of a manganese–salen complex into apo sperm whale myoglobin has been recently presented by Lu and tested in enantioselective sulfoxidation.[16]

Based on the highly successful use of monodentate phosphorus ligands in asymmetric catalysis,[17] we focused on the challenging goal of building a hybrid enzyme-bound rhodium catalyst with a single phosphorus donor ligand for hydrogenation and hydroformylation.[18] The phosphorus ligand would be attached directly



Scheme 1 Synthesis of the artificial enzyme. Reagents and conditions: (i) $Me(OCH_2CH_2)_3Cl$, Cs_2CO_3, CH_3CN, 80 °C, overnight, 80%.[22] (ii) $K_3[Fe(CN)_6]$, NaOH, H_2O/acetone, RT, 2 h, 60%.[23] (iii) PCl_3, Et_3N, toluene, 0 °C, 2 h. (iv) 3-Hydroxy-phenacyl bromide,[24] Et_3N, toluene, 0 °C, 2 h, 72% (over 2 steps).[25] (v) Papain (pre-treated with DTT), phosphate buffer pH 7.0/dioxane (4/1), RT, 3 h. (vi) $[Rh(COD)_2]BF_4$ (8 eq.) in water/dioxane (9/1).

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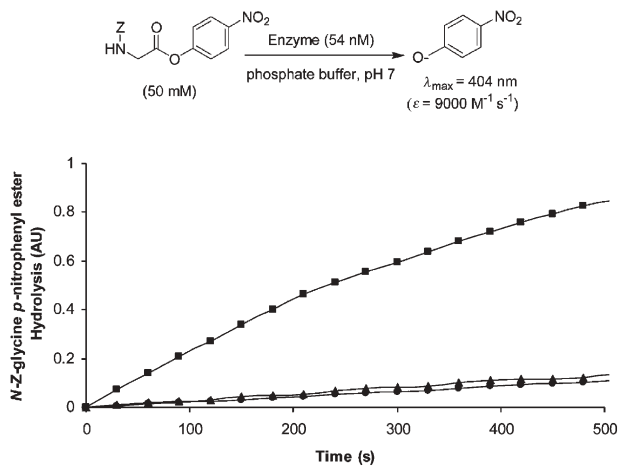
to the enzyme in a single well-defined position, preferably in or near the active site. Since cysteine proteases contain a highly nucleophilic SH group in the active site we decided to take this class of enzymes as a starting point. Our study has focused on papain, a readily available cysteine protease, which also has the advantage that many small molecules have been identified that bind selectively to Cys-25 in its active site.[19] Other advantages are that expression systems have been described[20] and the X-ray structure is known.[21]

Several requirements need to be met when designing such a catalyst. First of all, there is an unfavourable molecular weight ratio between the enzyme and the transition metal, demanding a highly active transition metal catalyst. For this reason, we have focused on the use of a bulky phosphite ligand, based on the principle that ligands with large cone angles may lead to unsaturated transition metal complexes, which have proven to be highly active catalysts in hydroformylation[26] and in aromatic substitution.[27] The second requirement is hydrolytic stability. This criterion can be met by the presence of bulky groups near the phosphorus centre.[28] Finally, the ligand needs to have sufficient water solubility to enable its coupling to the enzyme. For the attachment of the ligand to the enzyme we decided to use a phenacyl bromide moiety as a linker.

In Scheme 1 the synthesis of the ligand is depicted. 2-*tert*-Butylhydroquinone (**1**) was monoalkylated with the triethyleneglycol based synthon to provide **2**, which was oxidatively dimerised to bisphenol **3**. Reaction with PCl_3 followed by 3-hydroxy-phenacyl bromide furnished **4**, the desired ligand connected to an electrophilic moiety, ready for the reaction with papain.[29]

The reaction of **4** with papain was monitored by measuring the hydrolytic activity of papain as shown in Scheme 2.[30] After 3 h almost complete inhibition was observed; the residual activity shown in the graph is comparable to the hydrolysis of the substrate in the absence of the enzyme.



Scheme 2 Monitoring of the reaction of **4** with papain by measuring the hydrolytic activity of the enzyme (■ = native enzyme, ▲ = enzyme treated for 3 h with an excess of **4**, ● = in the absence of enzyme).

The successful modification of papain was also confirmed by ESI-MS analysis (Fig. 1). The analysis also showed that only a single molecule of ligand had been incorporated, as expected in consideration of the high reactivity of Cys-25 compared to other

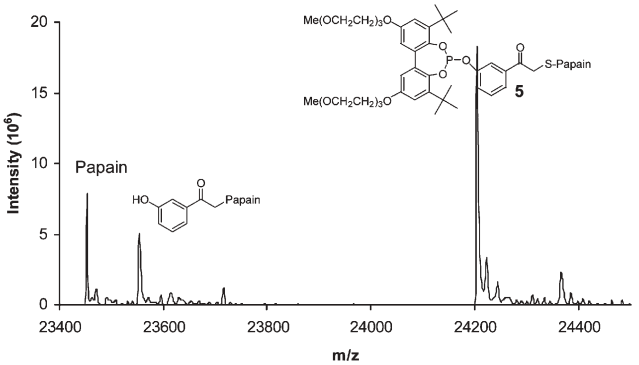


Fig. 1 ESI mass spectrum of the modified enzyme **5**.

residues.[31] In addition, a protein digestion experiment showed that modification occurred at Cys-25.

After treatment of **5** with an excess of $[Rh(COD)_2]BF_4$ the resulting yellow solution was purified on a desalting polyacrylamide column and the desired metallo-enzyme **6** was obtained.[32] Mass spectral analysis of the product confirmed the presence of a single rhodium and a single phosphite group; we thus assume that the rhodium is solely bound to the phosphite group (Fig. 2), this also being the best ligand for the metal.[33]

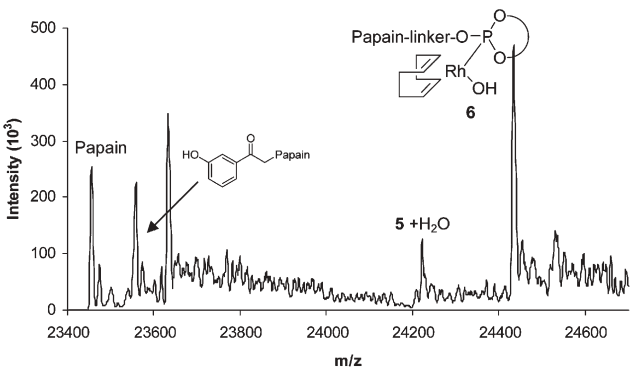
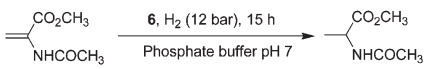


Fig. 2 ESI mass spectrum of **6** after purification.

The enzyme conjugate **6** is an active hydrogenation catalyst. At a low catalyst loading (substrate/catalyst ratio of 400), **6** was capable of hydrogenating methyl 2-acetamidoacrylate with 100% conversion in a buffered aqueous solution overnight at 12 bar H_2 (Scheme 3).[34]



Scheme 3 Hydrogenation of methyl 2-acetamidoacrylate.

As a control we have treated native papain with the rhodium precursor and purified it in exactly the same manner. Hydrogenation reaction under exactly the same conditions with the control gave less than 2% conversion. This result confirms that the phosphite-bound rhodium is responsible for the observed catalytic activity.

Although the hydrogenation reaction performed using **6** yielded the desired alanine derivative with 100% selectivity, no

enantioselectivity was observed. We thus conclude that the catalyst could be too flexible or too far removed from the chiral environment of the enzyme. Another possible explanation is that the cavity of papain, chosen as spacious enough to accommodate ligand, metal and substrate,[35] might be too large to guarantee enough secondary interactions necessary to ensure a more structurally defined metal complex. In all cases multiple conformations of the complex could be present. Such a possibility also seems to be suggested by the low enantioselectivity (<10% ee) reported by Reetz and co-workers while using papain with different ligands.[15]

In conclusion, we have shown that it is possible to convert a hydrolytic enzyme into a unique fully functional hydrogenation catalyst by attaching a single phosphorus ligand to its active site and treating it with a rhodium precursor. We are convinced that the concept is very powerful, allowing in principle the use of the same ligand with different proteins possessing a single reactive cysteine, either already present or engineered in the protein sequence. Nevertheless, further studies are necessary to establish satisfactory stereocontrol. In this respect the recent publication of Lu, showing the beneficial effect of double anchoring of a salen catalyst on the enantioselectivity of the sulfoxide product, seems to provide an interesting approach.[16] Ongoing research will also involve the use of different proteins and the use of this metalloenzyme as hydroformylation catalyst.

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