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A Genetically Encodable Ligand for Transfer Hydrogenation

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Simple tripeptides are shown here to be versatile ligands for iridium-catalyzed transfer hydrogenations affording large acceleration effects. A water-soluble iridium complex with Gly-Gly-Phe, for example, catalyzes the reduction of diverse ketones, aldehydes, and imines by formate with turnover frequencies rivaling or outperforming those of established ligand systems. Regioselective reduction of coenzyme NAD^+ to NADH illustrates the potential utility of this system for biotechnological applications. Because peptides are genetically encodable, they represent an attractive class of foldamer ligands for creating artificial metalloenzymes.

Introduction

Nature utilizes metal ions bound to protein scaffolds to effect a wide range of demanding chemical transformations with enviable ease.[1] The large rate accelerations and exacting selectivities achieved by such systems have inspired researchers to explore the feasibility of creating artificial metalloenzymes for reactions lacking biological counterparts.[2]

Both the peptidic backbone and amino acid side chains are useful for coordinating metal ions. Over the last two decades, computational search algorithms have been developed to design metal binding sites in structurally characterized proteins that take advantage of such interactions.[3] These programs have been successfully used to arrange constellations of residues in geometries appropriate for binding a range of metals ions, including zinc, copper, and iron.[4] De novo proteins have also been equipped with unique metal-binding capabilities and, as a result, appreciable hydrolytic and redox activities.[5] The ability to incorporate non-natural, metal-chelating amino acids site-selectively into proteins is likely to extend these strategies in the future.[6] Nonetheless, the design of functional metalloenzymes from scratch remains challenging. Avoiding unproductive interactions of the metal ions with residues elsewhere in the protein is one challenge. Tailoring the second and third coordination environments around the metal ion to fine-tune reactivity is another. Engineering a substrate binding site in proximity to the metal ion is a further hurdle.[2c]

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An alternative approach toward artificial metalloenzymes involves anchoring an organometallic complex to a protein scaffold.[2a,2b] Catalysts for alkene hydrogenation,[7] transfer hydrogenation of ketones and imines,[8] olefin metathesis,[9] and other activities[10] have been created by combining the intrinsic reactivity of a metal cofactor with the specificity of a protein in this way. Nevertheless, such hybrid catalysts are typically much less effective than their natural counterparts with respect to turnover number and selectivity. These shortcomings can largely be attributed to trade-offs made in the design process. The organometallic complex is usually attached to the scaffold protein through a flexible linker that is equipped either with a reactive chemical group for site-selective protein modification (covalent anchoring) or a small ligand such as biotin with unusually high affinity for a specific protein pocket (supramolecular anchoring).[2a,2b] The flexibility of the linker may limit accurate placement of the metal center in a defined environment, hampering effective preorganization of substrate and catalyst. Unlike natural metalloenzymes, residues at the active site of these hybrid constructs rarely interact productively with the cofactor (see recent work on C–H activation for a notable exception[10b]), making fine-tuning of metal properties difficult. As a consequence, the unbound metal complex usually displays higher activity than its bound form. In principle, artificial metalloenzymes can be improved by evolutionary approaches, but the optimization process is tedious and often unsuccessful.[11]

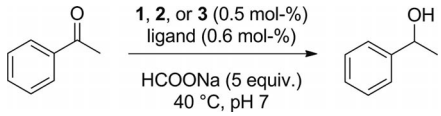
Short peptidic motifs with the ability to bind metal ions directly and thereby enhance catalytic prowess could obviate some of the problems associated with linkers in semisynthetic metalloenzymes.[12] As modular and genetically encodable elements, peptides can be easily embedded as tags in larger foldameric structures, potentially providing greater control over metal ion placement and reactivity. Transfer hydrogenations, important synthetic transformations in which unsaturated substrates are reduced by hydrogen do-

nors other than molecular hydrogen,[13] are attractive reactions for exploring this possibility. A myriad of transition-metal complexes with amino acids, proline derivatives, and pseudopeptides have been reported to promote this reaction under mild conditions in water.[14] In this work, we show that short peptide sequences also function as versatile ligands for iridium-based transfer-hydrogenation catalysts, providing large ligand-induced accelerations. The biocompatibility of such complexes could be advantageous for a range of biotechnological applications.

Results and Discussion

Despite the well-established capabilities of peptides to bind metals,[15] aqueous transfer hydrogenations have not been systematically studied with unmodified peptide ligands. Here, the tripeptide Gly-Gly-Phe was chosen as a simple test ligand. It contains a free amino group at its N terminus, a carboxylate at its C terminus, and an aromatic chromophore to facilitate purification and concentration determination. Our model reaction was transfer hydrogenation of acetophenone by formate catalyzed by the readily accessible, water-soluble, piano-stool complexes [Ru(*p*-cym)$(H_2O)_3]SO_4$ (**1**), [RhCp*$(H_2O)_3]SO_4$ (**2**), and [IrCp*$(H_2O)_3]SO_4$ (**3**) (*p*-cym = *p*-cymene, Cp* = pentamethylcyclopentadiene).

Experiments were carried out in water at neutral pH and at 40 °C with 50 μM catalyst, 10 mM substrate, and 50 mM sodium formate (Scheme 1). In the absence of peptide, the catalysts displayed no detectable activity (Table 1, entries 1–3; Supporting Information, Figure S1A). Addition of 1.2 equiv. of Gly-Gly-Phe to ruthenium and rhodium complexes **1** and **2** marginally increased the reaction rates (Table 1, entries 4 and 5). In contrast, adding the tripeptide to iridium complex **3** gave quantitative conversion and more than 140 turnovers per hour (Table 1, entry 6; Supporting Information, Figure S1B). Even higher turnover numbers were achieved by increasing the formate concentration. Thus, doubling the number of reducing equivalents afforded 200 turnovers per hour at pH 8 (Table 1, entry 7; Supporting Information, Figure S2). At pH 7 and a substrate/catalyst ratio of 2000:1, the reduction of acetophenone proceeded to >96% conversion after 16 h, corresponding to >1900 total turnovers (Supporting Information, Figure S3). Acetylation of the N terminus of the peptide ligand completely abrogated activity (Table 1, entry 8), whereas replacement of the C-terminal carboxylate with an amide did not significantly affect catalyst performance (Table 1, entry 9; Supporting Information, Figure S4A). Scrambling the tripeptide sequence also diminished catalytic efficiency.



Scheme 1. Transfer hydrogenation of acetophenone in water with peptidic ligands.

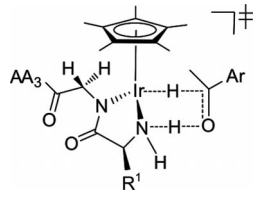
Table 1. Transfer hydrogenation of acetophenone with **1**, **2**, and **3** with peptidic ligands.

Entry	Complex[a]	Ligand	TOF[b] (h^{-1})
1	[Ru(*p*-cym)$(H_2O)_3]SO_4$ (**1**)	none	<1
2	[RhCp*$(H_2O)_3]SO_4$ (**2**)	none	<1
3	[IrCp*$(H_2O)_3]SO_4$ (**3**)	none	<1
4	[Ru(*p*-cym)$(H_2O)_3]SO_4$ (**1**)	Gly-Gly-Phe	2
5	[RhCp*$(H_2O)_3]SO_4$ (**2**)	Gly-Gly-Phe	1
6	[IrCp*$(H_2O)_3]SO_4$ (**3**)	Gly-Gly-Phe	142
7[c]	[IrCp*$(H_2O)_3]SO_4$ (**3**)	Gly-Gly-Phe	200
8[d]	[IrCp*$(H_2O)_3]SO_4$ (**3**)	Ac-Gly-Gly-Phe	<1
9[d]	[IrCp*$(H_2O)_3]SO_4$ (**3**)	Gly-Gly-Phe-NH_2	149
10	[IrCp*$(H_2O)_3]SO_4$ (**3**)	Gly-Phe-Gly	3
11	[IrCp*$(H_2O)_3]SO_4$ (**3**)	Phe-Gly-Gly	92
12[d]	[IrCp*$(H_2O)_3]SO_4$ (**3**)	Phe-Gly-Phe	193

[a] Acetophenone/formate/complex = 200:1000:1; 1.2 equiv. of ligand used in the reaction. [b] Turnover frequencies: determined after 60 min by reverse-phase HPLC. [c] Carried out at pH 8 with 10 equiv. of formate for 30 min. [d] Determined after 30 min.

Thus, Phe-Gly-Gly exhibits 35% lower activity than Gly-Gly-Phe, whereas Gly-Phe-Gly affords basal levels only (Table 1, entries 10 and 11; Supporting Information, Figure S4B).

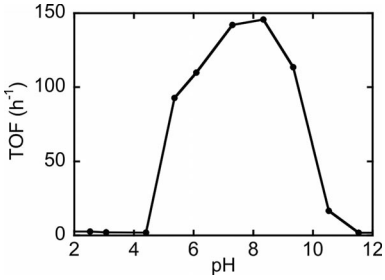
Taken together, these results suggest that tripeptides may act as bifunctional Noyori-type catalysts, binding to **3** through the N-terminal amine and adjacent amide group (Scheme 2).[13a] Analogous complexes have been previously characterized by X-ray crystallography but never tested as catalysts for transfer hydrogenation.[16] The first and third amino acids are tolerant to substitution, but steric considerations probably dictate the requirement for glycine as the second amino acid. Additional bulk at this position could hinder either formation of the peptide–iridium adduct or substrate binding. Consistent with this model, Phe-Gly-Phe still gives rise to large rate accelerations, even outperforming the original Gly-Gly-Phe ligand (Table 1, entry 12; Supporting Information, Figure S4A). Even more active catalysts might be generated by further sequence optimization.



Scheme 2. Proposed transition state for the transfer hydrogenation of ketones by **3** with peptidic ligands. R^1 = H, benzyl, AA_3 = Gly, Phe.

Transfer hydrogenation of acetophenone is pH dependent. In the presence of Gly-Gly-Phe and 50 mM sodium formate, the pH–rate profile for the iridium-catalyzed reaction is bell shaped with a broad maximum around pH 8 (Scheme 3). Turnover frequency (TOF) exceeds 100 h^{-1} be-

tween pH 6 and 9. As proposed by Xiao,[17] protonation of the ligand, the active metal hydride, or formate decreases activity. Above pH 9, replacement of a catalyst-bound water molecule by hydroxide prevents formate binding, thereby shutting down the catalytic cycle.



Scheme 3. pH–rate profile for the transfer hydrogenation of acetophenone with the Gly-Gly-Phe peptide–iridium complex.

The ligand acceleration effect observed here is similar in magnitude to that of highly active ligand complexes with diamines, aminosulfonamides, and amino alcohols.[14,18] Unlike many highly stereoselective transfer-hydrogenation catalysts, however, the peptide–iridium catalysts generate racemic products despite the presence of chiral phenylalanine(s) in the ligand. Embedding the catalytic tripeptide motif into larger foldameric structures will therefore be necessary to control substrate access to the metal center and achieve enantioselective hydride transfer. Toward this end, the catalyst must form and retain high activity in the presence of other protein functional groups. In fact, when 1 equiv. of **3** is added to a mixture of the tripeptide (60 μM) and bovine serum albumin (BSA) (0.1 mg mL^{-1}), a catalytically active complex is formed that converts substrate into product with a TOF of 77 h^{-1} (Supporting Information, Figure S5A). In the absence of tripeptide, no conversion is detected. If the amount of BSA is increased to 0.5 mg mL^{-1}, the TOF drops to 11 h^{-1}, indicative of competitive chelation by the protein (Supporting Information, Figure S6A).[19] Given that the BSA–iridium complex is inactive, though, the concentration of iridium can simply be increased to overcome this problem. For example, addition of 5 equiv. of **3** to the mixture of tripeptide and 0.5 mg mL^{-1} BSA affords a TOF of 88 h^{-1} (Supporting Information, Figure S6B). Such protein compatibility is unusual for many transition-metal catalysts and supports the feasibility of using this approach to construct artificial metalloenzymes.[20]

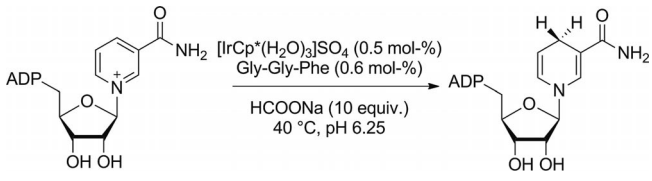
Broad substrate scope is another notable attribute of the Gly-Gly-Phe peptide–iridium catalyst. Initial TOFs for a variety of aldehydes, ketones, and imines are summarized in Table 2. Benzaldehyde (Table 2, entry 9) and activated acetophenone derivatives containing electron-withdrawing aryl substituents or a trifluoromethyl group adjacent to the carbonyl are particularly good substrates (Table 2, entries 1–4), whereas derivatives containing electron-donating groups and aliphatic ketones are converted less rapidly than acetophenone itself (Table 2, entries 5–8). Although transfer hydrogenation of imines is well documented,[21] the cyclic imine 1-methyl-3,4-dihydroisoquinoline proved to be a poor substrate for the peptide–iridium complex under our standard conditions (<1 h^{-1}). Lowering the pH to 6.25, however, afforded a large increase in activity (197 h^{-1}), presumably due to protonation of the imine. The resulting rate is comparable to that for reduction of acetophenone under our standard conditions.

Table 2. Turnover frequencies (TOFs) for transfer hydrogenation of different substrates catalyzed by **3** in the presence of Gly-Gly-Phe.[a]

Entry	Substrate	TOF (h^{-1})	Rel. act.[b]
1	acetophenone	200	1
2	4′-chloroacetophenone	391	1.96
3	2′-chloroacetophenone	361	1.81
4	2,2,2-trifluoroacetophenone	366	1.83
5	4′-methylacetophenone	198	0.99
6	4′-methoxyacetophenone	154	0.77
7	2′-methylacetophenone	138	0.69
8	4-phenyl-2-butanone	98	0.49
9	benzaldehyde	273	1.37
10[c]	1-methyl-3,4-dihydroisoquinoline	197	0.99

[a] Reaction conditions: substrate/formate/catalyst = 200:2000:1, Gly-Gly-Phe (1.2 equiv.), pH 8.0, 40 °C, 600 rpm. [b] Relative activity compared to acetophenone as substrate. [c] pH 6.25.

Regeneration of dihydronicotinamide adenine dinucleotide (NADH), an essential coenzyme in biological redox reactions, from nicotinamide adenine dinucleotide (NAD$^+$) is a biotechnologically important reduction reaction.[22] Although nicotinamide cofactors can be regenerated enzymatically, nonenzymatic approaches involving organometallic redox mediators represent increasingly attractive alternatives.[23] The iridium complex with Gly-Gly-Phe efficiently reduced NAD$^+$ at pH 6.25 with a TOF of 225 h^{-1} (Scheme 4). The reaction proceeded regioselectively with a >10:1 preference for the biologically relevant 1,4-dihydronicotinamide isomer over the 1,2- and 1,6-isomers. The iridium catalyst is thus more than twofold more efficient than [Rh(bpy)H$_2$O]SO$_4$ (bpy = 2,2′-bipyridine), which has previously been used for the regeneration of NADH (TOF = 96 h^{-1}); however, the rhodium catalyst is somewhat more selective (20:1 in favor of 1,4-NADH; Supporting Information, Figure S7). These results are particularly notable as iridium catalysts often display significantly lower activities than the corresponding rhodium and ruthenium complexes.[23a] The combination of high turnover frequencies and good protein compatibility makes this system attractive for multistep chemoenzymatic processes.



Scheme 4. Reduction of NAD$^+$ by formate catalyzed by **3** in the presence of Gly-Gly-Phe.

Conclusions

Our results show that peptide–iridium catalysts, which are readily formed under mild conditions in aqueous buffer, efficiently catalyze transfer hydrogenation of diverse ketones, aldehydes, and imines. Simple tripeptides such as Gly-Gly-Phe significantly enhance the reactivity of a water soluble d^6-piano-stool iridium complex, providing turnover frequencies that rival or surpass those of established high-performance ligand systems.[14] They are thus interesting alternatives to amino acids[24] and pseudopeptides[25] for transfer hydrogenations under biological conditions.

The peptides likely bind the iridium complex through their free N-terminal amine and the adjacent amide to afford bifunctional Noyori-type catalysts. Because the C-terminal carboxylate is not required for activity, it should be possible to append the tripeptides to the N terminus of virtually any protein or foldameric structure as simple coordination tags to create artificial transfer hydrogenases. Formation of a productive complex, even in the presence of excess competing protein functionality, augurs well for transplantation of this catalytic motif into larger, more complex structures. Although the peptides tested to date confer no selectivity, placing the organometallic complex within the chiral environment of a protein would be expected to enable enantioselective transformations, as observed for biotinylated transfer-hydrogenation catalysts bound to streptavidin.[8] In contrast to biotinylated systems, though, a wide range of structures, tailored to specific applications, can be considered as scaffolds. Utilization of a short peptide as opposed to a flexible linker to anchor the iridium complex to a protein is also likely to provide greater control over the placement of the catalytic center at the active site as well as a means of fine-tuning affinity and reactivity through systematic sequence variation. The defined structure of such complexes may facilitate computational design efforts as well.

The biocompatibility of the peptide–iridium complexes and the low background activity observed in the absence of peptide are important prerequisites for in vitro and in vivo applications. As for biotin-streptavidin-based artificial transfer hydrogenases,[8] it may be possible to combine these organometallic complexes with natural biocatalysts to generate productive reaction cascades. The fact that tripeptides are genetically encodable ligands also opens the possibility of generating artificial metalloenzymes in vivo. Coupling the activity of such species to the survival of a cell might then enable genetic selection of highly active and selective transfer-hydrogenation catalysts.[26]

Experimental Section

Typical Procedure for Transfer Hydrogenation: Stock solutions of **3** (2.0 mM in water) and the corresponding peptide (2.4 mM in water) were freshly prepared, and 25 μL of each was added to water (550 μL) in a 1.5 mL screw-cap Eppendorf tube. The resulting solution was allowed to incubate in a thermo mixer at 40 °C for 10 min while shaking (600 rpm) to facilitate formation of the peptide–iridium catalyst. Then, 250 mM phosphate buffer (200 μL) at the appropriate pH containing 500 mM sodium formate and the substrate (50 mM in water, 200 μL) were added. The reaction mixture was allowed to react for 30 to 60 min at 40 °C while shaking before stopping the reaction by cooling the samples to 4 °C. Aliquots were periodically removed and analyzed by reverse-phase HPLC (5 μL injection) to monitor reaction progress and estimate initial turnover frequencies.

Transfer Hydrogenation in the Presence of BSA: BSA (10 or 50 μL of a 10 mg mL^{-1} stock solution in 250 mM sodium phosphate buffer, pH 8) was mixed with the Gly-Gly-Phe tripeptide (60 μM). Following addition of **3** (25 to 125 μL of a 2.0 mM stock solution in water) and incubation at 40 °C for 10 min, the resulting mixture was used for transfer hydrogenation as described for the standard procedure.

Turnover Experiments: The total turnover number for the catalytic reduction of acetophenone was determined by treating 50 mM substrate with 25 μM **3**, 30 μM Gly-Gly-Phe, and 500 mM sodium formate in 250 mM sodium phosphate buffer at pH 7 for 16 h.

Supporting Information (see footnote on the first page of this article): Materials and methods, experimental details, representative HPLC chromatograms, characterization data, and ^{1}H NMR as well as ^{13}C NMR spectra for synthesized tripeptides.

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