8-Amino-5,6,7,8-tetrahydroquinoline in iridium (III) biotinylated Cp* complex as artificial imine reductase

*Giorgio Facchetti[a] and Isabella Rimoldi[*a]*

In the present study, four different diamine ligands I-IV were studied in coordination with an iridium metal complex with the biotin moiety anchored to the Cp*ring. This strategy, in contrast to the traditional biotin-streptavidin technology based on the use of a biotinylated ligand in the resulted artificial imine reductase, outcome particularly practical for envisaging how the enantiodiscrimination operated by different Sav mutants could influence the chiral environment of the metal cofactor. Only in the case of (R)-CAMPY IV the chirality at the metal centre and the second coordination sphere environment, dictated by the host protein, resulted to operate in a synergistic way, thus leading to a better enantioselectivity when the S112M Sav catalyst/ catalyst ratio was settled at 1.0:2.5. Under these optimized conditions the artificial imine reductase afforded a valuable enantiomeric excess (83 %) in the ATH of 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline.

Introduction

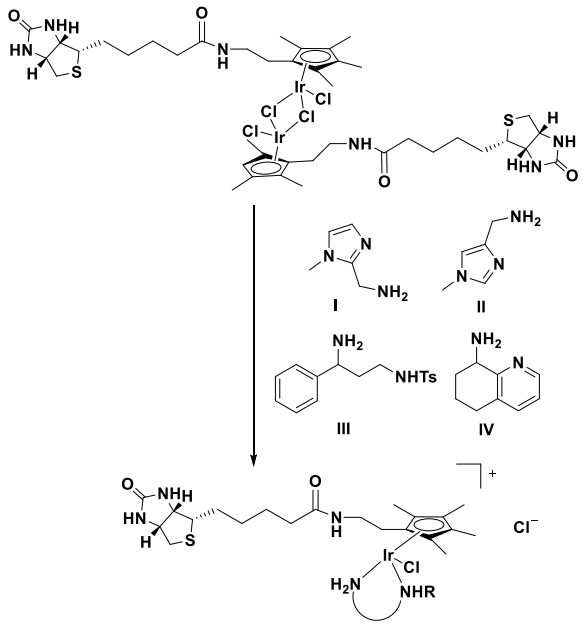
Enantioselective catalysis provides an efficient and versatile tool for the synthesis of many biologically active compounds in which chirality plays a critical role such as pharmaceuticals and agrochemicals.1, 2 In this regard both transition metal catalysis and biocatalysis have been the catalytic approaches more deeply exploited to produce enantiopure products. These synthetic methods have been considered as separated fields until recently when the so-called artificial metalloenzymes (Ar-Ms) have risen as a promising approach able to merge the attractive properties of the two worlds.3, 4 Ar-Ms are hybrid catalysts in which a transition metal complex is embedded within a biological environment. The idea is to join the broad scope of organic transformations catalysed by metal catalysts to the exquisite selectivity exhibited by enzymes.5-9 In the case of a conventional transition metal catalyst, the selectivity of the reaction relies totally on the first coordination sphere determined by the nature and the stereochemistry of the ligands around the metal centre. In this context, chiral phosphines and diamine ligands endowed with different steric and electronic properties have been extensively investigated for enhancing the performances of such a type of homogenous catalysis.10-15

Conversely, in the enzymatic catalysis the intricate network of interactions within the biological scaffold creates a chiral second coordination sphere environment around the catalytic site responsible for the high levels of enantio-discrimination as well as for the reduction in the energy barriers of the catalysed transformation thus resulting in extreme enantioselectivity along with accelerated reaction rates.16 In the of Ar-Ms, by incorporating an organometallic compound within a three dimensionally defined biological structure it is possible to synthesise a transition metal catalyst exhibiting the above-mentioned enzyme features.17-22

In the context of the Ar-Ms based on the biotin-streptavidin technology our research group recently developed an artificial imine reductase in which a chiral biotinylated 1,3 diamine ligand in coordination with an iridium (III) centre was anchored to a streptavidin mutant and employed in the stereoselective reduction of the salsolidine precursor.23 In that study the use of such a poorly investigated type of diamine ligands shed light on those protein residues that more deeply affected the stereoselectivity of the reaction. In the present work to expand the artificial cofactor library, a chemical optimization of the imine reductase was performed by exploring how a series of diamine ligands different in structure and electronic properties would impact on the ability of the second coordination sphere to induce selectivity in the asymmetric transfer hydrogenation of the model substrate. Indeed, we reasoned that this goal would be more easily appreciated by tethering the biotin anchor to the Cp* moiety so that the reactivity of the first coordination sphere would be exclusively dictated by the features of the chelating amines around the iridium centre.

Results and discussion

Starting from the pioneering work by Ward,24-26 in the present work the chemical optimization strategy was applied to the synthesis of the biotinylated catalyst precursor [IrCp*biotinCl2]2 then reacted in situ with different bidentate ligands to be employed in the preparation of the artificial metalloenzymes. In particular, we focused our attention on the use of a series of diamine chelating ligands different for both steric and electronic features: two achiral diamines based on imidazole moiety previously investigated by our group in platinum(II) complexes as anticancer agents,27 i.e. the (1-methyl-1H-imidazol-4-yl)methylamine I and the (1-methyl-1H-imidazol-2-yl)methanamine II, a chiral 1,3 diamine III23 already applied to the synthesis of Ar-Ms and the 8-Amino-5,6,7,8-tetrahydroquinoline, called CAMPY IV,28, 29 recently revealed an efficient chiral ligand in iridium complexes for the ATH of cyclic imines .30 (Scheme 1)



Scheme 1. Artificial imine reductases based on different selection of diamine ligands

A preliminary screening was performed in the ATH of the chosen substrate in the presence of achiral imidazole ligands I and II using different Sav mutants either bearing a single mutated residue at position S112 or K121 or with a double mutation at position S112 or K121 in addition to a mutation at residue L124. 31 (Table 1)

The choice of the positions of mutagenesis in aminoacids position was applied in function of estimated proximity to the catalytic metal determined by Ward and co-workers through x-ray crystal structure of the most selective studied ATHase reductase.32

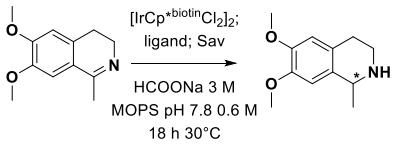
Moreover, knowing the sensitivity of the catalytic performance of such hybrid systems to many experimental parameters, different reaction conditions were evaluated: activation time, reaction temperature, substrate concentration and substrate/catalyst ratio (data not reported).

As a general trend emerged from the data reported in Table 1, in the presence of I and II a racemic mixture of the product was generally obtained. Only using in the case of Sav mutants S112A, S112T and S112Y+K121R the imine reductase bearing diamine II in its first coordination sphere was able to afford a really modest enantiomeric excess (up to 13 %). A significant effect deserving to be noted was the inversion of the configuration of the reaction product in function of the Sav mutant. (Table 1, entries 8, 10 and 19)

With the aim to study how the chirality of the diamine could improve the catalytic system in which the biotinylated piano stool complex represents the anchor factor to Sav-WT and Sav mutants, we decided to introduce in the first coordination sphere two chiral diamines, III and IV. (Table 2)

The results obtained with the chiral 1,3-diamine III, in which the coordination to the metal centre led to the formation of a labile six membered ring, confirmed the poor selectivity induced in the product formation. In this case, only in the presence of K121H and K121A mutants, a modest enantiomeric excess was achieved. (Table 2, entries 15 and 17)

Table 1. Screening of chemogenetic optimization of ATHases for asymmetric reduction of the salsolidine precursor with diamine I and II.



ENTRY	SAV	I[A]	II[A]
1	no	99 (rac)	99 (rac)
2	WT	8 (rac)	17 (6 S)
3	S112Q	45 (rac)	99 (rac)
4	S112R	57 (rac)	99 (3 S)
5	S112M	43 (4 R)	99 (rac)
6	S112C	5 (rac)	5 (rac)
7	S112H	49 (rac)	99 (rac)
8	S112A	59 (rac)	99 (10)
9	S112E	52 (rac)	99 (rac)
10	S112T	43 (rac)	94 (9 R)
11	S112Y	53 (5 R)	99 (rac)
12	S112K	66 (rac)	99 (rac)
13	S112W	68 (8 R)	99 (5 S)
14	K121R	55 (rac)	99 (6 S)
15	K121H	19 (rac)	99 (rac)
16	K121F	99 (4 R)	99 (5 S)
17	K121A	73 (rac)	99 (rac)
18	K121M	73 (3 R)	99 (3 S)
19	S112Y+K121R	83 (rac)	99 (13 R)
20	S112K+L124E	69 (rac)	99 (rac)
21	S112C+K121H	6 (rac)	9 (7 S)

Reaction condition: substrate 19 mM, 1 mol % ligand, 0.45 mol % [IrCp*biotinCl2]2, 4 mg/mL mutant Sav, MOPS 0.6 M pH 7.8, HCOONa 3 M, 18 h at 30°C. [a] conversion (e.e.%).

In the case of the 1,2 diamine CAMPY IV as ligand, better results in terms of enantioselectivity were obtained. Considering the good ability of CAMPY to induce in chirality of the corresponding metal complexes and the possibility to influence their catalytic activities depending on the selected Sav mutant, both the enantiomers of ligand IV were evaluated. Some considerations occurred:

In the presence of all the mutants the conversion of the reaction to the product resulted decreased (up to 77 % for (S)-IV in presence of S112K Sav mutant, table 2, entry 12).

The (S)-IV ligand gave always the S enantiomer of the product while the (R)-IV gave always the R enantiomer for all the Sav mutants, confirming that the enantiopure environment provided by the host Sav resulted to influence the chirality of the product at a less extent.

In all cases the enantioselectivity resulted equal or decreased in presence of Sav, demonstrating that because of the rigid backbone of the CAMPY ligand the corresponding metal co-factor was probably not sufficiently embedded within the host protein for steric reasons.

Table 2. Screening of chemogenetic optimization of ATHases for asymmetric reduction of the salsolidine precursor with diamine (S)-III, (S)-IV and (R)-IV.

ENTRY	SAV	(S)-III[A]	(S)-IV[A]	(R)-IV[A]
1	no	91 (3 R)	99 (59 S)	99 (65 R)
2	WT	39 (7 R)	8 (57 S)	12 (43 R)
3	S112Q	99 (rac)	42 (32 S)	30 (42 R)
4	S112R	99 (9 R)	32 (31 S)	32 (41 R)
5	S112M	85 (6 R)	35 (41 S)	27 (68 R)
6	S112C	20 (7 R)	9 (31 S)	5 (8 R)
7	S112H	52 (6 R)	25 (41 S)	17 (32 R)
8	S112A	94 (7 R)	35 (32 S)	45 (46 R)
9	S112E	91 (rac)	38 (30 S)	35 (43 R)
10	S112T	64 (rac)	27 (40 S)	25 (51 R)
11	S112Y	91 (rac)	39 (28 S)	19 (49 R)
12	S112K	87 (6 R)	77 (29 S)	27 (41 R)
13	S112W	49 (rac)	31 (39 S)	45 (45 R)
14	K121R	51 (rac)	34 (37 S)	33 (54 R)
15	K121H	44 (12 R)	22 (41 S)	28 (48 R)
16	K121F	88 (rac)	49 (38 S)	35 (36 R)
17	K121A	99 (13 R)	42 (29 S)	35 (34 R)
18	K121M	57 (5 R)	13 (54 S)	27 (54 R)
19	S112Y+K121R	99 (5 R)	34 (12 S)	45 (41 R)
20	S112K+L124E	64 (4 R)	59 (47 S)	32 (50 R)
21	S112C+K121H	21 (rac)	7 (40 S)	10 (25 R)

Reaction conditions: substrate 19 mM, 1 mol % ligand, 0.45 mol % [IrCp*biotinCl2]2, 4 mg/mL mutant Sav, MOPS 0.6 M pH 7.8, HCOONa 3 M, 18 h at 30°C. [a] conversion % (e.e.%).

Considering that the best results in terms of enantioselectivity for both the enantiomers of diamine IV were obtained in presence of S112M Sav mutant, other than the above-mentioned reaction conditions influencing the reactivity and the selectivity of the system were evaluated: different buffers at different pH and the S112M Sav mutant/ligand ratio. This last aspect was changed taking into consideration the tetrameric structure of Sav-WT, known for existing as a dimer of dimers each one providing a biotin binding site.33

The results are summarized in Table 3.

Table 3. Optimized results obtained using diamine IV and S112M Sav mutant.

ENTRY	BUFFER	S112M/LIGAND RATIO	(R)-IV[B]
1	acetate pH 5[a]	1.0:1.5	7 (53 R)
2	MES pH 6[a]	1.0:1.5	13 (60 R)
3	phosphate pH 8[a]	1.0:1.5	7 (17 R)

4	MES pH 7[a]	1.0:1.5	14 (59 R)
5	MOPS pH 7.8	1.0:1.0	23 (62 R)
6	MOPS pH 7.8[a]	1.0:1.5	27 (68 R)
7	MOPS pH 7.8	1.0:2.5	32 (83 R)

Reaction conditions: substrate 19 mM, 1 mol % ligand, 0.45 mol % 6[IrCp*biotinCl2]2, MOPS 0.6 M pH 7.8, HCOONa 3 M, 18 h at 30°C. [a] 4 mg/mL S112M Sav [b] conversion % (e.e. %).

From the results summarised in Table 3 using diamine(R)-IV, the following considerations could be made:
Taking into consideration the enantioselectivity but also the reactivity afforded by the system, MOPS buffer at pH 7.8 turned out as the best reaction medium compared to MES at pH 6 and pH 7. (Table 3, entry 6 vs entries 2 and 4)
When the Sav mutant/metal cofactor ratio was changed, the enantiomeric excess of the product showed a significant variation. In fact, bearing the ratio to 1.0:2.5, the enantioselectivity increased up to a significant 83 % e.e. along with a modest increase also in the reactivity (32 % yield). (Table 3, entry 7).
The observed changes in the enantioselectivity of the imine reductase activity as a consequence of a different Sav/metal cofactor ratio seems to fit the observation made above that because of the rigid structure of CAMPY ligand the metal cofactor probably fully occupied only one active site of the Sav, underlining that the steric hindrance played an important role in stabilizing a specific second coordination sphere environment thus dictating which prochiral face of the substrate can approach the hydride to afford the preferred enantiomer of the product. Under these conditions in fact, when two (R)-(R)-metal cofactors occupied simultaneously two close-lying biotin binding sites (the first in a dimer and the second in the opposite dimer of the tetrameric streptavidin for minimizing clashes between the two cofactors) each monomer is active and might behave independently with no interaction between the adjacent cofactors that allowed to increase the e.e. to an appreciable 83% in the case of S112M Sav mutant.

Experimental

General. Diamine I and II were purchased by Merck. The synthesis of diamine III and IV proceeded as reported in literature.11, 28 The synthesis of [IrCp*biotinCl2]2 was performed according to the literature.31, 34, 35 Catalytic reactions were monitored by HPLC analysis with Merck-Hitachi L-7100 equipped with Detector UV6000LP and chiral column (OD-H Chiralcel).

General procedure for asymmetric transfer hydrogenation.

[IrCp*biotinCl2]2 (1.9x10-5 mmol) and ligand (3.95x10-5 mmol) was dissolved in 100 µL of MOPS buffer 0.6 M pH 7.8 and stirred for 30 min at 30°C. The corresponding lyophilized Sav mutant (0.8 mg) was added and the mixture was agitated to ensure the binding between the precomplexed biotinylated metal system and the Sav. The substrate (final concentration 19 mM) was dissolved in 100 µl of buffer (HCOONa 6 M, MOPS 0.6 M, final pH 7.8) and added to the catalyst solution. The reaction was stirred for 18 h at 30°C. At the end of the reaction 10 µL of NaOH 10 N was added and the aqueous media was extracted with CH2Cl2. The organic layers were dried with anhydrous Na2SO4, filtered and the solvent was removed under vacuum for been analysed by HPLC equipped with chiral column. (eluent hexane/ethanol/DEA=95/5/0.1; λ=283 nm; flow=1.0 mL/min; retention time for starting material 10.9 min; enantiomers of the product: tS=17.2 min; tR=21.3 min)

Conclusions

In conclusion, the biotinylated piano stool complexes revealed to be a labile catalytic system with all the Sav mutants unless in the presence of chiral chelating ligand able to stabilise one enantiomer at the biotinylated Cp*metal precatalyst if compared to the conventional biotin-streptavidin technology in which the enantioselection

occurred also with an achiral biotinylated ligand. The difference between the rigid 1,2 diamine ligand IV and a flexible ligand as the 1,3 diamine III, despite both chiral, confirmed the importance of a five membered coordination ring at the metal centre also in presence of a structural different artificial metalloenzyme. Under optimized conditions (MOPS pH 7.8, Sav mutant/ligand ratio 1.0:2.5), the imine reductase formed by (R)-CAMPY bound to S112M Sav mutant showed an appreciable enantioselectivity in the ATH of the 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline (up to 83% e.e.).

Considering the wide versatility offered by this new approach in designing artificial metalloenzymes, in the future different ligands and metal complexes opportunely modified, i.e. in the spacer length and nature between the biotin anchor and the Cp* ring, could be investigated and optimized to improve the scope of the reaction.

Conflicts of interest

"There are no conflicts to declare".

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References

1. I. Rimoldi, M. Pellizzoni, G. Facchetti, F. Molinari, D. Zerla and R. Gandolfi, Tetrahedron Asymmetry, 2011, 22, 2110-2116.
2. P. Jeschke, Pest Management Science, DOI: doi:10.1002/ps.5052.
3. F. Schwizer, Y. Okamoto, T. Heinisch, Y. Gu, M. M. Pellizzoni, V. Lebrun, R. Reuter, V. Köhler, J. C. Lewis and T. R. Ward, Chem. Rev., 2018, 118, 142-231.
4. F. Rosati and G. Roelfes, ChemCatChem, 2010, 2, 916-927.
5. S. Pellegrino, G. Facchetti, A. Contini, M. L. Gelmi, E. Erba, R. Gandolfi and I. Rimoldi, RSC Advances, 2016, 6, 71529-71533.
6. J. C. Lewis, ACS Catal., 2013, 3, 2954-2975.
7. C. Mayer and D. Hilvert, Eur. J. Org. Chem., 2013, 2013, 3427-3431.
8. M. Basauri-Molina, C. F. Riemersma, M. A. Würdemann, H. Kleijn and R. J. M. Klein Gebbink, Chem. Commun., 2015, 51, 6792-6795.
9. I. Drienovska, L. Alonso-Cotchico, P. Vidossich, A. Lledos, J.-D. Marechal and G. Roelfes, Chemical Science, 2017, 8, 7228-7235.
10. G. Facchetti, E. Cesarotti, M. Pellizzoni, D. Zerla and I. Rimoldi, Eur. J. Inorg. Chem., 2012, 2012, 4365-4370.
11. G. Facchetti, R. Gandolfi, M. Fuse, D. Zerla, E. Cesarotti, M. Pellizzoni and I. Rimoldi, New J. Chem., 2015, 39, 3792-3800.
12. S. Pellegrino, G. Facchetti, R. Gandolfi, M. Fusè, E. Erba and I. Rimoldi, Canadian Journal of Chemistry, 2017, 96, 40-43.
13. A. J. Argüelles, S. Sun, B. G. Budaitis and P. Nagorny, Angew. Chem. Int. Ed., 2018, 57, 5325-5329.
14. D. Wang, A. Bruneau-Voisine and J.-B. Sortais, Catal. Commun., 2018, 105, 31-36.
15. Y.-M. Li, F.-Y. Kwong, W.-Y. Yu and A. S. C. Chan, Coord. Chem. Rev., 2007, 251, 2119-2144.
16. O. Pàmies, M. Diéguez and J.-E. Bäckvall, Adv. Synth. Catal., 2015, 357, 1567-1586.
17. P. Dydio, H. M. Key, A. Nazarenko, J. Y.-E. Rha, V. Seyedkazemi, D. S. Clark and J. F. Hartwig, Science, 2016, 354, 102-106.
18. F. Yu, V. M. Cangelosi, M. L. Zastrow, M. Tegoni, J. S. Plegaria, A. G. Tebo, C. S. Mocny, L. Ruckthong, H. Qayyum and V. L. Pecoraro, Chem. Rev., 2014, 114, 3495-3578.
19. J.-E. B. M. Dieguez, O. Pamies Artificial Metalloenzymes and MetalloDNAzymes in Catalysis: From Design to Applications, O. Pamies (eds) 1st edn edn., 2018.
20. M. M. Pellizzoni, F. Schwizer, C. W. Wood, V. Sabatino, Y. Cotelle, S. Matile, D. N. Woolfson and T. R. Ward, ACS Catal., 2018, 8, 1476-1484.

21.M. Filice, O. Romero, A. Aires, J. M. Guisan, A. Rumbero and J. M. Palomo, Adv. Synth. Catal., 2015, 357, 2687-2696.
22.Y. Yu, C. Hu, L. Xia and J. Wang, ACS Catal., 2018, 8, 1851-1863.
23.M. Pellizzoni, G. Facchetti, R. Gandolfi, M. Fusè, A. Contini and I. Rimoldi, ChemCatChem, 2016, 8, 1665-1670.
24.T. Quinto, F. Schwizer, J. M. Zimbron, A. Morina, V. Köhler and T. R. Ward, ChemCatChem, 2014, 6, 1010-1014.
25.M. Dürrenberger, T. Heinisch, Y. M. Wilson, T. Rossel, E. Nogueira, L. Knörr, A. Mutschler, K. Kersten, M. J. Zimbron, J. Pierron, T. Schirmer and T. R. Ward, Angew. Chem. Int. Ed., 2011, 50, 3026-3029.
26.M. Hestericová, T. Heinisch, L. Alonso-Cotchico, J.-D. Maréchal, P. Vidossich and T. R. Ward, Angew. Chem. Int. Ed., 2018, 57, 1863-1868.
27.I. Rimoldi, G. Facchetti, G. Lucchini, E. Castiglioni, S. Marchianò and N. Ferri, Bioorg. Med. Chem., 2017, 25, 1907-1913.
28.I. Rimoldi, G. Facchetti, E. Cesarotti, M. Pellizzoni, M. Fuse and D. Zerla, Curr. Org. Chem., 2012, 16, 2982-2988.
29.D. Zerla, G. Facchetti, M. Fuse, M. Pellizzoni, C. Castellano, E. Cesarotti, R. Gandolfi and I. Rimoldi, Tetrahedron: Asymmetry, 2014, 25, 1031-1037.
30.G. Facchetti, R. Bucci, M. Fusè and I. Rimoldi, ChemistrySelect, 2018, 3, 8797-8800.
31.J. M. Zimbron, T. Heinisch, M. Schmid, D. Hamels, E. S. Nogueira, T. Schirmer and T. R. Ward, J. Am. Chem. Soc., 2013, 135, 5384-5388.
32.M. Creus, A. Pordea, T. Rossel, A. Sardo, C. Letondor, A. Ivanova, I. LeTrong, R. E. Stenkamp and T. R. Ward, Angew. Chem. Int. Ed., 2008, 47, 1400-1404.
33.V. M. Robles, M. Dürrenberger, T. Heinisch, A. Lledós, T. Schirmer, T. R. Ward and J.-D. Maréchal, J. Am. Chem. Soc., 2014, 136, 15676-15683.
34.D. van Leusen, D. J. Beetstra, B. Hessen and J. H. Teuben, Organometallics, 2000, 19, 4084-4089.
35.R. Vallinayagam, J. Weber and R. Neier, Org. Lett., 2008, 10, 4453-4455.