Supporting Information

Artificial Metalloenzymes for Enantioselective Catalysis Based on Biotin-Avidin

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Materials and Methods

Production and purification of recombinant streptavidin

Streptavidin expression

BL21(DE3)pLysS *E. coli* strain was transformed by pET11b-SAV plasmid[1] containing a gene encoding for recombinant mature streptavidin[2] fused with the T7-tag (**Bold** arising from the plasmid pET11b, Novagen), followed by Asp and Gln (Underlined) and residues 15 to 159 of the wild-type mature streptavidin.

Protein Sequence:

ASMTGGQQMGRDQAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAESRY

VLTGRYDSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARINTQ

WLLTSGTTEANAWKSTLVGHDTFTKVKPSAASIDAAKKAGVNNGNPLDAVQQ

Transformed bacteria were plated on selective dishes containing glucose (1% w/v). A single colony was used for the preculture containing TP-medium[1] and glucose (1% w/v). The culture was inoculated with the preculture and was performed in TP-medium containing glucose (0.4% w/v). A ten liter cell culture was grown in a fifteen liter fermentor at 37 °C until 1.0< OD_{600}<1.4. Having reached this OD, □-D-thiogalactoside (IPTG) was added to a final concentration of 0.4mM. Three hours after induction, cells were harvested by centrifugation. The pellet was washed with Tris-Buffered Saline (TBS), and recentrifuged. The pellet was frozen at –80°C until purification.

Site-directed mutagenesis

Mutant S112G was obtained using Quick Change Site-Directed Mutagenesis Kit (Stratagene).[3,4] A PCR reaction (50 mL) was performed in presence of DMSO (5%). Following a single initial denaturation 94°C, 5 min., the optimized temperature profile for PCR cycles was: 94°C, 1 min., 65°C, 1 min. and 68°C, 15 min. This cycle was repeated 16 times followed by a final extension at 68°C for 1 hour.

For analysis, 2mL of the PCR mixture were loaded on a 0.6 % agarose gel containing TAE. The sample was used to transform the XL1-blue *E. coli* strain. Plasmids were extracted from bacteria and clones were sequenced.

Protein purification[2]

The pellet was thawed and resuspended in 20 m*M* Tris/HCl p*H* 7.4, 0.02 % w/v sodium azide, 10 m*M* $MgCl_2$. Benzonase was added and incubated for 2 hours.

The sample was sonicated, dialysed against 6*M* guanidium hydrochloride at p*H* 1.5, then against 20m*M* tris/HCl p*H* 7.4, followed by dialysis against 50m*M* sodium carbonate p*H* 9.8, 0.5*M* NaCl. The proteic extract was then centrifuged (47'000g for 20 min. at 4°C), the supernatant was filtered and applied on a 2-iminobiotin agarose column, washed and eluted according to the Sigma protocol. The purified protein was immediately dialysed against 10 m*M* tris/HCl p*H* 7.4), followed by two dialysis against water. After filtration, the purified protein was frozen at –80 °C, lyophilized and stored at 4°C until use. The number of active sites was determined using the biotin-fluorescein protocol developped by Gruber *et al.*[5] A ten liter fermentation typically yields 600 mg of streptavidin with *ca.* 3.65 active sites.

General procedure for the rhodium-catalyzed asymmetric hydrogenation of *N*-protected amino-acids acid with biotinylated catalysts and host proteins

All organic and aqueous solutions were degassed by flushing argon through the solutions for three hours. All operations were performed either using standard Schlenk techniques or carried out in a glove box.

The following buffers were screened: Formate, pH 3.0 0.1*M* final concentration (conc.); Acetate, p*H* 4.0 and p*H* 5.0 0.1*M* final conc.; MES: 2-(*N*-Morpholino)ethanesulfonic acid sodium salt, p*H* 6.0 0.1*M* final conc.; MOPS: 3-(*N*-Morpholino)propanesulfonic acid sodium salt pH 7.0 0.1*M* final conc.; Phosphate, p*H* 7.1 0.07*M* final conc.; TRIS: *Tris*-(hydroxymethyl)aminoethane, p*H* 8.0 0.1*M* final conc.

All ligands were synthesized using the CDMT peptide-coupling protocol[6] and purified by flash chromatography under argon. The appropriate ligand (13.1 mole) was dissolved in dichloromethane (5mL) and added to a solution of $[Rh(COD)_2]BF_4$ (4.1mg, 10 mole) in dichloromethane (5mL). The mixture was stirred and an aliquot (1.23mL) was evaporated to dryness. The resulting yellow residue was solubilized in dimethylsulfoxide (2mL).

A glass tube (volume *ca*. 2mL) was placed in an autoclave and charged with the *N*-protected dehydroaminoacid (acetamidoacrylic acid or acetamidocinnamic acid) solution in water (260 L of a 0.0238*M* solution, 6.2 mole). The protein solution was added (100 L of a 0.2064m*M* solution, 0.0207 mole). The buffer was then added to obtain a final volume of 1mL with the desired buffer composition. The catalyst solution in DMSO (100 L, 0.062 mole) was added last.

The autoclave was closed, purged under vacuum and charged with 5 atm. H_2. After stirring for 15 hrs at room temperature, the reaction was quenched by adjusting the p*H* to 2 with 2.0*N* aqueous HCl solution. The aqueous solution was continuously extracted with ethyl acetate for 2 hrs and the organic phase was evaporated to a minimum volume (1mL). The hydrogenation product (acetamidoalanine or acetamidophenylalanine) was converted *in situ*

to its methyl ester using trimethysulfonium hydroxyde before GC analysis on a capillarity column (Chirasyl-L-Val 25m x 0.25mm column –using pentadecane as internal standard– and/or Heptakis-2,3,6-perethyl-□-cyclodextrin, 22m x 0.32 mm column).

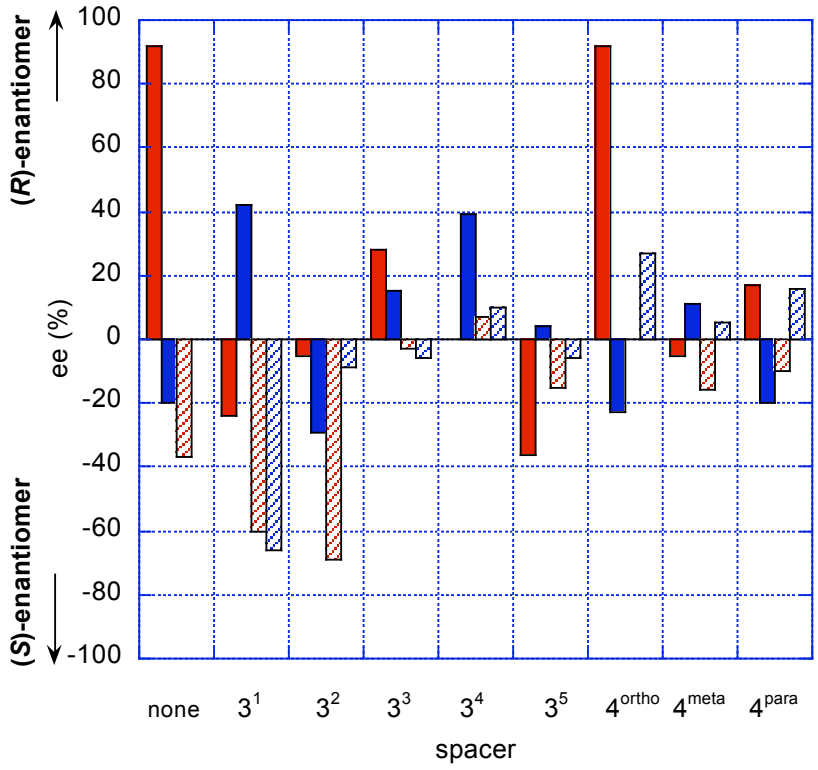

Figure 1 Graphical summary of the results for the enantioselective reduction of acetamidoacrylic acid with ligand scaffold **1** (red coding) and ligand scaffold **2** (blue coding) in streptavidin (solid bars) and in avidin (hatched bars).

Tables S1 and S2. Results for the enantioselective hydrogenation of acetamidoacrylic acid using biotinylated rhodium-diphosphine complexes in (strept)avidin (Data for Fig. 1)

	Ligand Scaffold **1**					
	Streptavidin			Avidin		
Spacer	Conv.	ee	buffer	Conv.	ee	buffer
none	100	92 (*R*)	Acetate	90	37 (*S*)	Phosph
3^{1}	100	24 (*S*)	Acetate	100	60 (*S*)	MOPS
3^{2}	100	5 (*S*)	Acetate	100	69 (*S*)	MOPS
3^{3}	100	28 (*R*)	Acetate	100	3 (*S*)	MOPS
3^{4}	100	0	Acetate	100	7 (*R*)	MOPS
3^{5}	82	36 (*S*)	Phosph	100	15 (*S*)	MOPS
4^{ortho}	100	92 (*R*)	Acetate	91	0	MOPS
4^{meta}	100	5 (*S*)	Acetate	100	16 (*S*)	MOPS
4^{para}	100	17 (*R*)	Acetate	100	10 (*S*)	MOPS

	Ligand Scaffold **2**					
	Streptavidin			Avidin		
Spacer	Conv.	ee	buffer	Conv.	ee	buffer
none	55	20 (*S*)	Acetate	94	0	MOPS
3^{1}	100	42 (*R*)	Acetate	100	66 (*S*)	MOPS
3^{2}	100	29 (*S*)	Acetate	100	9 (*S*)	MOPS
3^{3}	100	15 (*R*)	Acetate	100	6 (*S*)	MOPS
3^{4}	100	39 (*R*)	Acetate	100	10 (*R*)	MOPS
3^{5}	100	4 (*R*)	Acetate	100	6 (*S*)	MOPS
4^{ortho}	100	23 (*S*)	Acetate	100	27 (*R*)	MOPS
4^{meta}	100	11 (*R*)	Acetate	100	5 (*R*)	MOPS
4^{para}	82	20 (*S*)	Acetate	100	16 (*R*)	MOPS

References and Notes

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