Communication

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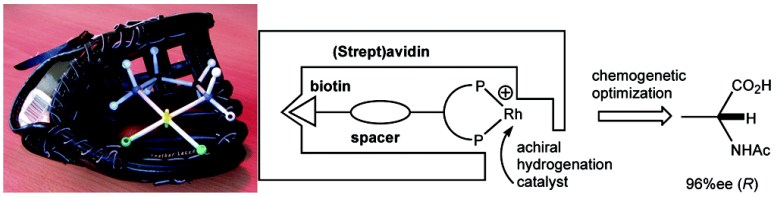

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Artificial Metalloenzymes for Enantioselective Catalysis Based on Biotin−Avidin

Jérôme Collot, Julieta Gradinaru, Nicolas Humbert, Myriem Skander, Andrea Zocchi, and Thomas R. Ward*

Institute of Chemistry, University of Neuchâtel, Av. Bellevaux 51, CP 2, CH-2007 Neuchâtel, Switzerland

Received April 9, 2003; E-mail: thomas.ward@unine.ch

Enantioselective catalysis is one of the most efficient ways to synthesize high-added-value enantiomerically pure organic compounds.[1] As the subtle details which govern enantioselection cannot be reliably predicted or computed, catalysis relies more and more on a combinatorial approach.[2] Biocatalysis offers an attractive and often complementary alternative for the synthesis of enantiopure products.[3] From a combinatorial perspective, the potential of directed evolution techniques in optimizing an enzyme's selectivity is unrivaled.[4] The present Communication outlines our efforts to create artificial metalloenzymes by incorporating an achiral rhodium−diphosphine moiety into a protein environment to yield efficient enantioselective hydrogenation catalysts.

Inspired by the seminal work of E. T. Kaiser,[5] several groups have recently developed methods to covalently modify proteins by incorporating transition-metal catalysts to yield hybrid catalytic systems with promising properties.[4d,6,7]

The approach delineated herein relies on noncovalent interactions between the metal catalyst and the protein. As no chemical coupling step is required upon addition of the catalyst precursor to the protein, the integrity of organometallic species is warranted.[8] As proposed by Whitesides, the biotin−avidin system[9] offers an attractive scaffold to perform such experiments.[10]

Initial enantioselective hydrogenation of acetamidoacrylic acid was performed with biotinylated amino-diphosphine ligands **Biot-1** and **Biot-2** using avidin as the host protein (Scheme 1).[11] The modest levels of enantioselection obtained with both ligand scaffolds (Table 1, entries 1[10a] and 2) led us to question the affinity of the catalyst precursors [Rh(COD)(**Biot-1**)]$^+$ and [Rh(COD)(**Biot-2**)]$^+$ for avidin. Indeed, the Coulomb repulsion caused by the cationic character of avidin at neutral pH (pI = 10.4) may dramatically decrease the affinity of the biotinylated cationic catalysts for avidin. We thus tested neutravidin (pI = 6.3) with catalysts [Rh(COD)(**Biot-1**)]$^+$ and [Rh(COD)(**Biot-2**)]$^+$ (7% ee (*S*) and 10% ee (*S*), respectively, entries 3 and 4).

As compared to avidin, streptavidin (pI = 6.2) possesses a similar affinity for biotin (K_a ca. 10^{14} M^{-1}) but is endowed with a deeper binding pocket.[12] In the spirit of E. Fischer's lock-and-key principle, streptavidin may thus offer a more propitious environment for an enantiodiscrimination event. To our delight, catalyst precursor [Rh(COD)(**Biot-1**)]$^+$ displayed good enantioselectivity, in favor of the (*R*)-enantiomer (92% ee, entry 5). The catalyst derived from [Rh(COD)(**Biot-2**)]$^+$ performed sluggishly to afford acetamidoalanine in 20% ee (*S*) with only 55% conversion after 15 h (entry 6).

To optimize the enantioselectivity of the reduction, two complementary strategies can be pursued: chemical modification of the first coordination sphere of the biotinylated catalyst or genetic modification of the gene coding the protein. So far, our efforts have focused primarily on the introduction of an amino acid spacer between biotin and the aminodiphosphine. This allows one to probe the topography of the host protein's binding pocket in search of a favorable chiral environment. Achiral alkylamino acid spacers **3**^{n} (n = 1−5) and arylamino acid spacers **4**^{q} (q = *ortho*-, *meta*-, *para*-) were inserted between the biotin anchor and the amino-diphosphine moieties **1** and **2** to afford ligands **Biot-3**^{n}**−1** and **Biot-3**^{n}**−2** as well as **Biot-4**^{q}**−1** and **Biot-4**^{q}**−2** (Scheme 1).[11]

Using avidin as the host protein, introduction of a glycine spacer has a beneficial effect both on the activity and on the selectivity of catalysts [Rh(COD)(**Biot-3**^{1}**−1**)]$^+$ and [Rh(COD)(**Biot-3**^{1}**−2**)]$^+$ (60% ee (*S*) and 66% ee (*S*), respectively, entries 7 and 8). In streptavidin, the sense of enantioselection for catalysts [Rh(COD)(**Biot-3**^{1}**−1**)]$^+$ and [Rh(COD)(**Biot-3**^{1}**−2**)]$^+$ is inverted as compared to the catalysts devoid of spacer (24% ee (*S*) and 42% ee (*R*), respectively, entries 9 and 10). The best result for avidin as the host protein is obtained with [Rh(COD)(**Biot-3**^{2}**−1**)]$^+$ (69% ee (*S*),

Scheme 1

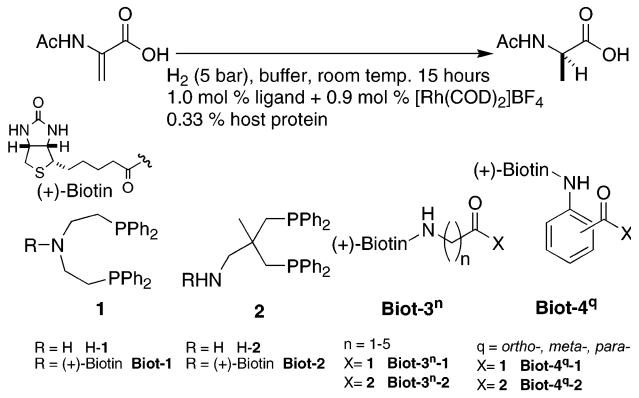

Table 1. Enantioselective Hydrogenation of the Acetamidoacrylic Acid Using Biotinylated Complexes in (Strept)avidin[a]

entry	ligand	host protein	buffer	ee (%)
1	**Biot-1**	avidin	phosphate	37 (*S*)[b]
2	**Biot-2**	avidin	MOPS	0[c]
3	**Biot-1**	neutravidin	phosphate	7 (*S*)[d]
4	**Biot-2**	neutravidin	MOPS	10 (*S*)[d]
5	**Biot-1**	streptavidin	acetate	92 (*R*)
6	**Biot-2**	streptavidin	acetate	20 (*S*)[e]
7	**Biot-3**^{1}**−1**	avidin	MOPS	60 (*S*)
8	**Biot-3**^{1}**−2**	avidin	MOPS	66 (*S*)
9	**Biot-3**^{1}**−1**	streptavidin	acetate	24 (*S*)
10	**Biot-3**^{1}**−2**	streptavidin	acetate	42 (*R*)
11	**Biot-3**^{2}**−1**	avidin	MOPS	69 (*S*)
12	**Biot-4**^{ortho}**−1**	streptavidin	acetate	92 (*R*)
13	**Biot-1**	strept. S122G	acetate	96 (*R*)
14	**Biot-4**^{ortho}**−1**	strept. S122G	acetate	94 (*R*)

[a] Unless otherwise stated, conversions (conv.) were quantitative after 15 h. [b] 90% conv. [c] 94% conv. [d] 87% conv. [e] 55% conv. The ee and conversion were determined by GC (after 15 h at room temperature, 5 atm H_2, buffer).[11] Hydrogenation with all biotinylated catalysts in the absence of a host protein produced essentially racemic material (ee < 8%, quantitative).

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entry 11). For streptavidin, [Rh(COD)(**Biot-4**^{ortho}**−1**)]$^+$ catalyzes the reduction of acetamidoacrylic acid to (*R*)-acetamidoalanine in 92% ee (entry 12). All other ligand−spacer−protein combinations yielded acetamidoalanine with an ee < 40%.[11]

The catalytic experiments reported herein reveal several noteworthy features:

(i) As the host protein, streptavidin is generally a better chiral inducer than avidin. Good levels of enantioselection, that match or exceed those obtained with catalysts devoid of spacers, can be achieved with either a glycine **3**^{1} or a β-alanine **3**^{2} spacer for avidin and an anthranilic acid **4**^{ortho} spacer in streptavidin. Although both enantiomers can be obtained with both proteins, streptavidin produces preferentially the (*R*)-enantiomer, and avidin produces preferentially the (*S*)-enantiomer.

(ii) The flexible ligand skeleton **1** generally catalyzes the reduction with a higher enantioselectivity than the more rigid ligand skeleton **2**.[13] Along the lines of the quadrant rule,[1b] we suggest that the conformation of the biotinylated chelating ligand (which exists as a racemic mixture in solution, but may be biased in favor of one enantiomer, λ or δ, upon incorporation in the host protein) plays a determinant role in the enantioselection event.

(iii) Buffer-screening experiments reveal that the fastest reduction rates and slightly higher enantioselectivities are obtained at neutral pH for avidin (0.1 M MOPS, pH 7.0 or 0.07 M phosphate buffer, pH 7.1) and acidic pH for streptavidin (0.1 M acetate buffer, pH 4.0 or 0.1 M MES, pH 6.0). This suggests that the Coulomb repulsion between the biotinylated catalyst and the cationic protein is not responsible for the modest performance with avidin as a host protein.

(iv) To test the substrate specificity of these hybrid catalysts, the reduction of acetamidocinnamic acid was carried out using [Rh(COD)(**Biot-1**)]$^+$⊂streptavidin and [Rh(COD)(**Biot-4**^{ortho}**−1**)]$^+$⊂streptavidin to yield (*R*)-acetamidophenylalanine with 86% ee (pH 6.0, MES buffer) and 83% ee (pH 6.0, MES buffer), respectively (as compared to 92% for (*R*)-acetamidoalanine, Table 1, entries 5 and 12). The substrate tolerance displayed by these hybrid catalysts is thus more typical of homogeneous than of enzymatic catalysis.[1e] Because the host protein was by no means designed to stabilize the transition state of a hydrogenation reaction, it is more tolerant toward substrates of varying steric requirements.

(v) These artificial metalloenzymes are amenable to a chemogenetic optimization procedure. Having identified by chemical modification the most promising organometallic fragments (entries 5 and 12), we subjected the streptavidin to site-directed mutagenesis. Preliminary experiments were performed by substituting single amino acids by a glycine[4d] in the flexible regions of streptavidin close to the biotin-binding site. Substitution of serine 112 by a glycine residue in the L7,8 loop of streptavidin (S112G) yields an improved host protein for the reduction of acetamidoacrylic acid both with [Rh(COD)(**Biot-1**)]$^+$⊂streptavidin S112G and with [Rh(COD) (**Biot-4**^{ortho}**−1**)]$^+$⊂streptavidin S112G (96% ee (*R*) and 94% ee (*R*), entries 13 and 14, respectively).

In the field of homogeneous catalysis, the steric and the electronic control of a catalytic moiety is mostly limited to the first coordination sphere of the metal. This contrasts with enzymes which take advantage both of the first and of the second coordination sphere to produce catalysts with exquisite activity and selectivity. The artificial metalloenzymes presented herein offer an attractive way to exploit the second coordination sphere provided by a host protein to produce versatile enantioselective catalysts with features reminiscent both of enzymatic and of organometallic catalysts. We have demonstrated that the enantioselectivity may be optimized either chemically or genetically (i.e., chemogenetic), thus offering an ideal scaffold for high-throughput optimization of enantioselective catalysts.

Finally, these hybrid enantioselective catalysts offer interesting perspectives toward "greener" organometallic catalysis as they operate in water and should prove easy to recycle either by immobilization or by size-selective filtration.

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Supporting Information Available: Materials and methods, complementary data tables and figures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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