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Note

Artificial metalloenzymes for enantioselective catalysis: the phenomenon of protein accelerated catalysis

Jérôme Collot, Nicolas Humbert, Myriem Skander, Gérard Klein, Thomas R. Ward *

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

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Abstract

We report on the phenomenon of protein-accelerated catalysis in the field of artificial metalloenzymes based on the non-covalent incorporation of biotinylated rhodium–diphosphine complexes in (strept)avidin as host proteins. By incrementally varying the [Rh(COD)(**Biot-1**)]$^+$ vs. (strept)avidin ratio, we show that the enantiomeric excess of the produced acetamidoalanine decreases slowly. This suggests that the catalyst inside (strept)avidin is more active than the catalyst outside the host protein. Both avidin and streptavidin display protein-accelerated catalysis as the protein embedded catalyst display 12.0- and 3.0-fold acceleration over the background reaction with a catalyst devoid of protein. Thus, these artificial metalloenzymes display an increase both in activity and in selectivity for the reduction of acetamidoacrylic acid.

Keywords: Biotin–avidin; Streptavidin; Enantioselective catalysis; Second coordination sphere; Hydrogenation; Artificial metalloenzyme; Bioinorganic chemistry; Protein-accelerated catalysis

1. Introduction

Enantioselective catalysis is one of the most efficient ways to synthesize high-added value enantiomerically pure organic compounds [1]. In recognition for their pioneering accomplishments in transition metal mediated enantioselective catalysis, the 2001 Nobel Prize in chemistry was awarded to Knowles [2], Noyori [3] and Sharpless [4].

As the subtle details which govern enantioselection cannot be reliably predicted or computed, catalysis relies more and more on a combinatorial approach. In the past couple of years, several groups have made important contributions in this field [5].

Biocatalysis offers an attractive alternative for the synthesis of enantiopure products [6]. In many aspects, enzymatic catalysis is complementary to transition metal-based systems, although the scope of reactions is limited to enzymatic processes. From a combinatorial perspective however, the potential of directed evolution techniques in optimizing an enzyme's selectivity is unrivalled [7].

We have recently demonstrated that incorporation of an achiral rhodium–diphosphine moiety into a protein environment can yield highly enantioselective hydrogenation catalysts [8].

Inspired by the seminal work of Kaiser [9,10], several groups have developed methods to covalently modify proteins by incorporating transition-metal catalysts to yield hybrid catalytic systems with promising properties [7,10].

The approach we follow relies on non-covalent (i.e., supramolecular) 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

* Corresponding author. Tel.: +41 32 718 2516; fax: +41 32 718 2511.
E-mail address: thomas.ward@unine.ch (T.R. Ward).

warranted [11]. As suggested by Whitesides, the biotin–avidin system [12] offers an attractive scaffold to perform such experiments [13,14].

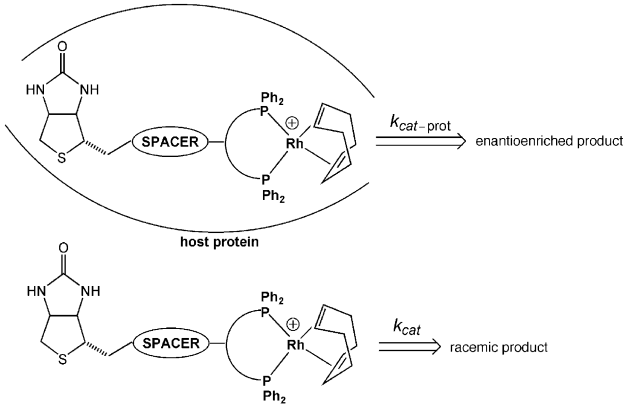
The principle of the biotin–avidin technology (often referred to as molecular velcro) relies on the extraordinary affinity of biotin for either avidin or streptavidin ($K_a \sim 10^{14}$ M^{-1}) [12]. Most importantly, it is generally accepted that derivatization of the valeric acid side chain of biotin does not affect significantly the strength of the biotin–avidin interaction as most stabilizing contacts are located on the bicyclic framework of (+)-biotin [12]. In the past twenty years, the biotin–avidin technology has found numerous applications in various fields of biotechnology including ELISA, immunolabeling, affinity targeting, drug delivery etc. [12]. In the context of this work, it should be emphasized that both avidin and streptavidin are tetrameric proteins constituted by four identical subunits which can bind up to four biotin molecules with no binding cooperativity.

Performing the reduction of acetamidoacrylic acid in avidin as host protein, the rhodium coenzyme $[Rh(COD)(\mathbf{Biot\text{-}1})]^+$ affords (*S*)-acetamidoalanine in quantitative yield with 39% ee.

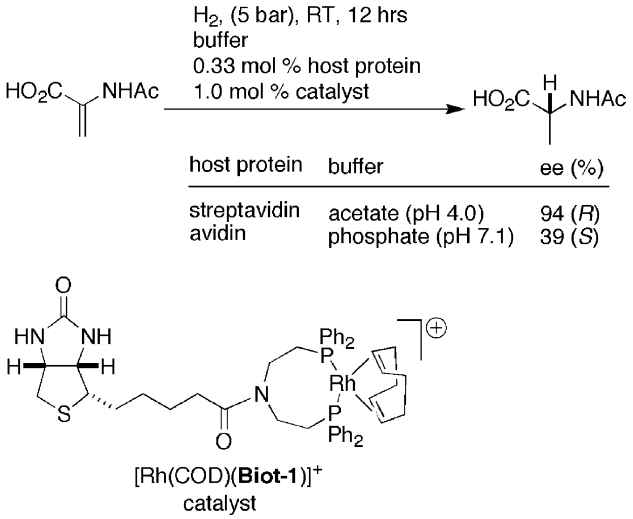
As the catalyst devoid of its protein environment yields essentially racemic product under identical conditions, we reasonned that it is imperative to ensure that catalysis proceeds exclusively within the protein (Scheme 1). As avidin possesses an isoelectric point p*I* = 10.4, we speculated that at pH 7.0, the cationic catalyst may display a significantly reduced affinity for the positively charged avidin. The modest ee may thus be a reflection of two competing catalytic cycles:

(i) inside the protein which affords an highly enantioenriched product;
(ii) outside the protein which affords racemic product.

Streptavidin, which displays only 30% sequence homology with avidin possesses a much lower isolectric point (p*I* = 6.4) [12]. Using streptavidin may thus solve



Scheme 1.



Scheme 2.

the problem of Coulomb repulsion between the host protein and the cationic catalyst precursor. The reaction performed under identical conditions afforded (*R*)-acetamidoalanine in nearly quantitative yield with 94% ee [8]. Upon lowering the pH to 4.0, the reaction proceeded quantitively (Scheme 2).

2. Results and discussion

Both in the fields of homogeneous [15] and heterogeneous [16] catalysis, the concept of ligand acceleration has proven very valuable. In ligand-accelerated catalysis, the addition of a ligand increases the reaction rate of a catalytic transformation which proceeds even in the absence of added ligand.

In the area of artificial metalloenzymes, the same concept may apply as the "coenzyme" $[Rh(COD)(\mathbf{Biot\text{-}1})]^+$ was shown to be active (and unselective) in the absence of host protein (Scheme 1). As the interaction between the biotinylated moiety and the host protein is noncovalent, it is inherently reversible. The amount bound and free catalyst (abbreviated μ_{bound} and μ_{free}, respectively) is determined by the stability constant of the supramolecular edifice $[Rh(COD)(\mathbf{Biot\text{-}1})]^+$–(strept)avidin and the individual concentrations (We use Lehn's notation for host–guest interactions [17]; (strept)avidin denotes both avidin and streptavidin) [18]. Because of the very low solubility of acetamidoacrylate, we could not perform a thorough kinetic analysis to determine the individual rate constants $k_{cat-prot}$ and k_{cat} for the reaction within the protein and outside the protein, respectively. Assuming that both competing catalytic cycles (within and outside (strept)avidin) proceed according to the same mechanism, we reasonned that we could determine the relative rates $k_{cat-prot}$:k_{cat} by

incrementally varying the [Rh(COD)(**Biot-1**)]$^+$ to (strept)avidin ratio. By plotting the enantiomeric excess of the product as a function of this ratio, it is should be possible to estimate the extent of protein-acceleration (Fig. 1).

Hydrogenation experiments were performed using both avidin and streptavidin loaded with varying amounts of [Rh(COD)(**Biot-1**)]$^+$. The [Rh(COD)(**Biot-1**)]$^+$-to-protein ratio was varied incrementally from 1:1 to 10:1 and the enantiomeric excess is plotted against this ratio, Fig. 1. The reactions were performed as described in [8]. Two noteworthy features emerge:

(i) protein acceleration and
(ii) cooperativity between the four catalytic sites.

(i) Both avidin and streptavidin display protein accelerated catalysis. The extent of protein acceleration in streptavidin (where the (*R*)-product is produced preferentially) can be estimated by

$$\%(R)_{\text{calculated}} = \frac{k_{\text{cat–prot}} \cdot \mu_{\text{bound}} \cdot a + k_{\text{cat}} \cdot \mu_{\text{free}} \cdot b}{k_{\text{cat–prot}} \cdot \mu_{\text{bound}} + k_{\text{cat}} \cdot \mu_{\text{free}}}, \qquad (1)$$

where $k_{\text{cat–prot}}$ and k_{cat} are the rate constants for the reaction within the protein and outside the protein, respectively, μ_{bound} and μ_{free} are the number of protein-bound complexes ([Rh(COD)(**Biot-1**)]$^+$–(strept)avidin) and protein-free [Rh(COD)(**Biot-1**)]$^+$ moieties; a and b are the % (*R*) produced by [Rh(COD)(**Biot-1**)]$^+$–(strept)avidin and by [Rh(COD)(**Biot-1**)]$^+$, respectively.

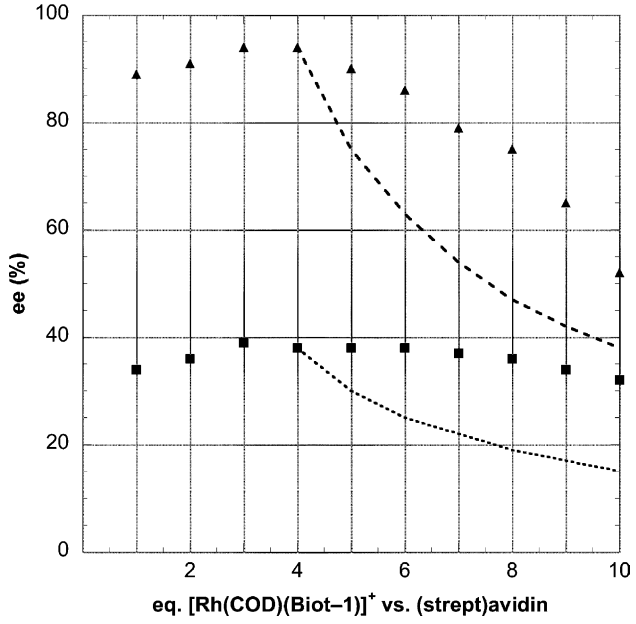


Fig. 1. Enantioselectivity as a function of the [Rh(COD)(**Biot-1**)]$^+$-to-(strept)avidin ratio. The triangles correspond to the ee (*R* enantiomer) obtained with streptavidin as host protein; the squares correspond to the ee (*S* enantiomer) obtained with avidin as host protein. The broken lines represent the theoretical ee with no protein acceleration, emphasizing the effect of the protein on the rate of the reduction.

As this procedure produces a single equation with two unknowns $k_{\text{cat–prot}}$ and k_{cat}, we can only extract the ratio rather than the individual rate constants.

If no protein acceleration is operative, $k_{\text{cat–prot}} = k_{\text{cat}}$. At eight equivalents [Rh(COD)(**Biot-1**)]$^+$ vs. protein, $\mu_{\text{bound}} = \mu_{\text{free}} = 4$ as (strept)avidin possesses four binding sites. Since $a = 0.97$ (i.e., 94% ee (*R*) for [Rh(COD)(**Biot-1**)]$^+$–streptavidin) and $b = 0.50$ (racemic material produced by [Rh(COD)(**Biot-1**)]$^+$), the % (*R*) predicted at eight equivalents and with no protein acceleration is % (*R*) = 73.5 (i.e., 47% ee (*R*)). The broken lines in Fig. 1 correspond to the ee calculated with no protein acceleration, assuming that both reactions proceed according to the same mechanism.

By performing a least square minimization on the calculated and measured % ee for a given $k_{\text{cat–prot}}$:k_{cat} ratio, we estimate the relative rates. For streptavidin and avidin, we compute a $k_{\text{cat–prot}}$:k_{cat} ratio of 2.95 and 11.96, respectively.

(ii) Increasing the [Rh(COD)(**Biot-1**)]$^+$-to-protein ratio from 1:1 to 4:1 (i.e., all four biotin binding sites occupied) leads to a slight increase in enantioselectivity (from 89% to 94% ee (*R*) for streptavidin and from 34% to 39% (*S*) for avidin). This suggests that there is a slight cooperativity on the enantioselectivity between the four biotin binding sites.

3. Conclusion

By incrementally varying the [Rh(COD)(**Biot-1**)]$^+$-to-(strept)avidin ratio, we have unraveled the phenomenon of protein-accelerated catalysis, similar to ligand accelerated catalysis. We have shown that the hydrogenation of acetamidoacrylic acid within artificial metalloenzyme proceeds at a faster rate than the corresponding hydrogenation outside of the protein.

We believe that this phenomenon is caused by the affinity of the hydrophobic substrate for the hydrophobic catalytic pocket within the host protein. Although the "active site" was by no means optimized to accomodate an enantioselective hydrogenation event, it is pleasing to witness an increase both in activity and in selectivity for this reaction, prototypical of the homogeneous catalysis kingdom. Current efforts in the group are directed towards the determination of individual rate constants using a more soluble substrate.

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