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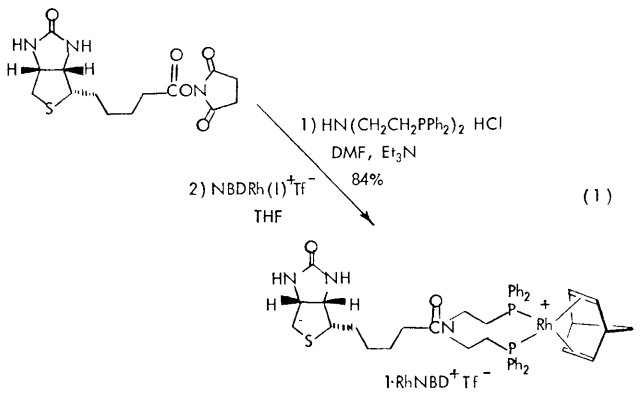
Conversion of a Protein to a Homogeneous Asymmetric Hydrogenation Catalyst by Site-Specific Modification with a Diphosphinerhodium(I) Moiety[1]

Sir:

We wish to describe an approach to the construction of asymmetric hydrogenation catalysts based on embedding an (effectively) achiral diphosphinerhodium(I) moiety at a specific site in a protein: the protein tertiary structure provides the chirality required for enantioselective hydrogenation. Although it is presently difficult to predict the enantioselectivity of any hydrogenation from knowledge of the structures of catalyst and substrate, phosphine–rhodium(I) complexes having rigid, conformationally homogeneous structures seem generally to be more effective catalysts than those which are conformationally mobile.[2] A globular protein modified by introduction of a catalytically active metal at an appropriate site could, in principle, provide an exceptionally well-defined steric environment around that metal, and should do so for considerably smaller effort than would be required to construct a synthetic substance of comparable stereochemical complexity.

Our initial efforts have focused on avidin. This well-characterized protein is composed of four identical subunits, each of which binds biotin and many of its derivatives sufficiently tightly that association is effectively irreversible (K_d = 10^{-12}–10^{-15} M).[3,4] Biotin was converted to a chelating diphosphine and complexed with rhodium(I) by the sequence outlined in eq 1 (NBD = norbornadiene, Tf = triflate).[5,6] The intermediate *N*,*N*-bis(2-diphenylphosphinoethyl)biotinamide (**1**) was fully characterized;[5] the rhodium complex **1**·RhNBD$^+$Tf$^-$ was prepared in situ and used without characterization.[6] The enantioselectivity of catalysis by complexes of avidin with **1**·RhNBD$^+$Tf$^-$ was tested by the reduction of α-acetamidoacrylic acid (**2**) to *N*-acetylalanine (**3**) (eq 2). This reduction has been used frequently in estimating the enantioselectivity of other asymmetric hydrogenation catalysts.[7]



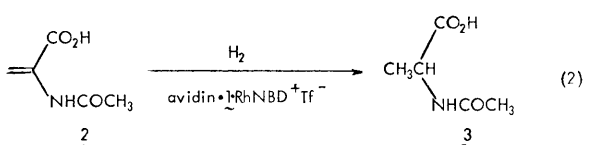


Table I. Catalytic Reduction of α-Acetamidoacrylic Acid (**2**) to *N*-Acetylalanine (**3**) using **1**·RhNBD$^+$Tf$^-$ (Alone and Mixed with Proteins) as Catalyst[a]

Protein added (mg)	Turnover no.[b] **2** → **3**[c]	Turnover no.[b] **4** → **5**[d]	Enantiomeric excess (**3**)[e] Polarimetric (±5%)[f]	Enantiomeric excess (**3**)[e] NMR (±10%)
None	475	10.3	<2	<2
Lysozyme (15)	450		<1	<2
Bovine serum albumin (15)	150		<5	
Carbonic anhydrase (15)	50	3.3	<10	
Avidin (10; 1 equiv[g])	>500[h]	12.6	41	44
Avidin (20; 2 equiv)	>500[h]		35	33
Avidin (10)·biotin[i]	200	3.5		<4
Avidin (10)·biotin[j]	160			<5
Avidin (10) + bovine serum albumin (15)[k]	480		34	34

[a] All hydrogenations were run with 0.50 μmol of **1**·RhNBD$^+$Tf$^-$ in 6.0 mL of water (0.1 M Na_2HPO_4 buffer, pH 7.0) at 0 °C for 48 h under 1.5-atm pressure of H_2. [b] Molecules of **2** or **4** hydrogenated per rhodium atom. [c] All experiments were run with 0.25 mmol of **2** and 0.25 mmol of Na_2HPO_4. [d] All experiments were run with 0.25 mmol of allyl alcohol. [e] The *S* enantiomer was in excess. [f] Calculated on the basis of the reported values for optically pure *N*-acetyl-(*R*)-alanine: $[\alpha]^{25}_D$ +66.5° (*c* 2, H_2O) (S. M. Birnbaum, L. Levitow, R. B. Kingsley, and J. P. Greenstein, *J. Biol. Chem.*, **194**, 455 (1952)). No rotations were observed for values denoted (<): low turnover numbers for CA and BSA led to the large experimental uncertainty. [g] The quantity of avidin added was 125 U, and was sufficient to bind 0.50 μmol of biotin.[8] [h] This value represents complete hydrogenation: these turnover numbers are thus lower limits. [i] The avidin was incubated with a 10% excess of biotin (0.55 μmol, 0.13 mg) before exposure to the solution containing **1**·RhNBD$^+$. The excess of biotin precluded polarimetric assay. [j] The avidin was incubated with a 10-fold excess of biotin (1.2 mg). [k] The avidin and bovine serum albumin were mixed before adding to the solution of **1**·RhNBD$^+$.

A representative hydrogenation was conducted as follows. α-Acetamidoacrylic acid (32 mg, 0.25 mmol) and Na_2HPO_4 (36 mg, 0.25 mmol) were degassed in a 20-mL pressure reaction bottle (Lab Glass) with argon, and 5 mL of aqueous 0.1 M Na_2HPO_4 buffer (pH 7.0) was added. The solution was cooled to 0 °C and 1.0 mL of a similarly buffered solution of avidin (~10 mg, 125 U, binds 0.50 μmol of biotin[8]) was added. The bottle was swept with dihydrogen (welding grade) and the pressure adjusted to 1.5 atm with dihydrogen. A solution of **1**·RhNBD$^+$Tf$^-$ in THF (25 μL of a 20 mM solution, 0.50 μmol) was injected: the resulting solutions were pale yellow and homogeneous. The reaction was stirred for 48 h (0 °C, 1.5 atm of H_2). The reaction was worked up by adjusting the pH to 2.0 with 2.0 N aqueous HCl solution and filtered through Celite to remove any precipitated **1** and through an Amicon Diaflo PM 10 ultrafiltration membrane (10 000 mol wt cutoff) to separate avidin and avidin·**1** complexes from **2** and **3**. The optical rotation of the resulting clear colorless filtrate was combined with an NMR measurement of the extent of con-

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version of **2** to **3** to give one estimate of the enantiomeric excess for **3.** Turnover numbers for the reduction (molecules of **2** reduced per atom of rhodium in 48 h) were also calculated from these conversions. The mixture of **2, 3,** and buffer was concentrated to a paste. The **2** and **3** were extracted into methanol, and converted to methyl esters with diazomethane. Examination of the NMR spectrum of this mixture in the presence of the chiral europium shift reagent $Eu(hfc)_3$[9] provided a second estimate of the enantiomeric excess for **3.** Values from optical rotation and NMR were in good agreement. Hydrogenations carried out in the absence of avidin, and in the presence of other proteins, were conducted and assayed by analogous procedures. Results are summarized in Table I. For comparison, this table also lists turnover numbers for conversion of the less hindered substrate allyl alcohol (**4**) to 1-propanol (**5**).

Compound **1**·$RhNBD^+Tf^-$, by itself, was a moderately active hydrogenation catalyst which shows no enantioselectivity in production of **3.** The presence of lysozyme, bovine serum albumin (BSA), and carbonic anhydrase (CA) in solutions of **1**·$RhNBD^+Tf^-$ had no significant influence on enantioselectivity, although BSA and CA markedly lowered the activity of the catalyst (CA by approximately a factor of 10). The presence of 1 equiv of avidin in solution (assuming each avidin subunit to be associated with 1 equiv of **1**) resulted in a definite *increase* in activity, and in the production of **3** with ~40% *S* enantiomeric excess. When the ability of avidin to bind **1** was blocked by prior exposure to ether a 10% excess or a tenfold excess of biotin, the enantioselectivity of the reduction was eliminated. Unexpectedly, solutions containing 1.0 equiv of **1**·$RhNBD^+$ per avidin subunit showed significantly higher enantioselectivity than those containing 0.5 equiv. The origin of this difference is not evident, but may reflect interaction between the biotin binding sites in different subunits.[3,4] The reduction in enantioselectivity observed on addition of **1**·$RhNBD^+$ to a mixture of avidin and BSA may indicate either slow dissociation of **1**·Rh^+ from a complex with BSA or interaction between BSA and avidin·**1**·Rh^+.

The observations summarized in Table I are compatible with the hypothesis that the active catalyst in solutions of **1**·$RhNBD^+Tf^-$ and avidin is a complex in which **1** is associated with the protein at the biotin-binding site. The observation that the turnover numbers for **2** → **3** and **4** → **5** are roughly parallel suggests little gross structure sensitivity to the system. The catalyst system composed of **1**·Rh(I) bound to avidin is not a practical asymmetric catalyst: although avidin is commercially available, it is expensive by the standards of transition metal catalysis; the enantioselectivity displayed by avidin·**1**·Rh(I) in hydrogenation of **2** to **3** is only modest.[7] Nonetheless, the experiments summarized here establish two principles. First, it is possible to carry out homogeneous hydrogenation using a diphosphinerhodium(I) catalyst associated with a protein: neither the aqueous solution nor interactions between the metal and the protein necessarily deactivate the catalyst. Second, the chirality of the protein is capable of inducing significant enantioselectivity in the reduction. It may be possible to apply these principles to the development of other combinations of proteins and transition metals capable of effecting practical enantio- or regioselective hydrogenation.[10] Further, the techniques developed to bind transition metals to specific sites in proteins may find uses in biological and clinical chemistry unrelated to asymmetric synthesis. We will describe further studies in this area in subsequent publications.

References and Notes

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