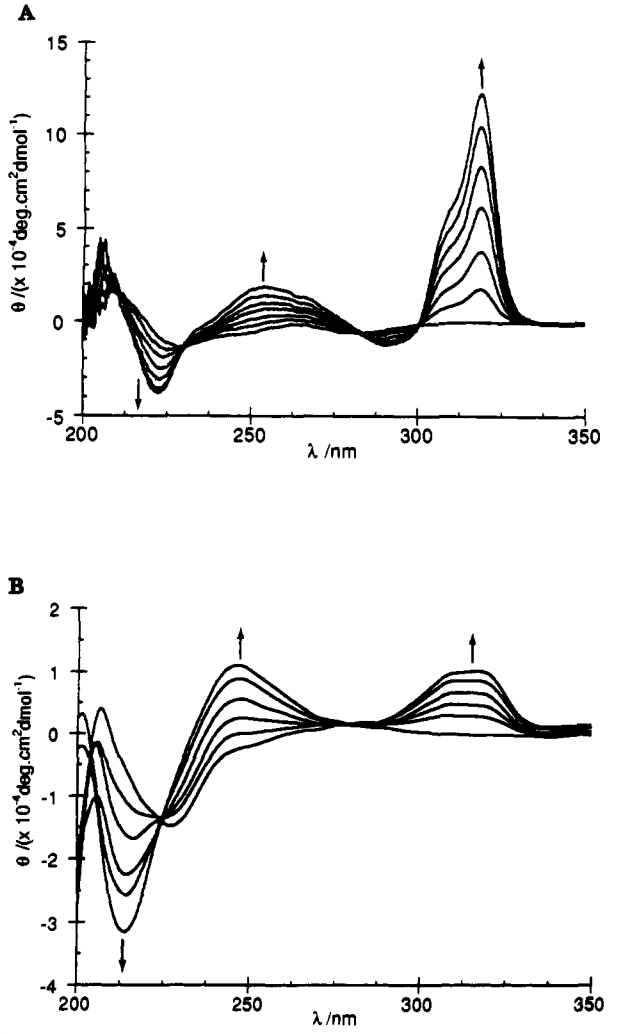

Figure 1. (A) Circular dichroism spectra of the titration of **2** (40 μM) with $CuCl_2$ (pH 6.6, 200 mM NaCl, 25 °C). $CuCl_2$ was added in 7.5-μM portions. (B) Circular dichroism spectra of the titration of **3** (40 μM) with $CuCl_2$ (pH 6.6, 200 mM NaCl, 25 °C). $CuCl_2$ was added in 7.5-μM portions.

formation of a chiral complex involving both bipyridyl ligands of **2**.

The coordination number of the metal in the peptide complexes was investigated through examination of the electronic spectra of the complex formed between **2** and Co(II).[13] Addition of Co(II) is accompanied by a weak absorption at 450 nm ($\epsilon_{max} <$ 100), which is suggestive of a five-coordinate complex. However, CD spectra of the Co(II) complex lacked the strong ellipticity observed with Cu(II) and this peptide. Thus, the conclusions from the Co(II) studies cannot provide general information regarding the coordination state of the other metal complexes, and in particular, alternative spectroscopic studies will be needed to establish the identity of the complex formed between **2** and Cu(II).

In conclusion, incorporation of the bipyridyl moiety into the polyamide framework of polypeptides should prove to be useful in de novo design of metalloproteins for both structural and functional roles, since it combines the wide scope of coordination chemistry available to this ligand with the versatility of protein biopolymers as templates for the assembly of organized three-dimensional structures.

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Registry No. 2, 136391-82-7; **3**, 136391-83-8; Zn(II), 23713-49-7; Co(II), 22541-53-3; Cd(II), 22537-48-0; Ni(II), 14701-22-5; Cu(II), 15158-11-9.

Supplementary Material Available: UV and CD spectra from titrations of **2** and **3** with $CuCl_2$ and of **2** with $CoCl_2$ and long-wavelength visible spectra from titration of **2** with $CoCl_2$ (5 pages). Ordering information is given on any current masthead page.

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Enantiofacial Protonation by Catalytic Antibodies

Ikuo Fujii, Richard A. Lerner,* and Kim D. Janda*

Departments of Chemistry and Molecular Biology
The Scripps Research Institute
10666 North Torrey Pines Road
La Jolla, California 92037
Received June 13, 1991

Several enzymes generate enolates as reaction intermediates.[1-4] Strategies for efficient generation of various substituted enols in an antibody binding site should expand the scope of antibody catalysis. Herein, we report on catalytic antibodies which not only accelerate hydrolysis of enol esters but also influence enantiofacial protonation of the "enolate"[5] intermediate to afford an optically enriched α-substituted cyclohexanone.

Hapten **1** was utilized as the antigen for induction of antibodies (Chart I). Notable features within this structure include (a) the phosphonate moiety as a direct mimic of the tetrahedral transition state anticipated in the hydrolysis of the enol ester **2**[6] and (b) application of our abzyme–substrate destabilization principle[7] via the placement of the methyl group in the substrate adjacent to its original point on hapten **1**. Phosphonate **1** was synthesized using the Michaelis–Becker reaction[8] and conjugated to the protein keyhole limpet hemocyanin (KLH) for antibody induction.[9]

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(8) The required phosphite and alkyl bromide were prepared as follows: To a solution of phosphorus trichloride (1.0 equiv) in dry benzene was added a mixture of benzyl alcohol (0.8 equiv) and pyridine (0.8 equiv) dropwise at room temperature. After 8 h, a mixture of 1-methylcyclohexanol (0.8 equiv) and pyridine (0.8 equiv) was added and the reaction mixture stirred overnight. The resulting solution was treated via aqueous workup and purified by flash chromatography (50/50 ethyl acetate/hexane; $R_f = 0.31$) to give 1-methylcyclohexyl benzyl phosphite (28% yield). The phosphite was reacted with 4-[2-[[(benzyloxy)carbonyl]amino]ethyl]benzyl bromide, which was prepared from 4-(2-aminoethyl)benzyl alcohol via protection of the amino group ($ClCO_2CH_2Ph/NaHCO_3/H_2O$) followed by bromination (Br_2PPh_3/CH_3CN), in the presence of sodium hydride in dry THF to afford the protected phosphonate. After deprotection of both benzyl groups ($H_2/Pd(OH)_2$–C/MeOH), the phosphonate was coupled with activated linker [[(succinimidooxy)-carbonyl]butyrl]chloride] to give hapten **1a**, which was purified by HPLC on a RP-C18 column eluting with 90/10 H_2O (0.1% TFA)/CH_3CN; room temperature, 17 min. All new compounds gave satisfactory spectroscopic and combustion analyses.

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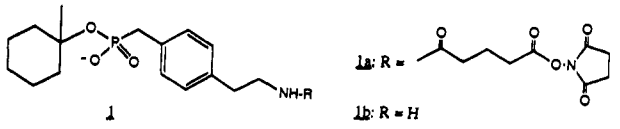
Chart I. Hapten with Activated Linker (**1a**) and Inhibitor (**1b**) Used in Assay Described in Table I



Scheme I. Simplified Mechanistic Picture for Reaction Catalyzed by Antibodies in Table I

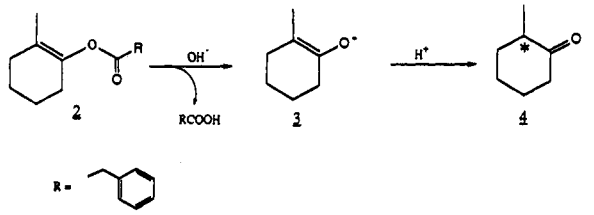


Table I. Kinetic Parameters for the Hydrolysis of Enol Ester **2** by Antibodies (27B5, 7F9, and 32B11)[a]

antibody	K_m, 10^{-6} M	k_{cat}, min^{-1}	k_{cat}/k_{uncat}[b]	K_i,[c] 10^{-6} M	% ee[d]	config[e]
27B5	994	0.01	300	4.3	42	*R*
7F9	909	0.02	680	6.5	<5	*R*
32B11	436	0.01	300	15.2	<5	*R*

[a] Conditions: Assay conditions [pH 9.0, 10% DMSO/ATE (0.1 M ACES, 0.052 M Tris, and 0.052 M ethanolamine), 20 μM antibody, 25 °C]. Hydrolysis rates were measured by following the generation of phenylacetic acid via HPLC on a RP-C18 column eluting with water–acetonitrile (85:15) at a flow rate of 1 mL/min with UV detection at 260 nm. [b] Background hydrolysis rate 3.4×10^{-5} min^{-1}. [c] Inhibition data determined using **1b** as the inhibitor. [d] Determined by GC analysis with the chiral cyclodextrin column. Retention times: (*S*)-**4**, 36 min; (*R*)-**4**, 37 min. Oven temperature 65 °C, He_2 gas. An authentic sample of (*S*)-**4** was prepared by the method described by Meyers:[12] $[\alpha]^{21}_D$ −45.6° (*c* = 2.3, MeOH). [e] The configuration of that fraction of product which is in enantiomeric excess.

An initial screen for simple hydrolytic activity was accomplished by HPLC (high-pressure liquid chromatography). The reaction consisted of 20 μM highly purified antibody[10] and 1500 μM 1-(phenylacetoxy)-2-methylcyclohexene (**2**)[11] (Scheme I) in 10% DMSO/ATE buffer (ACES 0.1 M, Tris 0.052 M, ethanolamine 0.052 M), pH 9.0, 25 °C. From 26 monoclonal antibodies screened, three were found to accelerate the hydrolysis of **2**. These three IgG antibodies were studied as a function of variable concentrations of **2** (50–1500 μM). All exhibited saturation kinetics (Table I) and could be completely inhibited by the addition of free hapten **1b** (Chart I). Next we studied whether any of these abzymes could direct protonation of the hydrolytic reaction product, enolate **3** (Scheme I), in an enantioselective manner.

Determination of the enantioselectivity of this reaction was accomplished through the use of a microcapillary gas chromatograph column (Chrompack, CD-(optically pure)-cyclodextrin-B-236-M-19), which completely resolved both enantiomers. The *S* isomer of ketone **4** was synthesized using the procedure of Meyers.[12] Each of three catalytic antibodies (20 μM) was reacted with 500 μM enol ester **2**. When 70 μM product **4** had formed, the assay mixture was extracted with diethyl ether and concentrated, and the enantiomeric enrichment was determined. One of the three abzymes (27B5) provided an optically enriched mixture of *R* ketone **4** (Table I).[13] Although the optical purity obtained was less than that achieved by natural enzymes for some substrates,[4] it is the *first demonstration of an antibody which influences chirality via enantiofacial protonation.*

In asymmetric induction by antibody catalysis, the enantiomeric excess can be a function of the interaction between complex variables. These may include the extent of solvent exclusion from one face of the substrate, the off rate of the enolate leaving group from the protein, and the availability of a proton or its rate of transfer in the antibody binding site.[14] Since antibodies are highly diverse and molecules from individual clones will bind substrates differently, we can expect to improve the enantiomeric excess by selecting antibodies with a more favorable balance between controlling parameters.

Future studies will include screening of additional antibodies and the design of new haptens for the induction of abzymes capable of retarding the escape of **3** into bulk solvent during protonation. This may be a first step toward a rational approach for improving abzyme-mediated asymmetric induction.

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(13) No racemization of ketone **4** was observed (assay conditions reported, Table I) when (*S*)-**4** was allowed to stand overnight. No asymmetric induction was detected when racemic ketone **4** (50 μM) was incubated with antibody 27B5 (50 μM). This result rules out the possibility that antibody 27B5 binds the *S* isomer of racemic product **4** to generate an enantiomeric excess.

(14) While we have implied the reaction mechanism to proceed through a stepwise process (i.e., enol/enolate), we cannot rule out a concerted mechanism of hydrolysis–protonation.

Solution and Solid-State Structures of a Cyclic ψ[CH_2SO] Pseudopentapeptide

Sougen Ma, John F. Richardson, and Arno F. Spatola*

Department of Chemistry, University of Louisville
Louisville, Kentucky 40292
Received February 19, 1991

Amide-bond surrogates within peptides can lead to effective enzyme inhibitors, enzyme-resistant peptide mimetics, or occasionally, potent hormone antagonists.[1] We have been interested in defining the conformational effects of various backbone replacements systematically in order to apply backbone modifications more judiciously. Our previous NMR studies have shown that the ψ[CH_2S][2,3] and ψ[CSNH][4] replacements are generally compatible with reverse turn structures[5] when incorporated into the model cyclic pentapeptide cyclo[Gly-Pro-Gly-D-Phe-Pro].[6-12]

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