here) is α to a ketone or a thiol ester are invariably syn,[14] whereas those where the abstracted proton is α to a carboxylic acid are universally anti.[14] Further, a chairlike aldol transition state is most reasonable in the light of the preferred chair conformation of the final product, dehydroquinate.[15]

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A New Semisynthetic Esterase

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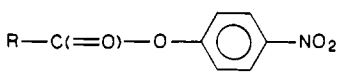
In recent years there has been considerable effort to mimic the catalytic action of enzymes. Most attempts have concentrated on ester hydrolases. Binding cavities based on macrocyclic structures[1,2] provide high rate accelerations for ester hydrolysis; however, these catalysts remain permanently acylated and no turnover is possible. Poly(ethylenimines) with attached imidazole groups[3] and micellar systems[4] have been shown to catalyze ester hydrolysis with turnover for some substrates, but the rate enhancement and the binding capability are only moderate. In the present paper we describe a new semisynthetic esterase that (1) exhibits some of the highest acylation rate enhancements observed with synthetic catalysts, (2) supplies an excellent binding site with binding free energies similar to those of enzymes, and (3) provides fast overall turnover.

The semisynthetic enzyme comprises a nonenzymatic protein, sperm-whale Met-myoglobin, from which the heme group has been removed by the acid/acetone method.[5] The apoprotein thus obtained possesses a deep hydrophobic cavity which serves as an enzymatic binding site for hydrophobic substrates. Within the heme pocket of myoglobin there are two imidazole residues, an established ester hydrolyzing catalyst. As expected, this combination of cavity and catalytic groups exhibits excellent hydrolyzing capability for esters with the structure **1**.



1

1a, R = C_5H_7, PNPcaproate
1b, R = C_9H_{11}, PNPcaprate
1c, R = CH_3, PNPacetate

The rates of hydrolysis in the presence of either apo-Mb or free imidazole (0.05 M Tris, pH 8.0, at 25 °C) measured by following the formation of *p*-nitrophenolate ion (PNP) at 400 nm were corrected for spontaneous hydrolysis in the buffer. PNPcaproate (**1a**) (1×10^{-5} M) hydrolyzes in the buffer with a first-order rate constant of 2.6×10^{-5} s^{-1}. In the presence of the apoprotein the hydrolysis is greatly accelerated and exhibits saturation behavior. The data obtained with 2×10^{-5} to 11×10^{-5} M of apo-Mb fit a Lineweaver–Burk plot, thus demonstrating 1:1 complex formation. These results are consistent with the first two steps in a mechanism analogous to that of hydrolytic enzymes (Scheme I). The value of k_2 for **1a** (4.9×10^{-2} s^{-1}) is 1900 times faster

Scheme I

$$\text{Apo-Mb} + \text{PNPAc} \overset{K_s}{\rightleftharpoons} \text{Apo-Mb-PNPAc}$$

$$\text{Apo-Mb-PNPAc} \xrightarrow{k_2} \text{Apo-Mb-Ac} + \text{PNP}$$

$$\text{Apo-Mb-Ac} \xrightarrow{k_3} \text{Apo-Mb} + \text{acid}$$

than k_2 in the buffer, and the Michaelis constant, K_m, is 7.4×10^{-5} M. The "catalytic constant", $k_2/K_m = 660$ M^{-1} s^{-1}, is 3000 times larger than $k_{imidazole}$ (0.22 M^{-1} s^{-1}) and 15 times larger than the one observed in the hydrolysis of the same substrate by the PEI–imidazole "synzyme".[3] The more hydrophobic substrate **1b**, studied under identical conditions, manifested the same kinetic behavior. Similar k_2 and K_m values were observed, 0.030 s^{-1} and 6.1×10^{-5} M, respectively; however, the enhancements achieved for this ester, $k_2/k_{buffer} = 4900$ and $(k_2/K_m)/k_{imidazole} = 3780$, are even higher than with **1a**.

The hydrolysis of PNPacetate (**1c**) by the apo-Mb semisynthetic enzyme was studied with ester concentrations in large excess over that of the enzyme. The initial rates of PNP release obtained with 3.6×10^{-5} M apo-Mb and 2.0×10^{-4} to 13.0×10^{-4} M **1c** fit a Lineweaver–Burk plot and yielded $k_2 = 5.8 \times 10^{-3}$ s^{-1} and $K_m = 4.3 \times 10^{-4}$ M. The rate enhancement ($k_2/k_{buffer} = 42$ or $(k_2/K_m)/k_{imidazole} = 27$) achieved by the semisynthetic enzyme for hydrolysis of **1c** is only moderate; however, it is almost 5 times larger than the one obtained with β-cyclodextrin.[6,7] From comparison of the "catalytic constants", k_2/K_m, under similar conditions, the apo-Mb is 287 times more efficient than cyclodextrin in hydrolyzing PNPacetate. Moreover, while PNPacetate permanently acetylates cyclodextrin, thus disabling the catalyst, the apo-Mb hydrolysis of PNPacetate is fully catalytic with a deacylation rate faster than acylation. Turnover was demonstrated by comparing the rate of hydrolysis of 1×10^{-5} M **1c** by 5×10^{-5} M apo-Mb to that of 1×10^{-5} M **1c** by a reaction mixture of 5×10^{-5} M apo-Mb and 5×10^{-5} M **1c** which had been allowed to react to 95% completion. The rates were identical, verifying that acylation rather than deacylation is the rate-determining step, i.e., $k_3 \gg k_2$. In the case of **1a** and **1b**, the same type of experiment revealed a decrease in catalysis rate, suggesting that with these two esters the deacylation is rate limiting. Preliminary studies of the absorbance changes at 240–270 nm, the acylated imidazole peak, indicate that the acyl group is not permanently attached and hydrolyzes slowly.

It is well established that peripheral nucleophilic residues of proteins like histidine, lysine, or serine can catalyze PNP release from PNP esters.[8] In fact, met-Mb in its native and denatured forms has been shown to react with **1c** but with a very slow rate.[9] In order to ascertain that our remarkable catalytic rates result from one active site only, namely, the empty heme pocket, we reinvestigated those myoglobin ester interactions under our experimental conditions. When **1a** was hydrolyzed in the presence of either native Mb or 8 M urea-denatured Mb, only a marginal increase in rate compared to the buffer was observed. In both cases the rate of hydrolysis was more than 3 orders of magnitude slower than in the presence of apo-Mb. This result establishes the heme cavity as the active site of the semisynthetic esterase. The existence of turnover supports the claim that one or both of the imidazole groups in the pocket are responsible for the hydrolase activity.

The apoprotein ester dissociation constants of (6.1×10^{-5})–(4.3×10^{-4}) M are significantly lower than those observed with cy-

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clodextrins or even chymotrypsin.[6,10,11] With cyclodextrin, only part of the ester, either the *p*-nitrophenyl or the aliphatic chain, is inserted in the pocket.[12] The apo-Mb cleft is large enough to accommodate the whole ester, at least in the case of **1a** and **1c**, resulting in a binding that is tighter by about 1 kcal. We have made no attempt to optimize the geometry of the transition state by tailoring the substrate to fit the active site. It is therefore conceivable that higher rate accelerations might be achieved with this new semisynthetic esterase.

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Adsorption of Formamide on the Ru(001)-p(1×2)-O Surface: The Spectroscopic Identification of η^2(N,O)-NHCHO

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Despite their importance as prototypes for studying amino acid chemistry, the interaction of amides with well-characterized single-crystalline metal surfaces has not been studied previously. A variety of transition-metal-formamido compounds have been synthesized and characterized, however, and a number of formamide-derived ligands have been observed, including η^1(C)-$CONR_2$ (R = H, alkyl or aryl),[1] η^2(N,O)-NRCRO,[2] η^1(N)-NHC(R)O,[3] η^2(C,O)-NR_2CO,[4] η^2(C,N)-OCNHR,[5] and η^2(C,N)-HOCNH.[6] In this paper, we present preliminary results of an electron energy loss spectroscopic (EELS) and thermal desorption mass spectrometric (TDMS) study of formamide adsorption on the Ru(001) surface on which an ordered p(1×2) overlayer of oxygen adatoms is present. This study provides evidence for the formation of an η^2(N,O)-NHCHO species, analogous to the η^2-formate formed from formic acid decomposition on the initially clean Ru(001) surface.[7,8]

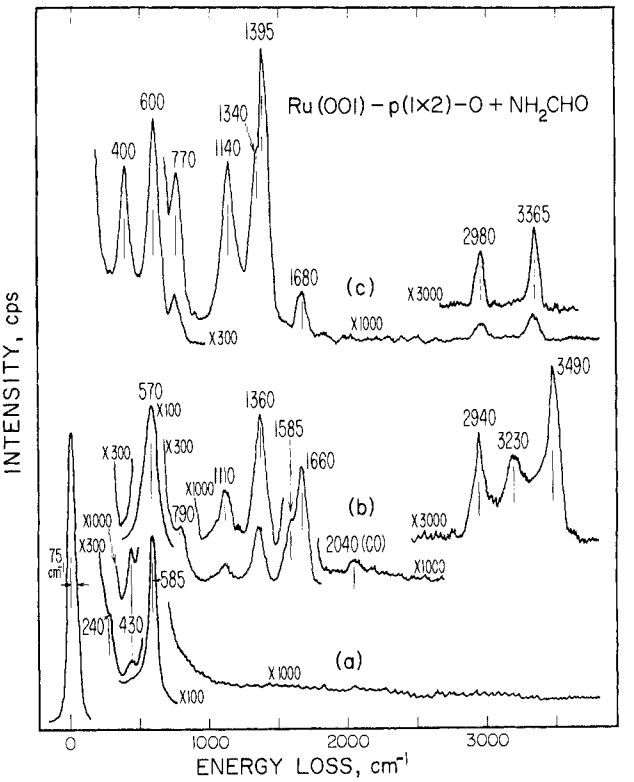

Figure 1. (a) EEL spectrum of the Ru(001)-p(1×2)-O surface. (b) The EEL spectrum that results following a 5×10^{-7} torrs exposure of NH_2-CHO to the Ru(001)-p(1×2)-O surface at 80 K, showing the characteristic features of molecular chemisorbed formamide. (c) The EEL spectrum that results when the surface of (b) is annealed briefly to 400 K and recooled to 80 K, showing the characteristic features of η^2(N,-O)-NHCHO.

Table I. Vibrational Frequencies in cm^{-1} and Mode Assignments of Molecularly Chemisorbed Formamide on the Ru(001)-p(1×2)-O Surface at 80 K and of Gas-Phase and Liquid Formamide

mode	NH_2CHO/Ru(001)-p(1×2)-O	NH_2CHO (gas)[13,14]	NH_2CHO (liquid)[13,15]
$\nu_a(NH_2)$	3490	3545	3388
$\nu_s(NH_2)$	3230	3451	3207
ν(CH)	2940	2852	2881
ν(CO)	1660	1734	1681
$\delta(NH_2)$	1585	1572	1611
ν(CN)	1360	1255	1309
δ(CH)	n.o.	1378	1391
π(CH)	n.o.	1030	1050
NH_2 deformations[a]	1110, 790	1059, 602, 289	1090, 750, 200
δ(NCO)	~525[b]	565	595
ν(Ru-NH_2CHO)	310[c]		

[a] The assignment of the NH_2 rocking, wagging, and twisting modes for the molecular formamide is somewhat controversial. thus, we have not attempted to assign these modes. [b] Overlaps with ν_s(RuO) of oxygen adatoms. [c] Not well resolved in Figure 1b; sharpens with annealing. n.o. = not observed, a = asymmetric, s = symmetric.

The ultrahigh vacuum chamber in which the EELS and TDMS experiments were performed has been described previously,[9] as have the properties and method of preparation of the Ru-(001)-p(1×2)-O surface.[10-12] The p(1×2)-O overlayer corre-

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