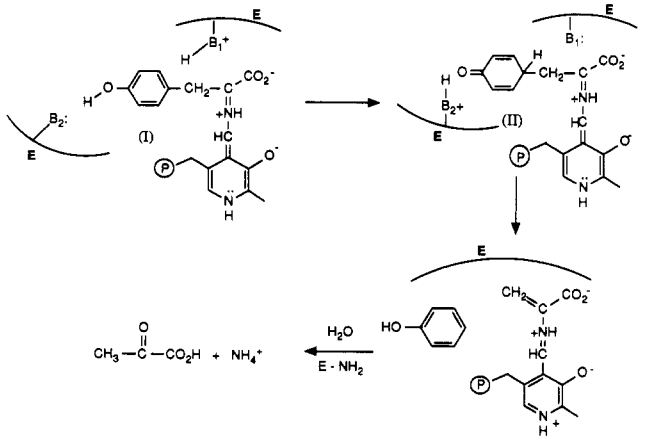

Figure 1. Part of the mechanism for the β-tyrosine phenol-lyase-catalyzed elimination reaction of tyrosine. P is a phosphate group and E the enzyme.

reaction mixture. The vials were then centrifuged at 5000 rpm for a few minutes; 5–10 μL of the quenched reaction mixtures were injected on an HPLC column,[9] and the separated labeled substrate and product were collected in liquid scintillation bottles. The radioactivity of the fractions was measured immediately, and after total decay of the ^{11}C, the ^{14}C-radioactivity was measured with a liquid scintillation counter. The ratio $k_{11}/k_{14} = \ln(1 - f_{11})/\ln(1 - f_{14})$, where f is the fraction of reaction, was calculated for each reaction point.

The results from three KIE experiments, performed in phosphate buffer pH 6.8 at 18 °C,[10] were 1.068 ± 0.017 (n = 10), 1.083 ± 0.013 (n = 11), and 1.051 ± 0.012 (n = 14), where n is the number of reaction points and the standard deviation is reported. The mean $^{11}C/^{14}C$ KIE value is 1.067 ± 0.009.

Several contributions to the elucidation of the mechanism of tyrosine phenol-lyase action have recently been reported.[11] For reviews, see, e.g., Snell and DiMari[12] and Miles.[13] The chemical mechanism involves the formation of an aldimine between L-tyrosine and pyridoxal 5-phosphate (PLP). The substrate α-proton is abstracted by an enzyme-bound base (see Figure 1; B_1, pK_a = 7.6[11c]) with the formation of a quinonoid structure (I). Another base (B_2, pK_a = 8.0[12c]) then abstracts the hydroxyl proton, and the first base (B_1) returns a proton to the aromatic C-4 position with the formation of a cyclohexadienone moiety (II). The activated carbon–carbon bond now breaks with simultaneous electron-push from the PLP, and electron-pull when the hydroxyl proton is returned by the base B_2. Phenol is released, and after transamination and hydrolysis, pyruvic acid and ammonia are released from the enzyme. In a study of the pH dependence of kinetic parameters and the primary deuterium KIE of tyrosine phenol-lyase from *C. freundii*, it was concluded that the α-proton abstraction was a partially rate-limiting step.[11c]

For the nonenzymatic malonic acid decarboxylation, a reaction in which carbon–carbon bond breaking (as in the present case) is accompanied by formation of a double bond to the isotopic carbon atom,[14] k_{12}/k_{14} for acid labeled in the 2-position was determined to be 1.076 by Ropp and Raaen.[15] Using the relation $\ln(k_{11}/k_{14})/\ln(k_{12}/k_{14}) \cong 1.6$[5b] between the different carbon isotope effects, the $^{12}C/^{14}C$ KIE corresponding to our value may be estimated to be 1.04. Our results, in combination with earlier conclusions,[11c] therefore suggest that the C–C bond breaking is at least partially rate limiting.

Acknowledgment. We thank Dr. Petter Malmborg for his help with the radionuclide production and Prof. Y. Watanabe for supplying the tyrosine phenol-lyase. This project is supported financially by the Swedish Natural Science Research Council.

(9) A RP C-18 column was used, and the mobile phase was 0.01 M acetic acid, pH 3.5: methanol (75:25, v/v) isocratic flow 2.0 mL min^{-1}.

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One-Pot Synthesis of Aromatic Methyl Esters by Electrochemical Oxidation of Aldehydes Mediated by Biscoenzyme Catalysis

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The direct oxidation of aldehydes to esters under mild conditions is a useful transformation in organic synthesis.[1] Here we describe an efficient, one-pot synthesis of aromatic methyl esters by electrochemical oxidation of aldehydes, mediated by two coenzyme catalysts: the thiazolium ions **1a/b** and flavin **MeFl**. The use of the macrocyclic catalyst **1b** in electroorganic synthesis elegantly combines the principles of electrocatalysis[2] with molecular recognition (Chart I).[3]

Thiazolium ions are known to catalyze the oxidation of aldehydes to esters.[4] The thiazolium ylide **2**[5] reacts to give the "active aldehyde" **3** (Scheme I).[6] This reactive intermediate can condense with another aldehyde to give an acyloin or can be oxidized to give the 2-acylthiazolium ion **4**. This ion reacts readily in alcohol[7] to give an ester (Scheme I). Stoichiometric amounts of oxidizing agents like nitrobenzene,[8] potassium ferricyanide,[9] and flavins[10] cause solubility problems and complicate product isolation. Also, the thiazolium catalyst is destroyed oxidatively in basic solution by ferricyanide,[9,11] iodine,[12] and air.[11,13] Attempts to regenerate catalytic amounts of **MeFl** with air resulted in the oxidation of **1a/b** by air (Table II, entry e).[13]

Our investigation by cyclic voltammetry shows that the anodic peak potential of **1a/b** in a 0.05 M solution of NEt_4Br in MeOH is ~0.2 V (vs Ag/AgCl at 0.02V s^{-1}), while that of **MeFl** is ca. −0.47 V ($E_{1/2}$ ca. −0.52 V at 0.02V s^{-1}). Under argon atmosphere,

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Scheme I

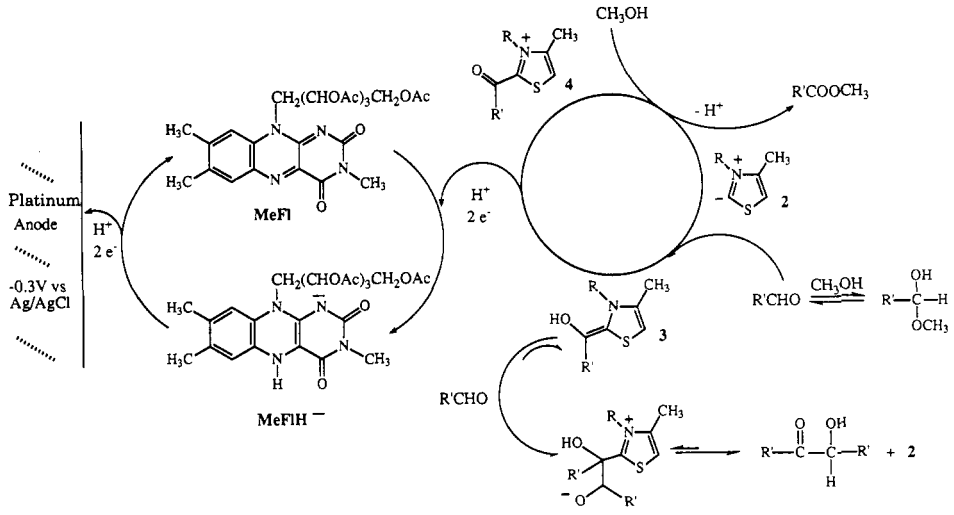
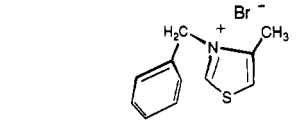


Table I. Electrochemical Oxidation of Aldehydes[a]

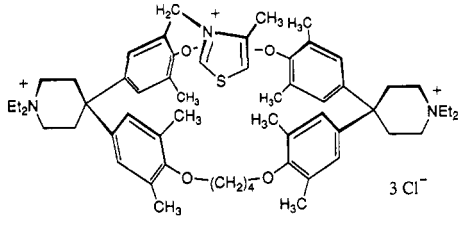
aldehyde	ratio of aldehyde to hemiacetal[c]	thiazolium catalyst	time (h)[d]	yield (%)[e]	current efficiency (%)[f]	turnover number[b]
valeraldehyde	1:16	**1a**	26	17	77	1.7
		1b	26	20	83	2.0
cyclohexanecarboxaldehyde	1:6.4	**1a**	24	7[g]	23	0.7
2-naphthaldehyde	1:0.074	**1a**	21	55	72	5.5
		1b	10	74 (76)	88	7.4
benzaldehyde	1:0.10	**1a**	21	54	60	5.4
4-cyanobenzaldehyde	1:2.3	**1a**	18	72 (69)	70	7.2
		1b	6	95 (88)	90	9.5
4-chlorobenzaldehyde	1:0.22	**1a**	18	78 (81)	84	7.8
methyl 4-formylbenzoate	1:0.85	**1a**	18	85 (83)	80	8.5

[a] The oxidation used an undivided cell equipped with a glassy carbon rod cathode (6 mm o.d.) and a platinum plate anode (12 mm × 50 mm) at −0.3 V vs a Ag/AgCl/3 M NaCl reference electrode at 308 K. The cell contained 11-mL solutions of electrolytes in MeOH with mole ratios of aldehydes (1.65 mmol, 150 mM):**1**:**MeFl**:NEt_3:NEt_4Br = 30:1:3:30:9. [b] Calculated as the number of moles of product per mole of **MeFl**, the catalyst with the highest concentration. [c] Determined by 200-MHz NMR of 150 mM solutions of aldehydes in 150 mM NEt_3 in CD_3OD. [d] The reactions were stopped when the current dropped to <10%. [e] Determined by gas chromatography. Numbers in brackets are isolated yields. [f] Calculated by (2 × moles of product × 100)/Faradays passed. [g] Approximately 80% of aldehyde remained unreacted.

Chart I



1a



1b

MeFl can be regenerated electrochemically at −0.3 V without causing oxidative destruction of **1a**/**b**. Therefore, we developed an unprecedented electrochemical regeneration cycle of the two coenzymes (Scheme I).

Using inexpensive **1a** and **MeFl** as catalysts, aromatic aldehydes give a 55–85% yield of methyl esters with high current efficiency (Table I). The much lower yields of aliphatic esters (<20%) are presumably due to the competing formation of hemiacetals, which significantly lowers the equilibrium concentration of aldehydes and hence the rate of formation of the "active aldehyde" intermediate, the slow step under the chosen reaction conditions (Table I). In the case of 4-cyanobenzaldehyde, the electron-withdrawing effect of the cyano group increases the reaction rate significantly and compensates for the decrease in equilibrium concentration of the aldehyde due to hemiacetal formation.

Table II. Control Experiments Performed with 4-Cyanobenzaldehyde (1.65 mmol) and Thiazolium Ion **1a** (0.055 mmol)

entry	**MeFl** (mmol)	atmosphere	working electrode potential (V vs Ag/AgCl)	time (h)	yield (%)[a]
a	0.165	argon	−0.3	18	72
b		argon	−0.3	20	19
c	0.165	argon		20	12[b]
d		argon		20	4
e	0.165	air		20	35
f		air		20	15[c]

[a] Determined by gas chromatography. [b] Theoretical yield from **MeFl** is 10%. [c] 0% yield in the absence of **1a**.

The supramolecular catalyst **1b** significantly enhances the rate[9b,13] and yield of aromatic ester formation (Table I). This catalyst forms tight inclusion complexes with benzene and naphthalene substrates in methanol. The increased rates are probably a result of (i) entropically favorable orientation and proximity effects and (ii) microenvironmental effects[14] in the apolar cyclophane cavity. Rates of thiazolium-catalyzed reactions increase with reduced solvent polarity since the relevant reaction

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transition states are less polar than the ground states.[15] The acidity of the macrocyclic thiazolium ring is enhanced by the apolar environment provided by the cavity.[14a] In deuterated acetate buffer (pD 4.7), the rate of H/D exchange at C-2 of the thiazolium ring in **1b** is about 2.6 times faster than that measured for **1a**.

The results of control experiments (Table II) show that the high yield obtained in entry a is due to the efficient regeneration of **MeFl** at the anode. Direct oxidation at the anode (entry b) is not an efficient enough process to trap all of the "active aldehyde" intermediates.[16,17]

The full scope of the supramolecular electrochemical process mediated by **1b** and the very useful oxidation of aldehydes to carboxamides are now under investigation.

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Facilitation of the $\Delta^2 \rightarrow \Delta^1$ Pyrroline Tautomerization of Carbapenem Antibiotics by the Highly Conserved Arginine-244 of Class A β-Lactamases during the Course of Turnover

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The hydrolytic action of β-lactamases is the primary mechanism of bacterial resistance to β-lactam antibiotics.[1] Within the past several years a variety of new β-lactam drugs have been developed that show resistance to the action of these enzymes.[2] Carbapenems constitute a group of such β-lactamase-resistant molecules, and they possess potent activity against a wide spectrum of bacteria.[3] Studies on the mechanism of action of class A β-lactamases with carbapenems by Knowles and colleagues have indicated a biphasic profile for hydrolysis of carbapenems, with an initial fast phase for substrate turnover leading to a slower one within minutes.[4,5] It was demonstrated that subsequent to active site acylation of a β-lactamase (at Ser-70) by these molecules, the Δ^2-pyrroline analogue **2** may either undergo deacylation or tautomerize to the corresponding Δ^1-derivative (**3**). The ester bond of **3** is kinetically more resistant to hydrolysis because of a less favorable substrate positioning in the active site (Scheme I). We present evidence here that the highly conserved arginine-244[6] is the essential source of proton for the $\Delta^2 \rightarrow \Delta^1$ tautomerization of carbapenem antibiotics, as depicted in Scheme I.

High-resolution crystal structures for two class A β-lactamases from *Staphylococcus aureus* PC1[7] and *Bacillus licheniformis* 749/C[8,9] have been reported recently. The information from crystal structure, in conjunction with kinetic findings from our laboratory, indicated that the substrate carboxylate forms hy-

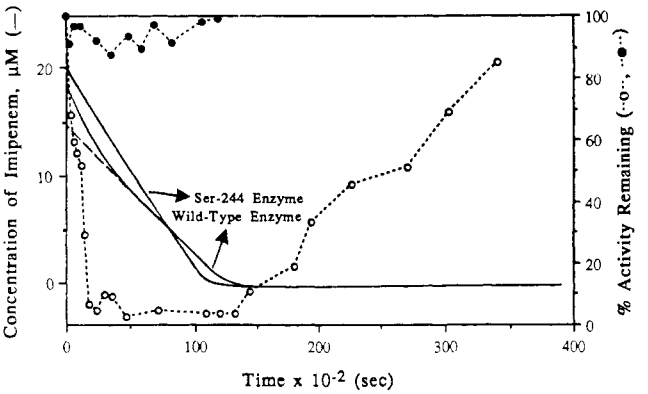


Figure 1. Hydrolysis of imipenem (20 μM) by the wild-type TEM-1 (2 μM) and the Arg-244-Ser mutant (2 μM) β-lactamases, in 0.1 M potassium phosphate buffer, pH 7.0, at room temperature (—). Extrapolation of the linear second phase of hydrolysis by the wild-type enzyme to time zero (- - -) and inhibition of activity of the wild-type (O) and the Ser-244 mutant (●) TEM-1 β-lactamases (as monitored for the turnover of benzylpenicillin) are indicated.

Scheme I

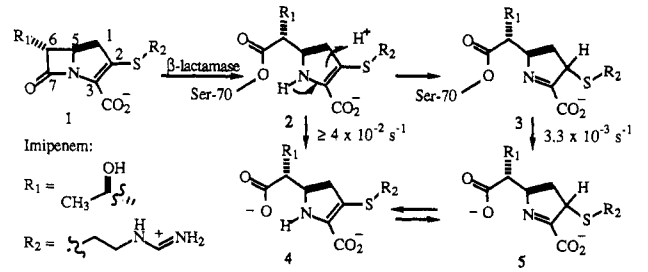
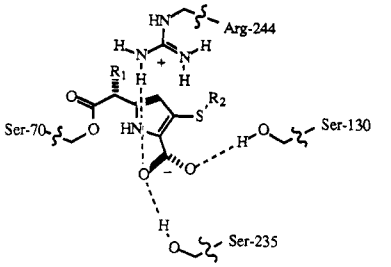


Chart I



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