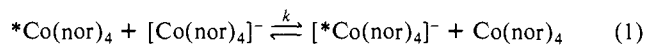


was associated with this reaction, which may also be effected with chemical oxidants. Addition of 1 equiv of AgBF_4 to a solution of **2** in THF resulted in immediate precipitation of a black solid (Ag), and from the brown solution crystalline $[\text{Co}(\text{1-norbornyl})_4]^+\text{BF}_4^-$ (**3**) could be isolated in 84% yield (Scheme I).⁹ **3** contains Co in the formal oxidation state V. It is expected to be a low spin tetrahedral complex like its precursor **2**.³ With a d^4 electronic configuration, it should be diamagnetic, and in accord with this prediction, the ^1H NMR and ^{13}C NMR spectra of **3** are those of a normal closed-shell compound containing norbornyl groups.

Some speculation on the origin of the stability of unusually high oxidation states of cobalt in this system seems appropriate. We believe that the tetrahedral geometry, in concert with the four strong field ligands, destabilizes **3** of the **5** metal d-orbitals (the σ -antibonding t_2 set) sufficiently to make their occupation energetically unfavorable. In other words, Co(V) in a tetrahedral environment is stable for the same reason that makes Co(III) the preferred oxidation state in a strong octahedral ligand field.

Given the rapid and reversible nature of the interconversions between **1**, **2**, and **3**, we decided to examine these electron-transfer reactions more closely. The room temperature ^1H NMR spectrum of a 1:1 mixture of **1** and **2** (0.014 M in **1** and 0.016 M in **2**, THF- d_8) is an averaged spectrum rather than the sum of the two individual spectra.¹⁰ At this temperature the electron self-exchange reaction (eq 1), which equilibrates the two species, is



apparently fast on the time scale of the NMR experiment. Upon cooling the sample, the resonances first broaden and finally separate into two sets assignable to **1** and **2**. This phenomenon is reversible and the coalescence temperature was determined to be 279 ± 2 K. From this and the independently measured temperature-dependent chemical shifts of the two reactants, the rate constant of the self-exchange reaction at the temperature of coalescence could be determined ($k = 4.1 (\pm 1.0) \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$).¹¹ A decrease in concentration of **1** and **2** (0.0062 M in **1**, 0.0071 M in **2**) increased the coalescence temperature to 292 ± 2 K, consistent with the bimolecular nature of the reaction. The rate of the exchange was not affected significantly by substitution of the $[\text{Na}(18\text{-crown-6})]^+$ salt of **1**. This lack of involvement of the counterion in the reaction is consistent with a simple outer-sphere electron transfer. Preliminary ^1H NMR experiments with mixtures of **2** and **3** show that this self-exchange is also fast on the NMR time scale. Our attempts to freeze out this reaction as observed by ^1H NMR have been unsuccessful and work is in progress to determine the exchange rate by other techniques.

The above electron transfers are very fast in comparison with the more standard Co(II)/Co(III) couples.¹² In both cases described herein the transferred electron occupies a σ -antibonding molecular orbital, leading us to expect a sizable reorganizational barrier and thus a slow exchange rate. However, there is little experimental precedent for the present situation, making a comparison of rate constants difficult.¹³

The chemistry of high valent alkyl complexes of the late transition metals is proving fascinating. These compounds and their interconversions via electron-transfer reactions are of particular interest as models for metal-catalyzed oxidations of hydrocarbons. We are now extending our experiments to other ligands and metals.

(9) **3**: IR (Nujol) 1053 cm^{-1} ; ^1H NMR (CD_2Cl_2) 2.37 (s, 4 H), 1.98 (m, 8 H), 1.80 (m, 16 H), 1.66 (s, 8 H), 1.47 (m, 8 H) ppm; ^{13}C NMR (CD_2Cl_2) 91.6, 44.3, 35.8, 32.5, 32.1 ppm. Anal. Calcd for $\text{C}_{28}\text{H}_{44}\text{BCoF}_4$: C, 63.89; H, 8.43; Co, 11.2. Found: C, 63.77; H, 8.53; Co, 12.2.

(10) **2**: ^1H NMR (THF- d_8) 10.4 (br s, 4 H), 7.2 (br s, 16 H), 4.3 (br s, 16 H), -3.4 (br s, 8 H) ppm. See ref 5 for data on **1**.

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Membrane-Enclosed Enzymatic Catalysis (MEEC): A Useful, Practical New Method for the Manipulation of Enzymes in Organic Synthesis¹

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We wish to describe a useful technique for the efficient manipulation of enzymes in organic synthesis,³ in which the enzyme in soluble form is enclosed in commercially available dialysis membranes. We have tested this technique (membrane-enclosed enzymatic catalysis, MEEC) in a number of representative enzyme-catalyzed reactions (Scheme I)⁴⁻¹² and have found that it combines the simplicity of use of soluble enzymes with certain of the advantages of immobilized enzymes. This technique may not be applicable to all enzymes, but it provides the simplest and most effective methods of using many of them in organic synthetic applications.

Enzyme-catalyzed reactions can use either soluble or immobilized enzymes.^{13,14} Procedures based on soluble enzymes are, in general, more convenient than those using immobilized enzymes, but the enzymes are not easily recovered for reuse, and their lifetime may be shortened by shear or interfacial deactivation. Immobilization allows the separation and reuse of enzymes and often protects them from deactivation by organic cosolvents, shear, interfacial adsorption, and proteases,^{15,16} but it is experimentally inconvenient and even under ideal conditions can cause significant deactivation of sensitive proteins.

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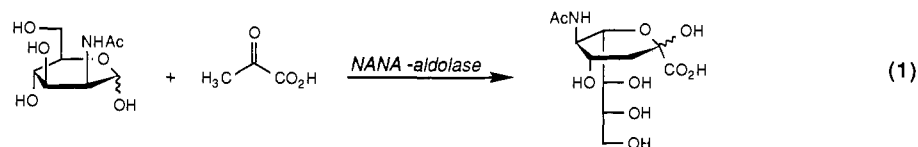
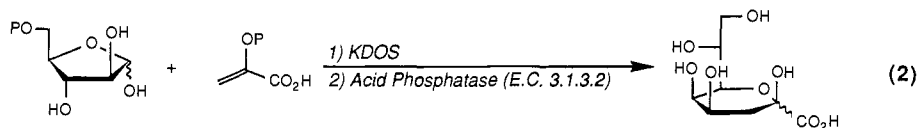
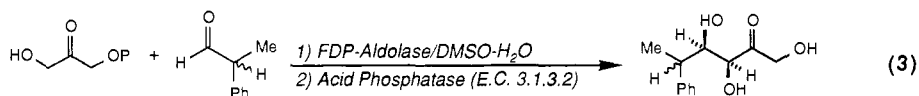
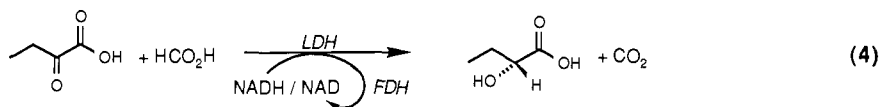
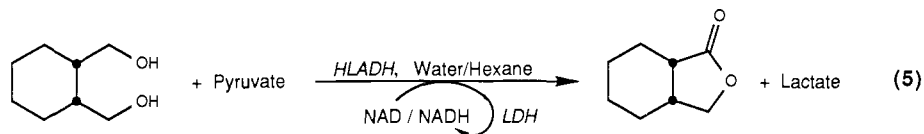
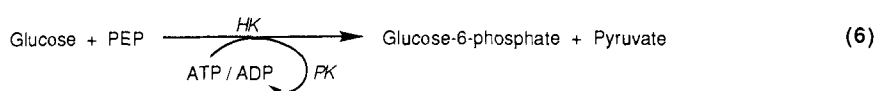
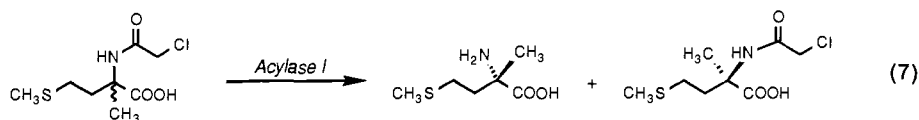
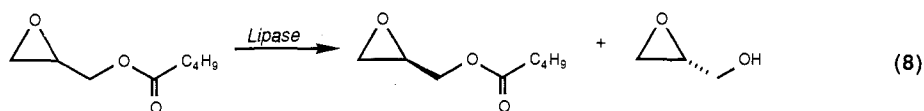
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Scheme I. Reactions Tested by Membrane-Enclosed Enzymatic Catalysis (MEEC)^aN-Acetylneuraminate pyruvate-lyase (NANA-aldolase; E.C. 4.1.3.3);⁴ 6.4 mmol:3-Deoxy-D-manno-octulosonate-8-phosphate synthetase (KDOS; E.C. 4.1.2.16);⁵ 7.8 mmol:Fructose-1,6-diphosphate aldolase (FDP-aldolase; E.C.4.1.2.13);⁶ 7.2 mmol:D-Lactate dehydrogenase (D-LDH; E.C. 1.1.1.28), Formate dehydrogenase (FDH; E.C. 1.2.1.2);⁷ 6.4 mmol:Horse liver alcohol dehydrogenase (HLADH; E.C.1.1.1.1), L-Lactate dehydrogenase (LDH; 1.1.1.27);⁸ 30 mmol:Hexokinase (HK; E.C. 2.7.1.1); Pyruvate kinase (PK; E.C. 2.7.1.40);⁹ 4 mmol:Acylase I (E.C. 3.5.1.14);¹⁰ 6 mmol L-amino acid, 10 mmol D-acylamino acid:Lipase (E.C. 3.1.1.3);¹¹ 17 mmol ester: α 2,6Sialyltransferase (α 2,6ST; E.C. 2.4.99.1);¹² 0.03 mmol:^aThe quantity of product isolated in each reaction is given as a measure of the scale on which the reaction was carried out.

We reasoned that dialysis membranes^{17,18} could be used to contain and, perhaps, to stabilize enzymes during use in organic synthesis. Containment would permit the separation of protein from the reaction medium. In all of the cases studied (Scheme I), separation of the products from the protein was straightforward and recovery of enzymatic activity was high. In the reactions summarized by eq 1, 3, 4, and 9, we demonstrated that the membrane-enclosed enzymes could be stored at 4 °C and reused for further catalysis. In these systems, the stabilities of the membrane-enclosed enzymes equalled or exceeded those of enzymes immobilized in PAN¹⁵ gel. Confinement of soluble lactate dehydrogenase in dialysis tubing (eq 5) prevented its contact with an immiscible organic phase (hexane) and protected it from deactivation. Such protection of soluble enzymes from interfacial deactivation in two-phase water-organic systems should be general. The MEEC technique was compatible with the presence of a miscible organic cosolvent (Me₂SO, eq 3) and with crude enzyme preparations (eq 2 and 8). Regeneration of nicotinamide (eq 4 and 5)¹⁹ and nucleoside triphosphate (eq 6)²⁰ cofactors was also possible by using this technique. MEEC was less effective with lipase (eq 8): the reaction proceeded at one-fifth of the rate expected on the basis of the usual emulsion-based procedures.¹¹ Lipases require a water-organic interface for activity, and we presume that the MEEC technique limits the interfacial area.

As an example of the method, we describe the oxidation of *cis*-1,2-bis(hydroxymethyl)cyclohexane (**1**) to (+)-(1*R*, 6*S*)-*cis*-8-oxabicyclo[4.3.0]nonan-7-one (**2**) (eq 5).⁸ A 1-L polyethylene bottle was charged with **1** (98%, 5.00 g, 34.0 mmol), sodium pyruvate (8.3 g, 75.0 mmol), Gly-Gly buffer (11 mL of 0.1 M solution), and 300 mL of water. A few drops of 4 N NaOH adjusted the pH of the mixture to pH 8.0. A 4-in. length of cellulose acetate dialysis tubing (Spectrapor 2, Spectrum Medical Industries, MW cutoff 12000-14000, 25-mm width) was washed with distilled water, and one end was tied shut. Horse liver alcohol dehydrogenase (250 mg, 350 units) and rabbit muscle lactate dehydrogenase (0.17 mL of a suspension in 2.1 M (NH₄)₂SO₄, 2 mg of protein, 800 units) were transferred in 4 mL of 0.1 M Gly-Gly buffer, pH 8.0, into the dialysis tubing. The open end of the tubing was tied shut with a string. The enzyme-containing bag was secured to a magnetic stir bar with a rubber band, washed

with water and submerged in the aqueous mixture; the reaction was stirred gently. NAD (0.060 mmol) was added, and 500 mL of hexane covered the aqueous layer. The enzyme-containing bag did not contact the hexane layer. Progress of the reaction was monitored by assaying the aqueous phase for pyruvate.²¹ After 32 h, the reaction was 98% complete. The hexane layer was removed by forced siphon with a cannula, and the aqueous phase was decanted. The dialysis bag was rinsed with water, and the washings were added to the aqueous layer. The enzyme bag could be prepared for reuse by dialyzing against buffer and was stored at 4 °C under buffer. The aqueous layer was extracted with ether. The ether and hexane layers were combined, dried overnight over CaCl₂, and evaporated under reduced pressure. The residue distilled through a short-path distillation head and gave **2** as a colorless oil (4.2 g, 88%): bp 59-61 °C (0.2 torr) lit.⁸ bp 86 °C (2 torr); α²³_D +43.8° (neat) (lit.²² α²³_D +43.8° (neat)); ¹H NMR (250 MHz, CDCl₃) δ 1.1-2.6 (m, 10 H), 3.90 (dd, *J*_{gem} 18.8, *J*_{vic} 1.2 Hz, 1 H), 4.15 (dd, *J*_{gem} 8.8, *J*_{vic} 5.0 Hz, 1 H); IR (neat) 1770 (C=O) cm⁻¹. All spectral data were in agreement with those in the literature.⁸

The major advantages of MEEC relative to immobilization to solid or gel supports is that it is operationally the much simpler and more convenient technique, it makes it possible to obtain high volume activities of enzyme in the reaction vessel,²³ it eliminates loss in enzymatic activity on immobilization, and it is applicable to crude enzyme preparations.²³ Its disadvantages are that reaction rates may be slow in circumstances in which mass transport across the membrane is rate-limiting and that enzyme deactivation due to protease contaminants is not prevented (although adding bovine serum albumin within the membrane seems to stabilize some enzymes). For most applications in organic synthesis, the advantages outweigh the disadvantages.

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Registry No. **1**, 15753-50-1; **2**, 65376-02-5; alcohol dehydrogenase, 9031-72-5; lactate dehydrogenase, 9001-60-9.

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