14C,3H,3H]acetate by this route gave methionine containing a methyl group which, if chiral, must have R configuration and R-14C,3H,3H]acetate gave (methyl-S)-[methyl-14C,3H,3H]methionine. The overall yields in these syntheses were about 10% and the T/14C ratios of starting material (e.g., 7.01 and 9.91) and product (e.g., 7.04 and 9.43) were essentially unchanged.

Previous work from this laboratory12 has shown that Streptomyces griseus (ATCC 12648) incorporates the methyl group of methionine into the C-methyl and N-methyl groups of the antibiotic, indomycin. This system was used to probe the stereic course of a C-methylation reaction, the transfer of the methyl group of adenosylmethionine to the methylene carbon of indole 3-pyruvate.13 Employing procedures described previously,12 we incubated (methyl-R)- and (methyl-S)-[methyl-14C,3H,3H]methionine (T/14C 7.04 and 7.08, respectively) with shake cultures of S. griseus and isolated the resulting indomycin (T/14C 7.12 and 6.94). Following removal of the N-methyl group by alkaline hydrolysis, the indomycin was further degraded by Kuhn–Roth oxidation to give acetic acid from the C-methyl group12 (T/14C 6.47 and 6.99, respectively). The chirality of the two specimens of acetate was determined as indicated above (39 and 63% tritium retention in the fumarase reaction, respectively) and the results were compared to those obtained in the chirality analysis of the original acetate samples used for the synthesis of the methionines (31 and 71% tritium retention in the fumarase reaction, respectively).

These results show that the above synthesis of methionine did produce material containing a chiral methyl group. The acetate obtained from the degradation of indomycin had the same configuration as the acetate used for the synthesis of the respective methionine. Since the synthesis of methionine from acetate involves one inversion of configuration, it follows that the C-methylation of indole 3-pyruvate proceeds with net inversion of configuration at the methyl group.14 This result complements our earlier finding15 of retention of configuration at the methylene carbon of indole 3-pyruvate in this reaction and allows us to describe the stereochemistry of the process as shown in Scheme II. The somewhat (40%) lower stereocchemical purity of the methyl group in the product compared to the starting acetate acid would indicate that some racemization takes place during one of the chemical steps, e.g., the alkylation of homocysteine or the Kuhn–Roth oxidation or, less likely, that the enzymatic methylation reaction is not entirely stereospecific.

Methyl transferase reactions are believed to proceed by nucleophilic attack on the methyl group of S-adenosylmethionine16 and Hegazi et al.17 have concluded on the basis of secondary isotope effect measurements that catechol-O-methyl transferase involves an S2-like transition state. Inversion of configuration at the methyl group, as observed in this study, indicates that an odd number of nucleophilic displacements takes place and thus suggests that the methyl group is transferred directly from the donor to the acceptor without generation of a methylated-enzyme intermediate.

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References and Notes


(10) Arigoni and co-workers have independently developed a similar method for the synthesis of chirally labeled methionine and have determined that this displacement does indeed occur with inversion of configuration.1

14) Arigoni and co-workers have fed chiral methionine to P. shermani and found that the incorporation of chiral methyl groups into the corrin system of vitamin B12 also occurred with inversion of configuration.11

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Application of a Novel Thermistor Mercury Electrode to the Study of Changes of Activity of an Adsorbed Enzyme on Electrochemical Reduction and Oxidation

Sir:

The strong adsorption of the amino acid cystine and proteins (e.g., albumin and insulin) from aqueous solutions onto a mercury electrode surface, presumably through interaction of the disulfide bond with mercury, has been reported.1–3 The question of whether enzymes adsorbed on mercury maintain their activity, how this activity is changed upon electroreduction, and the application of electrodes based on such adsorption for analytical purposes is the subject of this communication. It is known that enzymes covalently bound to surfaces or immobilized in gels can be employed as catalysts,4,5 but that metal ions, such as Hg2+, often deactivate enzymes. The activity of the adsorbed enzyme was determined in this study by measuring temperature changes resulting from the action of the enzyme urease on a substrate (urea) solution; similar techniques have previously been employed in studies involving dissolved enzymes.6

The thermistor mercury electrode (tme) was constructed by sealing a Veeco Engineering Co. 32A223 thermistor in a U-shaped glass tube with epoxy cement. A small drop of mercury (0.1 ml) covers the thermistor and serves as both the adsorption surface for the enzyme and to conduct heat from the electrode surface to the thermistor. Electrical contact to the mercury is made with a fine Pt wire. Electrodes containing thermists in close proximity to solid metals have previously been described for measurement of heats of electrode reactions.7 Temperature changes, determined using a dc Wheatstone bridge arrangement, followed previous practice.8 The thermostat bath at 25 °C was maintained constant to within 0.001 °C.

When urease is adsorbed on a tme by immersion for several minutes in a solution of 4 × 10–5 M urease, then washed and immersed in a pH 7.3 phosphate or Tris buffer alone and subjected to cyclic voltammetry, the characteristic reduction and oxidation peaks of an adsorbed material (similar to those found with cystine, insulin, and albumin1–3) are observed (Figure 1a). The peak currents are directly proportional to scan rate, c; Epc = –0.58 V vs. sce and Epwa = –0.52 V vs. sce. The
temperature changes occurring at the tme during the reduction and oxidation processes are measurable but very small. When the tme, prepared by equilibration with enzyme solution and washing is immersed in a pH 7.3 buffer solution containing urea, in a thermostat bath, the temperature, after a small drop upon transfer, increases and finally levels off at a steady state value governed by the rate of the hydrolysis reaction, the mass transfer of urea to the electrode, and the thermal conduction of heat by the solution and electrode material (Figure 2). The rate of temperature rise and the final steady state value increases with increasing urea concentration. For 0.5 M urea, however, much smaller temperature changes (ca. 0.87 °C) were found. At this concentration substrate inhibition 9 is not undergone electrochemical experiments. Trials with solutions containing higher concentrations of urea showed larger temperature changes. Upon removal from the urea solution, the tme showed cyclic voltammetric behavior virtually identical with that observed before the hydrolysis experiment (Figure 1c). This behavior indicates that enzyme activity is at least partially restored upon reoxidation. Reduction at -0.58 V following the oxidation scan again caused the temperature to return to the bath value. Similarly when an electrode which had been subjected to the reduction-oxidation cycle is immersed in water held in a thermostat (to allow it to come to the temperature of the bath) and then transferred into a 5 mM urea solution (pH 7.3), the temperature rises finally attaining a steady state value 1.9 × 10⁻³ °C above the bath temperature, just as observed with a tme with adsorbed urease which had not undergone electrochemical experiments. Trials with solutions containing higher concentrations of urea showed larger temperature changes. Upon removal from the urea solution, the tme showed cyclic voltammetric behavior virtually identical with that observed before the hydrolysis experiment (Figure 1a).

The results demonstrate that enzymes adsorbed at a mercury surface remain active, so that the active site of the enzyme is not significantly modified by the process. There is much current interest in chemically modified electrode surfaces,10 13 and the results presented here suggest that the strong adsorption of enzymes is a particularly simple method of modifying a mercury electrode. These electrodes can be used for
Paramagnetic Organometallic and Dialkylamide Complexes of Trivalent Titanium, Vanadium, and Chromium Stabilized by Diketonate Ligands

Sir:

Organometallic complexes of the early transition metals\(^1\) and, in particular, paramagnetic complexes are generally very reactive and unstable. Large, bulky ligands such as \(-\text{C(CH}_3\text{)}_3\)\(^-\), \(-\text{CH}_2\text{Si(CH}_3\text{)}_3\), and \(-\text{N}[\text{Si(CH}_3\text{)}_3]\text{)}_2\) have, however, yielded some very interesting and stable complexes. We are currently interested in preparing organometallic complexes of the early metals\(^2,3\) and sought new starting materials for these reactions. The most commonly used reagents are the insoluble polymeric anhydrous metal halides and their tetrahydrofuran derivatives, i.e., TiCl\(_3\)(THF)\(_3\), but these materials often lead to undesirable products.\(^4\) We have now discovered a new series of compounds of trivalent titanium, vanadium, and chromium \(1\), that are useful reagents for the syntheses of a variety of new and unusual paramagnetic, organometallic compounds.

The titanium and chromium derivatives are prepared directly from MCl\(_3\)(THF)\(_3\) and 1 equiv of the appropriate 1,3-diketone in tetrahydrofuran. For example, a suspension of anhydrous TiCl\(_3\) (35.0 g) in 500 ml of dry THF was refluxed under nitrogen for 2 h, and dipivaloylmethane (DPM-H) \((41.8\) g) was added dropwise. The solution was stirred magnetically for 1 h, cooled, and filtered and the solvent was removed to give red-purple crystals that were washed from the flask with pentane and dried. The yield of (DPM)TiCl\(_3\)(THF)\(_3\) was 97.0\% (96\%). The analogous vanadium complexes were prepared in only low yield by this procedure; however, good yields may be obtained by the addition of a THF solution of the sodium or potassium salt of the diketone (prepared in situ) to a refluxing solution of VCl\(_3\)(THF)\(_3\) in tetrahydrofuran. The diketonate complexes (diket)VCl\(_3\)(THF)\(_3\) were isolated as brown or yellow-green air-sensitive crystalline solids.

The magnetic moments of 1 were measured in solution by the Evans method\(^5\) and were in the range 1.7–1.9, 2.6–2.7, and 3.7–3.9 \(\mu\)B for the titanium, vanadium, and chromium complexes, respectively. These values are in the range expected for octahedral \(d^1\), \(d^2\), and \(d^3\) electronic configurations\(^6\) of trivalent Ti, V, and Cr.

The addition of 2 equiv of Li\(_2\)-C\(_6\)H\(_4\)CH\(_2\)N(CH\(_3\))\(_2\) to a solution of (DPM)TiCl\(_3\)(THF)\(_3\) in ether under an inert atmosphere at room temperature gave a dark green solution containing a suspension of lithium chloride. From this solution 2 was isolated in \(\sim70\%\) yield as a dark green, very air-sensitive solid. It decomposes over a period of several days when stored under nitrogen at room temperature but may be stored for months at \(-40\) °C without any apparent decomposition. Its electronic spectrum is typical of octahedral \(d^1\) complexes\(^7\) with an absorption band at 385 nm and a shoulder at 656 nm. The magnetic moment of 1.7 \(\mu\)B is also consistent with trivalent titanium. Hydrolysis of a benzene solution of 2 with D\(_2\)O gave C\(_6\)H\(_4\)DCH\(_2\)N(CH\(_3\))\(_2\), confirming the metalation of the benzylidimethylamine ligand. The vanadium analogue of 2 was synthesized in an analogous fashion and isolated as a dark maroon, air-sensitive crystalline solid. Unlike the titanium complex it may be stored under nitrogen at room temperature without decomposition. The magnetic moment \((2.7 \mu\text{B})\) was close to the spin only value of 2.83 \(\mu\text{B}\).

\[\text{R} \quad \text{H} \quad \text{O} \quad \text{MCl}_3(\text{THF})\text{)}_2 \]

\[\text{R} \quad \text{M} = \text{Ti, V, Cr}\]

\[\text{R} \quad \text{R} = -\text{CH}_2-\text{CCH}_3-c(\text{CH}_3)_2\]

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References and Notes

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