The solution was adjusted to pH 7.6 (no buffer). Polyacrylamide gel particles containing immobilized glycerol kinase (42 U, 30 mL of gel) and acetate kinase (E.C. 2.7.2.1, 42 U, 7 mL of gel) were added to the mixture.[8] An aqueous solution (60 mL, 1 M, pH 7.6) containing 120 mmol of diammonium acetyl phosphate[3] was added continuously to the stirred reaction mixture over 48 h at a rate of 2.5 mL/h (2.5 mmol/h).[9] The mixture was maintained between pH 7.4 and 7.8 by addition of 1.5 M NH_4OH, using an automatic pH controller. Reaction was carried out at ambient temperature, and the reaction mixtures and all reagent solutions were deoxygenated before use and maintained under argon. After addition of 120 mmol of AcP over 48 h, enzymatic assay[10] indicated that 100 mmol of GP had been formed. Stirring was stopped and the gel suspension allowed to settle for 6 h at ambient temperature. The supernatant was decanted under positive argon pressure using a stainless-steel cannula. The reactor was then reloaded with glycerol, ATP, DTT, and $MgCl_2$ and the addition of AcP continued for another 48 h. Three consecutive reactions (134 h of operation) generated a total of 318 mmol of GP. The combined supernatant from these reactions (3810 mL) was adjusted to pH 3.0 with concentrated HCl and concentrated under vacuum (10 Torr, 60 °C) to a volume of 40 mL. This concentrate was adjusted to a pH between 0.0 and 0.5 with concentrated HCl and 120 mL of methanol was added. The mixture was allowed to stand for 20 min at 4 °C, the precipitate (mainly inorganic phosphate) separated by filtration, and the filtrate treated with 2 equiv of cyclohexylamine (63 g, 636 mmol).[11] Any precipitate which formed at this point was separated by filtration and discarded. The mixture was poured slowly into 1000 mL of acetone with vigorous stirring. The resulting white, fluffy precipitate was filtered and washed with acetone (2 × 500 mL) and anhydrous ether (500 mL). The precipitate (115 g) was dried over Drierite for 12 h under vacuum: it contained 95% di(cyclohexylammonium) GP (238 mmol, 79% based on AcP, 76% based on glycerol). The activities of GK and AcK in the recovered gel after these three consecutive runs were 98 and 51%, respectively, of the activities of the original immobilized preparations.

This same enzymatic system has been used to prepare *sn*-glycerol-2-d_1 3-phosphate in 0.5-mol scale (213 g of the dicyclohexylammonium salt) and *sn*-glycerol-3-d_1 3-phosphate in 30-mmol scale.[12]

This synthesis illustrates the practicality of synthesizing chiral intermediates by enzymatic reactions which require ATP. The requirement for substantial amounts of isotopically and chemically substituted phospholipids in membrane biochemistry and the prospect that large quantities of enantiomerically pure phospholipids may be needed if liposomes prove useful as drug delivery systems justify the development of this synthesis of *sn*-glycerol 3-phosphate. A facile preparation of GP should also prove useful in syntheses of other substances (triglycerides, trichoic acids, cardiolipins)[5,13] derived from it biosynthetically.

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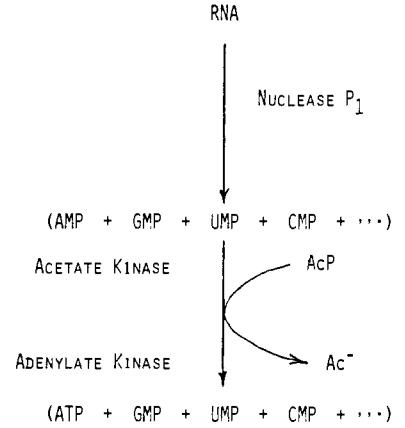
Conversion of the Adenosine Moieties of RNA into ATP for Use in Cofactor Recycling

Sir:

We report here a practical procedure for transforming the adenosine moieties of RNA into ATP (Scheme **I**). Yeast RNA and the requisite enzymes are commercially available and inexpensive, and diammonium acetyl phosphate (AcP) is easily prepared.[1] The mixture of nucleotides generated by this procedure (containing ATP, GMP, UMP, CMP, and other minor constituents) may be used without purification in synthetic schemes involving enzymatic catalysis with ATP recycling.[2,3] This method is the most practical one presently available for generating the ATP required in such schemes. The following details illustrate the manipulations involved in preparing the ATP-containing mixture, and in its use in ATP-requiring enzymatic synthesis.

In a representative procedure, RNA (100 g, 85% pure, from Torula yeast, Sigma Chemical Co.) was dissolved in 400 mL of water, and the solution adjusted to pH 5.6 with NaOH and to 0.1 mM in Zn(II).[4] Nuclease P_1[5] (E.C. 3.1.4.-, 0.20 mg, 93 U[6]) was added and the solution was allowed to stir at 65 °C.

Scheme I. Conversion of RNA into a Mixture of Nucleotides Containing ATP



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The progress of the digestion was followed by LC[7] and by enzymatic assay for AMP[8] and appeared to be complete in 65 h. The resulting solution contained (millimoles) AMP (80), GMP (60), UMP (81), and CMP (31). Active enzyme could be recovered from the digest by ultrafiltration and reused.

An aliquot of this solution (280 mL, 50 mmol of AMP) was cooled to room temperature and diluted to 2.25 L. The pH of the solution was adjusted to pH 7.6 with 2 M Na_2CO_3, and a pH-stat was employed to maintain this pH through the subsequent reaction by addition of 2 M Na_2CO_3. Acetate kinase (E.C. 2.7.2.1, 100 U) and adenylate kinase (E.C. 2.7.4.3, 300 U) immobilized in ~200-μm particles of cross-linked poly(acrylamide-*co*-*N*-acryloxysuccinimide) gel[9] were added, together with magnesium acetate (75 mmol), ATP (0.1 mmol), and dithiothreitol (0.3 g). Diammonium acetyl phosphate (1.0 M solution) was added at 2.2 mmol h^{-1} to the stirred solution. After 44 h of addition, the reaction mixture contained (millimoles) ATP (41), ADP (5), and AMP (1).[8] These quantities correspond to a 90% selectivity for conversion of acetyl phosphate into ATP and ADP. The remainder of the acetyl phosphate was lost by hydrolysis; no di- or triphosphates of other nucleotides were detected.

This mixture was used directly in enzyme-catalyzed reactions requiring ATP recycling. For example, an aliquot (250 mL) was used to provide 7 mmol of ATP for a reaction mixture originally containing, inter alia,[10] glucose (1.0 mol), hexokinase (E.C. 2.7.11, 120 U), and acetate kinase (100 U) in 1.6 L of solution. Addition of AcP over 136 h, followed by workup as described previously,[2] yielded 110 g of solid, of which 90% (by weight) was accounted for by enzymatic assay as Ba G-6-P·$7H_2O$. The yield of G-6-P based on AcP was 51%. Similarly, 170 g (420 mmol, 97% pure by weight, 70% based on acetyl phosphate) of dicyclohexylammonium *sn*-glycerol 3-phosphate was isolated following procedures described elsewhere[3] after 72 h of operation of a reactor originally containing glycerol (500 mmol), glycerol kinase (800 U, E.C. 2.7.1.30), acetate kinase (420 U), and a 90-mL aliquot of the nucleotide solution (1.7 mmol of ATP). Both of these reactions followed a course which was qualitatively indistinguishable from that observed with similar reactions carried out with pure ATP: this observation establishes that the crude, ATP-containing, nucleotide solution serves as a satisfactory substitute for pure ATP.

Much of the expense of commercial ATP is determined by its purification. High purity is not necessary for cofactors to be used in recycling schemes, but any impurities must not interfere with the reactions nor degrade the enzymes, cofactors, reactants, or products. The mixture of nucleotides generated by this procedure contains no more than 27 mol % ATP, but, since *no* purification steps are required prior to its use in procedures involving cofactor recycling, it provides a particularly convenient source of ATP for organic synthetic use. We note, however, that pure ATP can be isolated from this mixture by conventional techniques, if required. This preparation of ATP is superior to one reported earlier based on phosphorylation of adenosine:[10] the enzymes required are all commercially available, and the cost of the starting adenosine moieties is lower.

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Photoreaction of Hexacarbonylmolybdenum(0) and Tricarbonyl(η^5-cyclopentadienyl)hydridomolybdenum(II) in the Presence of Cyclopentadiene. Substitution, Oxidative Addition, and Hydrometalation

Sir:

We report here new results pertaining to photoreaction of low-valent metal carbonyls in the presence of olefins. The systems reported on here are $Mo(CO)_6$ and $(\eta^5\text{-}C_5H_5)$-$Mo(CO)_3H$ irradiated in the presence of cyclopentadiene, C_5H_6. These particular systems are important inasmuch as $Mo(CO)_6$ is a known catalyst precursor under photochemical conditions for the isomerization of olefins[1] and 1,4-hydrogenation of 1,3-dienes,[2] and $(\eta^5\text{-}C_5H_5)Mo(CO)_3H$ is reported to be a stoichiometric reducing agent for converting 1,3-dienes into alkenes.[3] Further, C_5H_6 is a source of $\eta^5\text{-}C_5H_5$ in metal complexes, when reacted with an appropriate precursor. Generally, using light to effect an individual step in a catalytic or stoichiometric process may provide for greater specificity or rate or change the course of events altogether. In the systems under consideration here, Mo–CO dissociation is likely a thermally rate-limiting process at room temperature which can be substantively altered by optical excitation.[4,5]

Our first interest in the $Mo(CO)_6/C_5H_6$ system actually began some years ago when we attempted the photoassisted 1,4-hydrogenation of C_5H_6 to cyclopentene under the conditions[2] giving efficient hydrogenation of those 1,3-dienes which can easily achieve, or are held in, an *s*-cis conformation. Little catalytic chemistry occurred using $Cr(CO)_6$ as the catalyst precursor in the attempted hydrogenation of C_5H_6, though large spectral changes obtained upon irradiation. We now present the results of a study of the irradiation of $Mo(CO)_6$ or $Cr(CO)_6$ in the presence of C_5H_6. Irradiation (355 ± 20 nm, 2×10^{-6} einstein/min) at 25 °C of 5×10^{-3} M $Mo(CO)_6$ in a degassed isooctane solution of freshly distilled 0.1 M C_5H_6 rapidly yields chemical reaction which can be monitored by IR spectroscopy in the CO-stretching region. In accord with very early findings associated with irradiation of $M(CO)_6$ (M = Cr, Mo, W) in the presence of olefins,[6] the IR spectral changes initially correspond to formation of $Mo(CO)_5(\eta^2\text{-}C_5H_6)$ with IR bands as given in Table I (eq 1). This primary

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