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 diaminomethylene acetyl phosphate (AcP) is easily prepared. 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. 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(ll). Nuclease P1 (E.C. 3.1.4.-, 0.20 mg, 93 U) was added and the solution was allowed to stir at 65 °C.

The progress of the digestion was followed by LC7 and by enzymatic assay for AMP8 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 Na2CO3, and a pH-stat was employed to maintain this pH through the subsequent reaction by addition of 2 M Na2CO3. 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-acryloxyseuccinimide) gel9 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 reaction, the mixture contained (millimoles) ATP (41), ADP (5), and AMP (1).10 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, 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 1.6 h, followed by workup as described previously, yielded 110 g of solid, of which 90% (by weight) was accounted for by enzymatic assay as Ba G-6-P+2 7H2O. The yield of G-6-P based on AcP was 51.0%. Similarly, 170 g (420 mmol, 97% pure by weight, 70% based on acetyl phosphate) of diacylhexammonium sn-glycerol 3-phosphate was isolated following procedures described elsewhere 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-μL 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; the enzymes required are all commercially available, and the cost of the starting adenosine moieties is lower.

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References and Notes

8. LC analyses were carried out using a C-18 μ-Bondapak reverse-phase column with 5 μm tetrabutylammonium phosphate, pH 7.2 (Waters Associates).
11. Metal ion and antioxidant concentrations were those described in more detailed procedures.2,3 Baughn, R. L.; Adalsteinsson, O.; Whitesides, G. M. J. Am. Chem. Soc. 1976, 100, 304-306.
12. Conacyt (Mexico) postdoctoral fellow.