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Note

A Rapid Enzymatic Preparation of [^{32}P]AMP from [α-^{32}P]ATP

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Adenosine 5′-mono[^{32}P]phosphate ([^{32}P]AMP) is used as a substrate for sensitive assays of 5′-nucleotidase (1, 2). It is commercially available from the Radiochemical Centre, Amersham, but the commercial product has a relatively low specific activity (0.5~3 Ci/mmol) and was provided only in a large package ($\geqq$1 mCi). When a relatively small amount of highly labeled [^{32}P]AMP is a demand, it seems convenient to prepare it from adenosine 5′-[α-^{32}P]triphosphate ([α-^{32}P]ATP) which is available with higher specific activity and in a relatively small quantity (0.5~250 Ci/mmol, $\geqq$250 μCi from the Radiochemical Centre or 10~30 Ci/mmol, $\geqq$100 μCi from New England Nuclear, Boston).

For the preparation of AMP from ATP, a chemical method (3) does not appear applicable to small-scale preparations. We recently developed a rapid and simple method using myokinase. Myokinase (adenylate kinase, ATP: AMP phosphotransferase, EC 2.7.4.3) catalyzes the following reaction;

$$\text{ATP} + \text{AMP} \rightleftharpoons 2\ \text{ADP}$$

The equilibrium constant at pH 7.4 and at 25° is reported to be 2.26 M (4). When the reaction starts with [α-^{32}P]ATP and 10 or 100 times more nonradioactive AMP, the equilibrium is expected to be attained when approximately 83% or 98%, respectively, of the radioactivity is distributed to AMP. The procedure is very simple and applicable to any scale, albeit lowering of specific activity by about one or two orders.

EXPERIMENTAL

[α-^{32}P]ATP (sodium salt, 250 μCi, 10 Ci/mmol) was obtained from the Radiochemical Centre. Myokinase (from rabbit muscle) was purchased from Boehringer/Mannheim-Yamanouchi, Tokyo. Polyethyleneimine cellulose (Polygram cel 300) was from Mesherey-Nagel and Co., Düren, and Dowex AG 1×2 from Bio-Rad Laboratories. Determination of radioactivity on thin-layer chromatograms was performed with Packard Radiochromatogram Scanner Model 7201.

[α-^{32}P]ATP, freed of vehicle solvents by evaporation, was dissolved in 1.0 ml

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of distilled water. A 0.7-ml portion (3×10^8 cpm, 26 nmol) was transferred to a small test tube, and again evaporated to dryness. The dried material was dissolved, in the same tube, in the mixture (0.1 ml) containing 10 μmol of Tris-HCl (pH 7.5), 2.6 μmol of AMP, 1 μmol of $MgCl_2$ and 7 units of myokinase. Incubation was carried out for 3 hours at 37°. Figure 1 shows radiochromatograms of aliquots

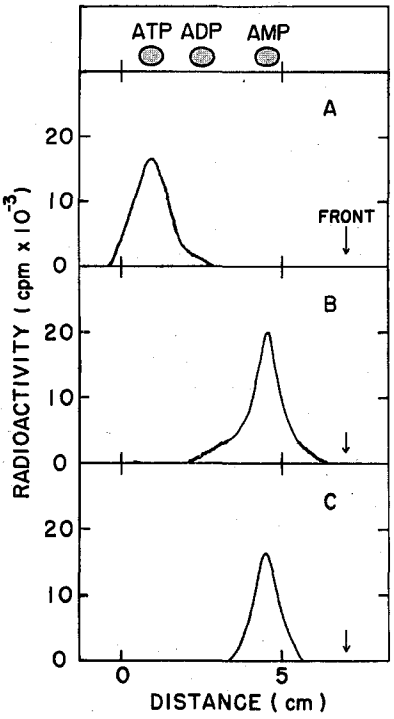

Fig. 1. Analysis of reaction products at various stages. Either the reaction mixture at zero time (A) or after 3 hour incubation (B) or AMP purified on Dowex AG 1×2 (C) was applied to a PEI cellulose sheet. Chromatography was performed with 1.0 M LiCl as a solvent system at room temperature (5). Authentic AMP, ADP, and ATP were cochromatographed as markers, and located on the chromatogram under UV light.

taken at zero-time (A) and after three hours of incubation (B). As judged by the distribution of radioactivity among three nucleotides, the reaction appeared to reach equilibrium within 3 hours. The reaction was terminated by adding 0.2 ml of 10% perchloric acid and the mixture neutralized with KOH. The supernatant fraction after brief centrifugation was applied to a Dowex AG 1×2 column (0.7×6 cm). The column was washed with 15 ml of water, and eluted with 30 ml of 0.3 N formic acid. Under this condition, neither ADP nor ATP elutes out from the column. The fractions containing [^{32}P]AMP were pooled and lyophilized. The recovery of radioactivity from [α-^{32}P]ATP to AMP was 79%. Purity of [^{32}P]AMP thus prepared was verified by PEI cellulose thin-layer chromatography (Fig. 1C); no radioactive impurity was detectable.

A similar method is applicable to a preparation of [β-^{32}P]ADP from [γ-^{32}P]ATP (6, 7).

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