A Rapid Enzymatic Preparation of $^{32}$PAMP from $[^\alpha-32P]$ATP

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Adenosine 5'-mono$^{32}$Pphosphate ($^{32}$PAMP) 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/mmoll) and was provided only in a large package ($\geq 1$ mCi). When a relatively small amount of highly labeled $[^32P]$AMP is a demand, it seems convenient to prepare it from adenosine 5'-$[^\alpha-32P]$triphosphate ($[^\alpha-32P]$ATP) which is available with higher specific activity and in a relatively small quantity (0.5--250 Ci/mmoll, $\geq$250 $\mu$Ci from the Radiochemical Centre or 10--30 Ci/mmoll, $\geq$100 $\mu$Ci 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;

$$ATP + AMP \rightleftharpoons 2 ADP$$

The equilibrium constant at pH 7.4 and at 25° is reported to be 2.26 M (4). When the reaction starts with $[^\alpha-32P]$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

$[^\alpha-32P]$ATP (sodium salt, 250 $\mu$Ci, 10 Ci/mmoll) 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 Radiocromaticogram Scanner Model 7201.

$[^\alpha-32P]$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 \((3 \times 10^8 \text{ cpm}, 26 \text{ nmol})\) was transferred to a small test tube, and again evaporated to dryness. The dried material was dissolved, in the same tube, in the mixture \((0.1 \text{ ml})\) containing 10 \(\mu\text{mol}\) of Tris-HCl \((\text{pH} 7.5)\), 2.6 \(\mu\text{mol}\) of AMP, 1 \(\mu\text{mol}\) of MgCl2 and 7 units of myokinase. Incubation was carried out for 3 hours at 37°. Figure 1 shows radiochromatograms of aliquots 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\( \times \)2 column \((0.7 \times 6 \text{ cm})\). 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 \([32\text{P}]\)AMP were pooled and lyophilized. The recovery of radioactivity from \([\alpha-32\text{P}]\)ATP to AMP was 79\%. Purity of \([32\text{P}]\)AMP thus prepared was verified by PEI cellulose thin-layer chromatography (Fig. 1C); no radioactive impurity was detectable.

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\( \times \)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.
Preparation of [32P]AMP

A similar method is applicable to a preparation of $[\beta^{32}\text{P}]\text{ADP}$ from $[\gamma^{32}\text{P}]\text{ATP}$ (6, 7).

REFERENCES

(7) K. Ueda and J. Oka, manuscript in preparation.