PREPARATION OF ^{14}C-LABELLED AMP, ADP AND ATP FROM ADENINE-8-^{14}C BY USING *BREVIBACTERIUM AMMONIAGENES*

V.N. PANDE
Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Bombay 400 085, India.

SUMMARY

High radiochemical yields of ^{14}C-labelled adenine nucleotides (AMP, 4.6%; ADP, 15.5% and ATP, 59.5%) could be obtained by growing the cells of *Brevibacterium ammoniagenes* in the presence of ^{14}C-adenine. The specific radioactivity of the adenine nucleotides almost reached that of ^{14}C-adenine indicating negligible dilution of the label. The procedure is convenient and especially suited for commercial preparation of the radiolabelled nucleotides directly from labelled adenine. Preliminary results indicate that the organism could also be used for the preparation of radiolabelled guanine nucleotides.

Key Words: Adenine, Adenine nucleotides, Guanine nucleotides, *Brevibacterium ammoniagenes*

Adenine nucleotides labelled with ^{14}C-radioisotope are useful compounds for biochemical research. The radioactive nucleotides are expensive since the commercial procedures for the preparation of these involve either a multistep chemical synthesis or an enzymatic synthesis using a number of purified enzymes and labelled precursors. The present communication describes a simple and inexpensive procedure for the preparation of adenosine-8-^{14}C mono-, di-, and triphosphate of high specific radioactivity from adenine-8-^{14}C by growing cells of *Brevibacterium ammoniagenes*. This bacterium is known to accumulate large amounts of adenine nucleotides in the medium when grown with adenine (1, 2).

A typical procedure used for the preparation of labelled adenine nucleotides using this bacterium is described as follows: *Brevibacterium ammoniagenes* (ATCC 6872) was maintained on nutrient agar slants. The bacterium was grown

0362–4803/85/040383–04$01.00

Received June 19, 1984
Revised September 24, 1984

overnight at 30° in a medium (40 ml in 500 ml Erlenmeyer flask) containing 1% glucose, 0.5% peptone, 0.5% yeast extract and 0.15% NaCl (pH 7.3). One and half ml of the overnight culture was inoculated in a 30 ml medium (contained in 500 ml Erlenmeyer flask) consisting of 3 mCi adenine-8-^{14}C (50 mCi/mmole), 10% glucose, 1% K_2HPO_4, 1% KH_2PO_4, 1% $MgSO_4$, 0.01% $CaCl_2$, 0.002% cysteine, 0.0005% thiamine, 0.001% calcium pantothenate, 0.5% yeast extract, 0.6% urea and 3 µg per 100 ml biotin (pH 8.3).

Incubation was carried out for 48 hr at 30° on a rotary shaker (250 rpm). At the end of incubation, cells were spinned off and discarded. The supernatant fluid was cooled in an ice-bath, adjusted to pH 3-4 with 5 M acetic acid and passed through an activated charcoal column (5 cm x 1.5 cm). The column was washed with 200 ml water and the adsorbed nucleotides were eluted with 50% ethanol containing 1.5% ammonia. The eluate was lyophilized, rehydrated with 200 - 300 µl water and subjected to preparative paper chromatography on a Whatman No.3 paper using 1 M ammonium acetate: 95% ethanol (30:70 V/V) as the solvent system. The nucleotide bands corresponding to those of standard markers were identified by visualization in ultraviolet light and autoradiography (Fig.1). The nucleotide bands were cut, eluted with water by descending technique, lyophilized and rehydrated with a small volume of 50% ethanol.

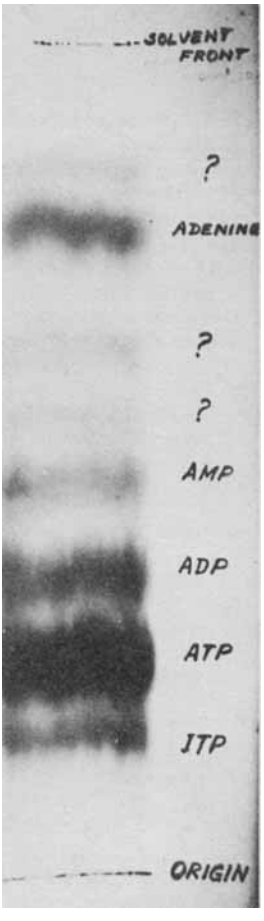

Fig.1. Autoradiogram of ^{14}C-labelled adenine nucleotides isolated from fermented broth (concentrated charcoal eluate) of Brevibacterium ammoniagenes.

The concentration of each isolated nucleotide was determined spectrophotometrically at 259 nm (3). For the determination of radioactivity, an aliquot of the solution was spotted on a Whatman No.1 paper strip, dried by hot air blower, placed in 10 ml scintillator (0.5% PPO in toluene) and counted for radioactivity in an LS-100 Beckman liquid scintillation system. The specific radioactivity was expressed as mCi per mmole concentration of each of the purified nucleotides. The total radiochemical yields as shown in Table 1 for ATP, ADP and AMP were 59.5%, 15.5% and 4.6%, respectively. The remaining 15 - 20% radioactivity was distributed in adenine, ITP and three unidentified bands on the chromatogram.

Table 1. Purification and isolation of ^{14}C labelled AMP, ADP and ATP from fermented broth of *Brevibacterium ammoniagenes*.

Purification steps	Radiochemical yield %			Specific radioactivity (mCi/mmole)		
	AMP	ADP	ATP	AMP	ADP	ATP
1. Supernatant	4.6	15.5	59.5	-	-	-
2. Charcoal absorption	4.2	15.0	59.1	-	-	-
3. Preparative paper chromatography	3.95	14.5	58.6	49.7	49.7	49.7

The 30 ml fermentation medium contained 3 mCi adenine-8-^{14}C at a final concentration of 2 mM. An aliquot of supernatant or of concentrated eluate of the charcoal steps was subjected to paper chromatography as described in the text and radioactivity in nucleotide spots determined.

The specific radioactivities of the isolated ^{14}C-adenine nucleotides were found to be approximately the same as that of the ^{14}C-labelled adenine. The Rf values obtained in thin-layer chromatography of the isolated labelled nucleotides with various solvent system were the same as those reported for the authentic ATP, ADP and AMP (4). The radiochemical purities of the nucleotides isolated by preparative paper chromatography were approximately 94 - 96% which could further be improved either by re-running the same chromatogram in the same solvent system before elution or by column chromatography using DEAE cellulose (5).

It was further observed that ^{14}C-labelled GMP, GDP and GTP could also be prepared similarly by growing this bacterium in the presence of ^{14}C-guanine in the medium (results not shown). Nara et al. have demonstrated that 5-phosphoribose pyrophosphokinase and purine nucleotide pyrophosphorylase were involved in the nucleotide synthesis from purine base (salvage pathway) by this organism (6). These authors have further shown that the actual ribotidation of adenine and guanine to their respective nucleotides takes place extracellularly through the action of these enzymes which leak out of the cells. The non-dilution of specific radioactivity of the isolated adenine nucleotides could be attributed to this phenomenon. These results suggest that the use of B.ammoniagenes cells could be a promising method for the commercial preparation of labelled purine nucleotides.

Acknowledgement: I am grateful to Dr. D.S. Pradhan and Dr. G.B. Nadkarni for their helpful suggestions and critical reading of the manuscript.

REFERENCES

1. Nara, T., Misawa, M. and Kinoshita S. Agr. Biol. Chem. 32: 561 (1968).
2. Tanaka, H., Sato, Z., Nakayama, K. and Kinoshita, S. Agr. Biol. Chem. 32: 721 (1968).
3. Ishiyama, J., Yokosuka, T. and Saito, N. Agr. Biol. Chem. 38: 507 (1974).
4. Randerath, K. and Randerath, E. Methods in Enzymol. Vol. 12: 323 (1967).
5. Stahelin, M. Biochim. Biophys. Acta. 49: 11 (1961).
6. Nara, T., Misawa, M., Komuro, T. and Kinoshita, S. Agr. Biol. Chem. 33: 358 (1969).