ENZYMATIC SYNTHESIS OF [RIBOSE-U-^{14}C]8-AZAGUANOSINE

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SUMMARY

8-Azaguanosine ^{14}C-labeled in the ribose moiety was enzymatically synthesized in two steps: 1) phosphorolytic cleavage of [U-^{14}C]inosine to yield [U-^{14}C]ribose-1-phosphate; 2) synthesis of 8-azaguanosine from [U-^{14}C]ribose-1-phosphate and 8-azaguanine catalyzed by purine nucleoside phosphorylase. The radiochemical yield was 70%, and no contaminants were present in the isolated radioactive nucleoside analog, as judged by HPLC analysis.

Key words: 8-azaguanosine, carbon labeling, [U-^{14}C]ribose-1--phosphate, enzymatic synthesis, purine nucleoside phosphorylase, nucleoside analogs.

INTRODUCTION

8-Azaguanine is a base analog widely used to study the mechanisms of cellular resistance to the effect of the cytotoxic nucleoside monophosphate derivative (1-4). 8-Azaguanosine is believed to undergo a phosphorolytic cleavage in the cell, making the base 8-azaguanine available for the synthesis, phosphoribosyl

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pyrophosphate dependent, of the cytotoxic mononucleotide. However, in several cell types, the presence of nucleoside kinase activities able to convert the nucleoside analogs to the corresponding monophosphates has been reported (5-7). Such activities may play an important role in determining the different sensitivities to purine analogs displayed by different cell types.

Investigations of the phosphorylation and further metabolism of compounds such as 8-azaguanosine can be greatly facilitated by the availability of isotopically labeled forms.

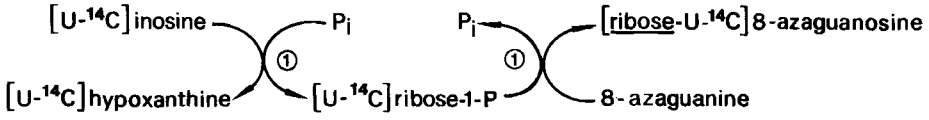
In this paper we describe a simple method of synthesis of 8-azaguanosine labeled in the ribose moiety, in a good yield. The nucleoside analog was isolated to a high degree of purity, making it available for studies on nucleoside kinases involved in the mechanisms mediating the cellular resistance to base and nucleoside analogs.

RESULTS AND DISCUSSION

A necessary prerequisite for the enzymatic synthesis of labeled 8-azaguanosine was to verify that 8-azaguanine was a substrate for purine nucleoside phosphorylase. For this reason, the purine base analog was incubated 30 min at 37°C with the enzyme and an excess ribose-1-phosphate. At 10 min intervals, 10 μl aliquots of the mixture were applied on a PEI cellulose plate and the chromatogram was developed with deionized water. Under these conditions, azaguanine was completely converted in an ultraviolet absorbing material showing an Rf of 0.55. The compound was eluted from the plate and identified as 8-azaguanosine by comparison of its spectral properties with those described for the chemically synthesized nucleoside analog: at pH 4 , λmax 255 nm, with a shoulder at 268 nm, and a ratio in absorbance 255 nm/268 nm of 1.3 (9).

The synthesis of 8-azaguanosine labeled in the ribose moitey was accomplished in two steps: 1) the formation of [U-^{14}C]ribose-1-phosphate and its purification, 2) the incorporation of [U-^{14}C]ribose into 8-azaguanosine (see scheme). The first step was performed by phosphorolytic cleavage of [U-^{14}C]inosine as described before (8). In our hands, the method gave a yield of 60%. [U-^{14}C]Ribose-1-phosphate was then converted into [ribose-U-^{14}C]8-azaguanosine by incubation with purine nucleoside phosphorylase in the presence of excess 8-azaguanine, as described in the Experimental section.

SCHEME



Enzymatic synthesis of [ribose-U-^{14}C]8-azaguanosine.
1=purine nucleoside phosphorylase.

8-Azaguanosine, specifically labeled in the ribose moiety, prepared with this procedure, had a concentration of 12 μM, with a specific radioactivity of 277 mCi/mmol. The yield was 70%. The thin layer chromatographic analysis of the synthesized radioactive 8-azaguanosine, performed after 3 months of storage at -20°C, showed that the sample was free of any radioactive degradation product (Fig. 1).

Since the Rf of 8-azaguanosine and guanosine are very similar (0.55 and 0.5, respectively), on the basis of the thin layer chromatographic analysis, it is difficult to exclude any contamination of the radioactive nucleoside analog by guanosine, derived from guanine, possibly present in the commercial 8-aza-

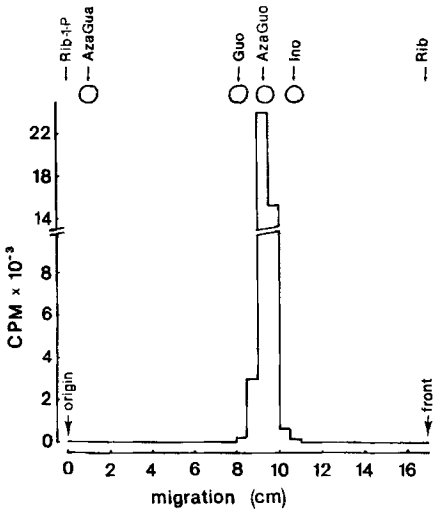


Fig. 1. Thin layer chromatographic analysis of [ribose-U-^{14}C]8-azaguanosine. Ten μl of the final product were applied on a PEI-cellulose thin layer plate. The chromatogram was developed with deionized water.

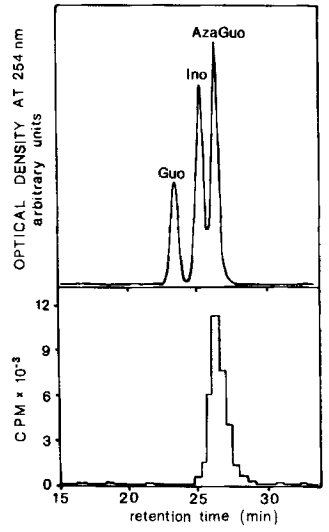


Fig. 2. HPLC analysis of [ribose-U-^{14}C]8-azaguanosine. The analysis was performed with a Beckman Model 332 HPLC system on a reverse-phase Supelco LC-18-DB (25 cm x 4.6 mm). The elution was performed using a gradient of 10 mM potassium phosphate pH 7/acetonitrile. The gradient was, in terms of percent acetonitrile, 1.5-2.0% (0-7.5 min), 2.0-2.5% (7.5-10 min), 2.5% (10-35 min). The flow rate was 1.5 ml/min. The retention time of the standard nucleosides was: Guo (23.2 min) Ino (25 min), AzaGuo (26.1 min). The analysis was performed on 5 μl of the synthesized [ribose-U-^{14}C]8-azaguanosine. For the radioisotope measurements, 0.5 ml fractions were collected directly into scintillation vials and counted after addition of 8 ml of Aquasol-2.

guanine. However, this contamination could be ruled out by the results of HPLC analysis, which allowed a better separation of 8-azaguanosine from guanosine (Fig. 2).

EXPERIMENTAL

Materials. 8-Azaguanine, ribose-1-phosphate and calf spleen purine nucleoside phosphorylase were obtained from Sigma Chemical Co.. [U-^{14}C]Inosine (554 mCi/mmol) was purchased from Amersham International plc. Polyethyleneimine (PEI) cellulose-precoated thin layer plastic sheets (0.1 mm thick) were obtained from Merck and prewashed once with 10% NaCl and three times with deionized water before use. Aquasol-2 liquid scintillation cocktail was from New England Nuclear. All other chemicals were of reagent grade.

Enzymatic synthesis of[U-^{14}C]ribose labeled 8-azaguanosine. For a typical preparation, 14 μCi of [U-^{14}C]ribose-1-phosphate (277 mCi/mmol), prepared as described before (8), were incubated at 37°C for 30 min, in a final volume of 1.4 ml, in the presence of 0.252 mM 8-azaguanine, 35 μg/ml of purine nucleoside phosphorylase and 50 mM Tris-HCl, pH 7.4. The mixture was dried under vacuum and resuspended in a minimal volume of 1 mM NaOH. The solution was applied as a streak on a PEI cellulose thin layer plate and the chromatogram was developed with deionized water. The ultraviolet absorbing material showing an Rf of 0.55, completely distinct from 8-azaguanine (Rf=0.062), was the only radioactive spot present in the chromatogram, and contained 75% of the total radioactivity. This spot, corresponding to 8-azaguanosine, was removed and eluted with three successive washings with 1 ml of water. The resulting solution contained, in a total volume of 3 ml, 12 μM in 8-azaguanosine with a specific radioactivity of 277 mCi/mmol.

CONCLUSIONS

The method presented in this paper allows an effective and rapid preparation of highly purified 8-azaguanosine labeled in the ribose moiety. The nucleoside analog prepared with this procedure is suitable for studies on substrate specificity of nucleoside kinases and on the pathways involved in the incorporation of 8-azaguanosine in the nucleotide pool. Modifications of the above approach might, moreover, allow the extension of this method to the preparation of other specifically labeled nucleosides.

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REFERENCES

1. Plagemann P.G.W. and Wohlhuter R.M. J. Cell Biochem. 27, 109-120 (1985).
2. Berman J.J., Tong C.W. and Williams G.M. J. Cell Physiol. 103, 393-398 (1980).
3. Van Diggelen O.P, Donahue T.F. and Shin S.-I. J. Cell Physiol. 98, 59-72 (1979).
4. Saunders P.P., Tan M.-T., Spindler C.D., Robins R.K. and Plunkett W. J. Biol. Chem. 261, 6416-6422 (1986).
6. Saunders P.P. and Lai M.M. Biochim. Biophys. Acta 761, 135-141 (1983).
7. Yamada Y., Goto H. and Ogasawara N. Biochim. Biophys. Acta 761, 34-40 (1983).
8. Camici M., Mura U., Del Corso A., Gini S. and Ipata P.L. Anal. Biochem. 166, 253-256 (1987).
9. Hutzenlaub W., Tolman R.L. and Robins R.K. J. Med. Chem. 15, 879-883 (1972).