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Short Communication

Rapid and quantitative separation of nicotinamide and its N^1-methylated metabolite by Dowex AG50-X4 chromatography

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ABSTRACT

The use of column chromatography with Dowex AG50-X4 resin has allowed the quantitative separation of nicotinamide from its primary metabolite, N^1-methylnicotinamide. Although the sensitivity is similar to earlier high-performance liquid chromatographic methods, this procedure allows multiple assays to be carried out simultaneously in a matter of minutes. This method should be useful to study nicotinamide methyltransferase activity in either whole cells or extracts, and is particularly well suited to screen column fractions for enzyme purification purposes.

INTRODUCTION

Although the use of nicotinamide (NA) to decrease cholesterol levels has gained widespread clinical acceptance, in isolated cases, acute liver toxicity results from chronic high dose niacin treatment [1]. NA has been shown to decrease DNA synthesis and cell growth of isolated hepatocytes [2]. Excess NA is primarily metabolized in the liver and kidney to form N^1-Methylnicotinamide (N^1-MN) by enzymatic methylation catalyzed by nicotinamide methyltransferase (NAMTase, E.C. 2.1.1.1). To facilitate the study of NAMTase, we developed a rapid, sensitive chromatographic separation of NA from N^1-MN. Previous chromatographic methods [3–6] are either very time consuming or lack sensitivity.

EXPERIMENTAL

To obtain purified ^{14}C-labeled N^1-MN, we enzymatically methylated [^{14}C]NA using rat liver NAMTase prepared and assayed by a modification of an existing procedure [6]. Changes included using 1 m*M* [^{14}C]NA (specific activity 0.5 mCi/mmol) and unlabeled S-adenosylmethionine (AdoMet), instead of [^{3}H]AdoMet and unlabeled NA in the 200 μl reaction mixture. [^{14}C]N^1-MN was separated from [^{14}C]NA by high-performance liquid chromatography (HPLC) on a Partisil SXC column as described previously [6]. Samples (1 ml) containing either 3000 cpm of [^{14}C]NA or 600 cpm of 1-MN (50 nmol; purified as above by HPLC) were made 0.1 *M* with respect to NH_3COO^- (pH 8.9) and loaded onto Dowex AG50-X4 (mesh size 200–400) columns (5 g pre-swollen) pre-equilibrated with 0.1 *M*

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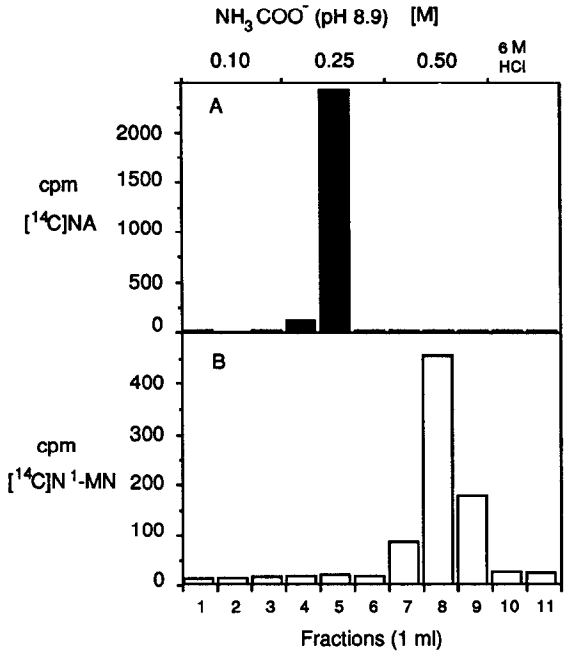

Fig. 1. Separation of NA and N^1-MN by Dowex AG50-X4 column chromatography. Purified [^{14}]N^1-MN (600 cpm or nmoles) or [^{14}C]NA (3000 cpm or 250 nmoles) were loaded as 1-ml samples in 0.1 *M* NH_3COO^- (pH 8.9) on Dowex AG50-X4 columns equilibrated with 0.1 *M* NH_3COO^- (pH 8.9). The columns were eluted with 3-ml step gradients of increasing NH_3COO^- (pH 8.9) at 0.1, 00.25 and 0.5 *M* [^{14}C]NA (A) and [^{14}]N^1-MN (B) were each completely eluted by 0.1 and 0.5 *M* NH_3COO^-, respectively. An additional 2 ml of 6 *M* HCl was unable to further elute any radioactivity.

NH_3COO^- (pH 8.9). The column was sequentially washed with 3-ml aliquots of 0.10, 0.25 and 0.50 *M* NH_3COO^- (pH 8.9) with gravity flow to collect 1-ml fractions and radioactivity determined by scintillation counting. NA and 1-MN were each completely eluted by 0.25 and 0.5 *M* NH_3COO^- (pH 8.9), respectively (Fig. 1), in a time frame of only minutes. Application of an acid-extracted NAMNTase reaction to the column allowed similar separation of the ^{14}C-labeled NA and N^1-MN [7].

RESULTS AND DISCUSSION

By taking advantage of the higher affinity of the positively charged 1-MN for Dowex AG50-X4 resin at high pH we have been able to effectively separate the product (N^1-MN) and substrate (NA) of the NAMTase reaction using differential buffer strength. Since all of the counts in the [^{14}]N^1-MN sample loaded on the column were recovered using this procedure, we offer it as a very sensitive measure of nmol amounts of this nicotinamide metabolite. The major advantage of Dowex chromatography over Partisil SXC HPLC is that it takes 5 min to perform multiple assays, where each HPLC column takes over 1 h to elute each sample [6]. Because of the high sensitivity and rapid nature of this procedure, it would be particularly useful to screen cytoplamic extracts for NAMTase activity in order to purify the enzyme or to study its kinetics. In addition, since this method employs labeled NA, rather than AdoMet, preliminary steps separating labeled AdoMet from the sample can be omitted.

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