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Note

Separation behaviour of nicotinamide and thionicotinamide derivatives on Dowex 1-X8

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In studies on nicotinamide-N^1-methyltransferase (E.C. 2.1.1.1), we tried to develop a method for the measurement of enzyme activity. Usually, the N^1-methylnicotinamide formed is determined in the protein-free supernatant of incubation mixtures after condensation with a ketone (*e.g.*, acetone or ethyl methyl ketone) by fluorescence assay[1–3]. Using radioactively labelled nicotinamide, we intended to determine the turnover rates in tissues that have lower enzyme activities than liver. Nicotinamide and its 1-methylated product can be separated by paper[4] or thin-layer chromatography[5]. However, we sought a method based on column chromatography, in which the labelled product obtained in the enzymatic reaction can conveniently be separated from the remaining substrate.

In the extensive publications on the separation of nucleotides by ion-exchange chromatography (for a review, see Caldwell[6]), little attention was paid to the free bases and nucleosides. Recently, some papers dealing with the separation of purine and pyrimidine bases and nucleosides have appeared[7–9].

Some purine derivatives are very strongly bound to polystyrene-based resins by non-ionic forces[6], which in most instances is an undesirable effect. We took advantage of these non-ionic interactions between ion exchangers and pyridine bases for their separation from the positively charged 1-alkylated derivatives, which are not retained by Dowex 1-X8. Based on the observations of Kašarov and Moat[10], who reported the separation of [^{14}C]nicotinamide riboside from nicotinamide on Dowex 1-X8, we modified this procedure for the separation of 1-methylnicotinamide from nicotinamide.

The fact that nicotinamide is retained on Dowex 1-X8 stimulated us to study the behaviour of various nicotinamide derivatives of biochemical interest with respect to their affinity for polystyrene-based resins.

EXPERIMENTAL

Materials

The compounds used are listed in Table I; 1a, 1b, 2a, 3a, 5a, and 6a were commercially available (E. Merck, Darmstadt, G.F.R.; Sigma, St. Louis, Mo., U.S.A.; EGA-Chemie, Steinheim, G.F.R.). The following derivatives were prepared

TABLE I

SEPARATION OF NICOTINAMIDE AND THIONICOTINAMIDE DERIVATIVES FROM THE CORRESPONDING 1-ALKYLATED PYRIDINIUM COMPOUNDS ON DOWEX 1-X8 USING TRIS–HYDROCHLORIC ACID BUFFER

Column height, 40.5 ± 1.5 cm; flow-rate, 20 ± 3 ml/h.

No.	*Nicotinamide derivative*	*Approx. elution peak (fraction Nos.)*
1a	Nicotinamide	11–14
1b	1-Methylnicotinamide chloride	3–5
1c	1-Methylnicotinamide iodide	3–4
1d	1-(2',6'-Dichlorobenzyl)nicotinamide iodide	4–5
2a	Thionicotinamide	—
2b	1-Methyl-3-thiocarbamoylpyridinium iodide	3–5
3a	6-Aminonicotinamide (6-AN)	19–23
3b	1-Methyl-6-amino-3-carbamoylpyridinium iodide	3–5
4a	6-Amino-3-thiocarbamoylpyridine	—
4b	1-Methyl-6-amino-3-thiocarbamoylpyridinium iodide	3–5
5a	Isonicotinamide	14–17
5b	1-Methylisonicotinamide iodide	4–5
6a	Isothionicotinamide	—
6b	1-Methyl-4-thiocarbamoylpyridinium iodide	4–5

according to the literature: 1c (ref. 11), 1d (ref. 12), 2b (ref. 13) and 5b (ref. 14). Compounds 3b, 4a, 4b, and 6b were synthesized in our laboratory by procedures reported recently[15].

Dowex 1-X8 ($HCOO^-$) (200–400 mesh), A grade, was supplied by Serva, Heidelberg, G.F.R.

For chromatography, glass columns with an I.D. of *ca.* 0.7 cm and a total length of 75 cm were used.

Methods

Dowex 1-X8 (*ca.* 15.8 g) was washed thoroughly with 0.01 *M* Tris–hydrochloric acid buffer (pH 7.0) and poured into a column, and final equilibration was achieved within the column by the buffer. The column height was 40.5 ± 1.5 cm. Nicotinamide (thionicotinamide) or the analogues and the corresponding 1-methylpyridinium compound, for example 6-aminonicotinamide (6-AN) and 1-methyl-6-amino-3-carbamoylpyridinium iodide, were dissolved in 0.01 *M* Tris–hydrochloric acid buffer (pH 7.0). The standard concentration was 1 mg/ml of each compound (sometimes, however, 0.5 or 2.0 mg/ml). The stock solutions were prepared immediately before loading. One millilitre of the mixture was applied to the column and elution was performed with 0.01 *M* Tris–hydrochloric acid buffer (pH 7.0) (see Figs. 1 and 2). For elution of the thiocarbamoylpyridines (2a, 4a and 6a), the buffer was removed from the top of the resin by suction and replaced with 1 *M* formic acid (see Fig. 2a).

Fractions of 2.5 ml were collected using an LKB fraction collector. The UV absorption was recorded continuously at 260 nm using a Uvicord absorptiometer and recorder. The flow-rate achieved was 20 ± 3 ml/h. Typical elution diagrams are shown in Figs. 1 and 2.

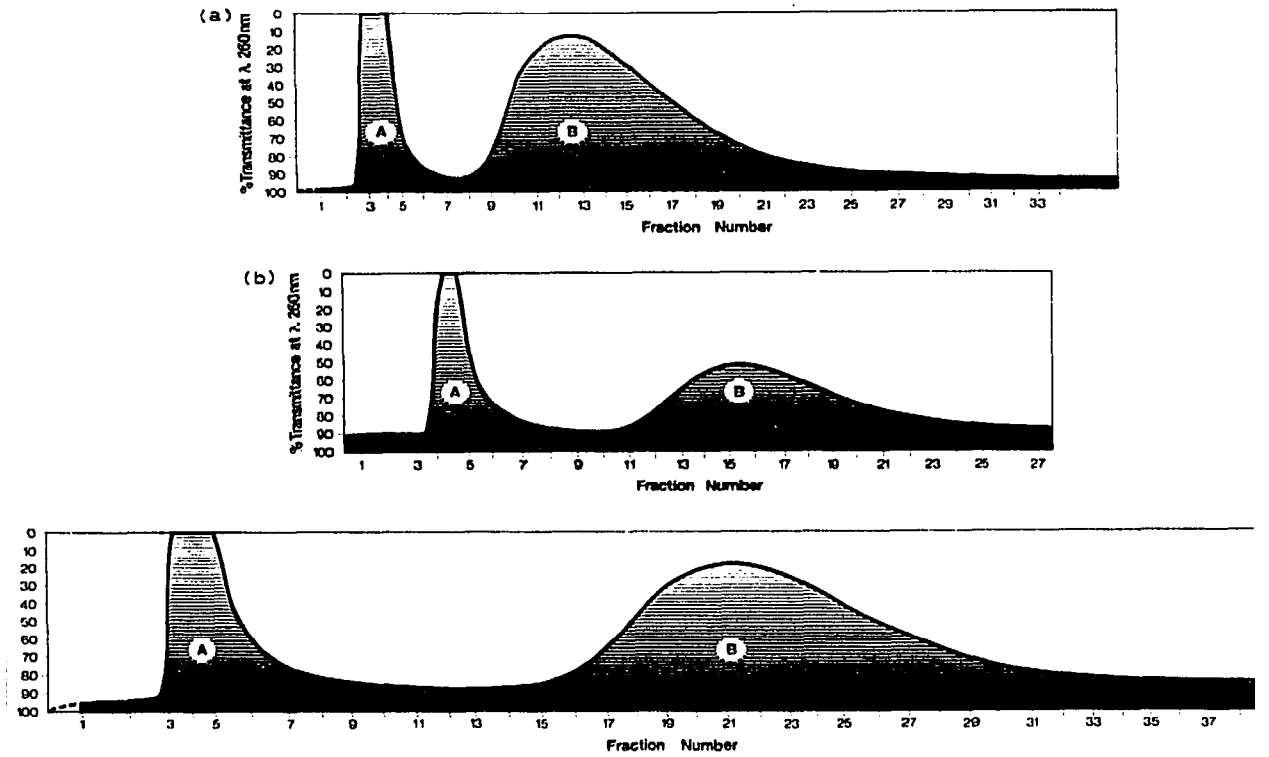


Fig. 1. Elution profiles of 1-methylcarbamoylpyridinium iodides and the corresponding carbamoylpyridines. (a) A, 1-methylnicotinamide iodide; B, nicotinamide (a load of 2 mg of each compound). (b) A, 1-methylisonicotinamide iodide; B, isonicotinamide (a load of 1 mg of each compound). (c) A, 1-methyl-6-amino-3-carbamoylpyridinium iodide; B, 6-aminonicotinamide (a load of 1 mg of 1-methylated compound and 0.5 mg of 6-AN).

The peaks were identified by recording the UV spectra (Beckman DK 2A spectrophotometer) and in some instances by TLC using pre-coated cellulose plates (solvent system: 1-butanol–methanol–glacial acetic acid–water (90:35:15:60, v/v).

RESULTS AND DISCUSSION

All 1-methylcarbamoylpyridinium and 1-methylthiocarbamoylpyridinium derivatives were eluted from the columns with Tris–hydrochloric acid buffer (pH 7.0) in the same fraction, the peaks appearing in fractions 4 and 5 (see Table I; elution profiles are illustrated in Figs. 1 and 2). The nature of the anion in the positively charged pyridinium compounds does not seem to influence the elution; 1-methylnicotinamide chloride (1b) and 1-methylnicotinamide iodide (1c) showed almost identical elution behaviour (see Table I).

In order to investigate the influence of the N^1-substituent, we eluted a load of nicotinamide and 1-(2′,6′-dichlorobenzyl)nicotinamide iodide (1d). Compound 1d showed an affinity for the column material similar to that of 1-methylnicotinamide.

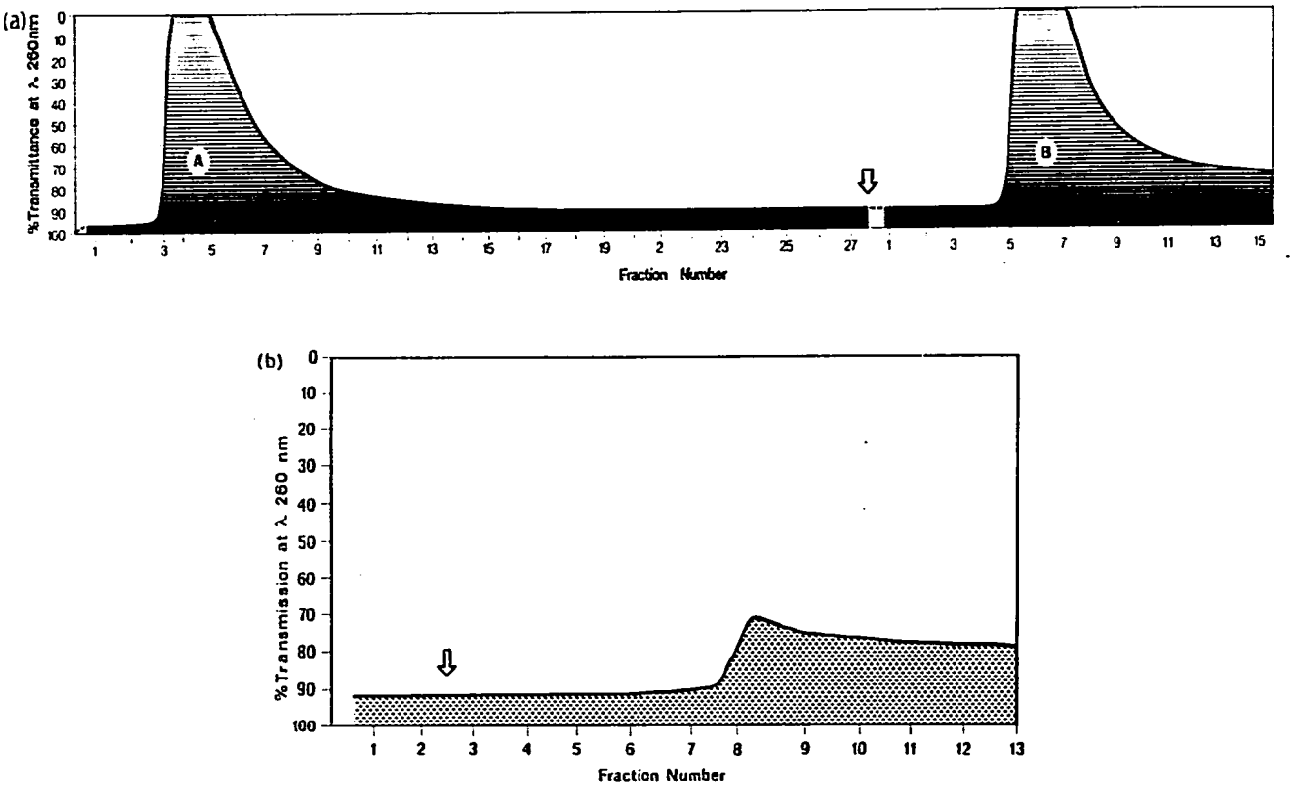
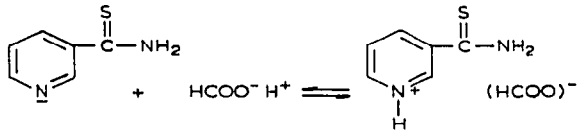


Fig. 2. (a) A typical elution profile of 1-methyl-3-thiocarbamoylpyridinium iodide and thionicotinamide. Fractions Nos. 1–27 were eluted with 0.01 *M* Tris-hydrochloric acid buffer. At the arrow, the buffer was removed from the top of the resin and replaced with 1 *M* formic acid. A, 1-Methyl-3-thiocarbamoylpyridinium iodide; B, thionicotinamide (a load of 2 mg of each compound). (b) Identical procedure as in (a) without any load of nicotinamide derivative (blank column).

It seems that the aromatic character of the N^1-substituent does not influence the elution of 1-alkylpyridinium derivatives.

All carbamoylpyridines investigated were eluted by Tris–hydrochloric acid buffer (pH 7.0). A progressive increase in affinity for the Dowex 1-X8 was observed in order nicotinamide < isonicotinamide < 6-aminonicotinamide (see Figs. 1a–1c).

It was very surprising, however, that all thiocarbamoylpyridines were retained so strongly by the resin material that they could not be eluted with Tris–hydrochloric acid buffer. For elution of these compounds, the application of 1 *M* formic acid was necessary (see Fig. 2a). The elution with acid can be explained by the addition of a proton to the nitrogen atom in the pyridine ring, converting the molecule into a pyridinium form:



These positively charged pyridinium compounds were no longer retained by Dowex 1-X8. They were eluted in the same fraction (the peaks appearing in fractions 5–8 after addition of formic acid). Thiocarbamoylpyridines seem to have special qualities with respect to their affinity for different column materials. In a previous paper[16], we reported an increase in adsorption to Sephadex G-25 if oxygen in the carbamoyl group of the nicotinamide is replaced with sulphur.

CONCLUSIONS

The interactions of nicotinamide and nicotinamide analogues with Dowex 1-X8 resin provide means by which product and substrate of the enzymatic reaction catalyzed by nicotinamide N^1-methyltransferase (E.C. 2.1.1.1) can be separated. This allows the use of a new method for the measurement of low enzyme activities when radioactively labelled nicotinamide is applied.

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