Effect of Alkali on Ribonucleoside-2′,3′-monophosphates

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The common method for the determination of the nucleotide composition of ribonucleic acid (RNA) includes a hydrolysis in potassium hydroxide followed by chromatographic separation of the resulting ribonucleoside-2′,3′-monophosphates. Cytidylic acid (CMP) is deaminated to uridylic acid (UMP) during the alkali treatment.[1-3] Röttger and Fritz [4] found that also adenylic (AMP) and uridylic acids decompose in 1.0 N potassium hydroxide. During studies on RNA in granuloma tissue, we encountered a similar decomposition of ribonucleotides and studied it quantitatively to evaluate the necessary correction coefficients.

Table 1. Recovery of individual ribonucleotides after incubation in 0.3 N KOH at + 37°C. The values are mean percentages. The standard errors are also indicated (n = 10).

Nucleotide	Not incubated in KOH solution	After 20 h in 0.3 N KOH	Correction coefficient
CMP	100.3 ± 1.2	90.3 ± 0.5	1.11
AMP	101.4 ± 1.0	98.2 ± 0.9	1.03
UMP	101.1 ± 0.7	99.7 ± 0.6	1.01
GMP	95.8 ± 0.4	95.5 ± 1.1	1.00

Commercial mononucleotides (Calbiochem, Los Angeles 63, Calif., U.S.A.) were used. Their purities were checked spectrophotometrically using extinction values given by Beaven *et al.*,[5] by paper chromatography,[6] and by ion-exchange chromatography.[7,8] CMP contained 1.5 % of UMP and was purified by anion-exchange chromatography.[8] About 5 mg of each nucleotide was dissolved in 10 ml of 0.05 N hydrochloric acid, potassium hydroxide was added to give the final concentration of 0.3 N, and the samples were kept at + 37°C for 20 h. The solutions were neutralized with 6 N perchloric acid at 0°C and the precipitated potassium perchlorate was removed by centrifugation in a refrigerated centrifuge. The supernates were made 0.05 N in hydrochloric acid and a 0.2 ml portion of each sample was then subjected to cation-exchange chromatography.[7] The fractions were located with a continuously recording Uvicord (LKB Produkter, Stockholm, Sweden) spectrophotometer and the amount of each nucleotide determined using a Beckman DU spectrophotometer.

The results are presented in Table 1. In addition, 6.0 ± 0.3 % of CMP was recovered as UMP after incubation in 0.3 N potassium hydroxide for 20 h. When UMP is determined in the presence of CMP, 6.0 % of the corrected CMP content should be deducted from the measured UMP content.

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