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Nucleosides, Nucleotides and Nucleic Acids

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HPLC as a Tool to Study the Kinetics of Parallel and Consecutive Reactions: Competitive Interconversion, Dephosphorylation, Deamination and Depyrimidination of Cytidine 2'- and 3'-Monophosphates in Aqueous Acid

Mikko Oivanen^a; Harri Lönnberg^a

^a Department of Chemistry, University of Turku, Turku, Finland

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HPLC AS A TOOL TO STUDY THE KINETICS OF PARALLEL AND
CONSECUTIVE REACTIONS: COMPETITIVE INTERCONVERSION,
DEPHOSPHORYLATION, DEAMINATION AND DEPYRIMIDINATION OF
CYTIDINE 2'- AND 3'-MONOPHOSPHATES IN AQUEOUS ACID

Mikko Oivanen and Harri Lönnberg

Department of Chemistry, University of Turku, SF-20500
Turku, Finland

Abstract. The applicability of HPLC as a method to study the kinetics of complicated reaction systems of nucleosides and nucleotides has been demonstrated by using the hydrolytic reactions of cytidine 2'- and 3'-monophosphates as an example.

HPLC with UV detection offers a convenient method to follow quantitatively complicated reaction systems of nucleosides and nucleotides, which consist several kinetically distinguishable parallel and/or consecutive steps. Chromatographic analysis of the aliquots withdrawn at appropriate intervals, and comparison of the peak heights or areas with those of calibration samples of known concentrations, gives time-dependent concentrations of the UV-absorbing intermediates and products, and hence provide enough data to calculate the rate constants of all the partial reactions involved.

As an example, cytidine 2'- and 3'-monophosphates have been shown to undergo in acidic solutions a mutual isomerization, with which dephosphorylation to cytidine and deamination to uridine monophosphates compete, in particular at low hydronium ion concentrations ($\text{pH} > 2$). Hydrolysis to cytosine and ribose phosphate may also take place, but is of minor importance. The subsequent reactions include interconversion of uridine 2'- and 3'-monophosphates, hy-

drolysis of cytidine to cytosine, and deamination of cytidine and cytosine to uridine and uracil. A complete kinetic description for this multistage process was obtained on the basis of the time-dependent product distributions determined separately for the solvolytic reactions of 2'-CMP and 2'-UMP. The chromatographic analyses were carried out on a Hypersil column (4x250 mm, 5 μ m), using an acetate buffer (0.025 M, pH 4.3, NH_4Cl 0.2 M) as eluant. The rate constants obtained at various hydronium ion concentrations were then employed to construct the pH-rate profiles for the different partial reactions involved. The rate constants referring to the reactions of different ionic forms were finally determined by fitting the kinetic equations, derived on the basis of the assumed mechanisms, to the experimental rate profiles. The results are briefly compared to those reported earlier for the interconversion and dephosphorylation of adenosine 2'- and 3'-monophosphates,¹ and deamination of cytidine.²

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