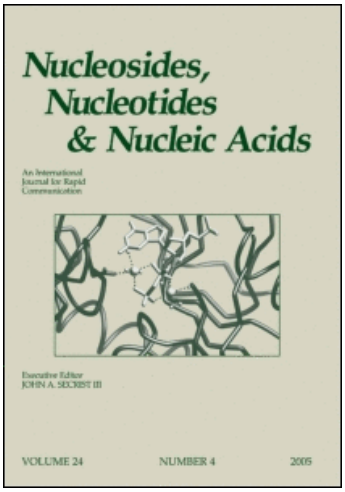

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Synthesis and Transmembrane Transport Studies of Lipophilic Adenosine 5'-Triphosphate Derivatives

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SYNTHESIS AND TRANSMEMBRANE TRANSPORT STUDIES OF LIPOPHILIC ADENOSINE 5′-TRIPHOSPHATE DERIVATIVES

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ABSTRACT: The preparation of acyl adenosine 5′-triphosphates as potential membrane permeable prodrugs is presented. The interaction of myristoyl- and cholesteryloxy-carbonyl-ATP with liposomes as model membranes and the release of ATP inside these vesicles was investigated using an enzymatic assay as well as ^{31}P-NMR spectroscopy.

Numerous studies to find prodrugs of nucleoside 5′-monophosphates have already been reported, but only little work has been described on carrier systems for nucleoside 5′-triphosphates.[1,2] In our ongoing program to design lipophilic membrane permeable nucleotides we have prepared various acyl adenosine 5′-triphosphates by acylating the terminal phosphate group of the nucleotide with a fatty acid or a cholesteryl moiety (Figure 1).[3] The resulting acylphosphates liberated ATP by selective cleavage of the mixed anhydride bond.

The interaction of myristoyl-adenosine 5′-triphosphate (Myr-ATP) with liposomes as model membranes was first investigated by an enzymatic assay containing luciferin / luciferase to mesure the amount of released ATP.[4] Although we have observed a 10% decrease of extravesicular ATP after incubation with Myr-ATP, its instability in the enzymatic medium led us to use ^{31}P-NMR spectroscopy in further experiments. In this case, the release of ATP from cholesteryloxycarbonyl-ATP[5] (Chol-ATP) was observed with the help of a pH gradient to distinguish between resonances of ATP molecules located inside and outside the liposomes.[6] ^{31}P-NMR spectra of ATP entrapped in

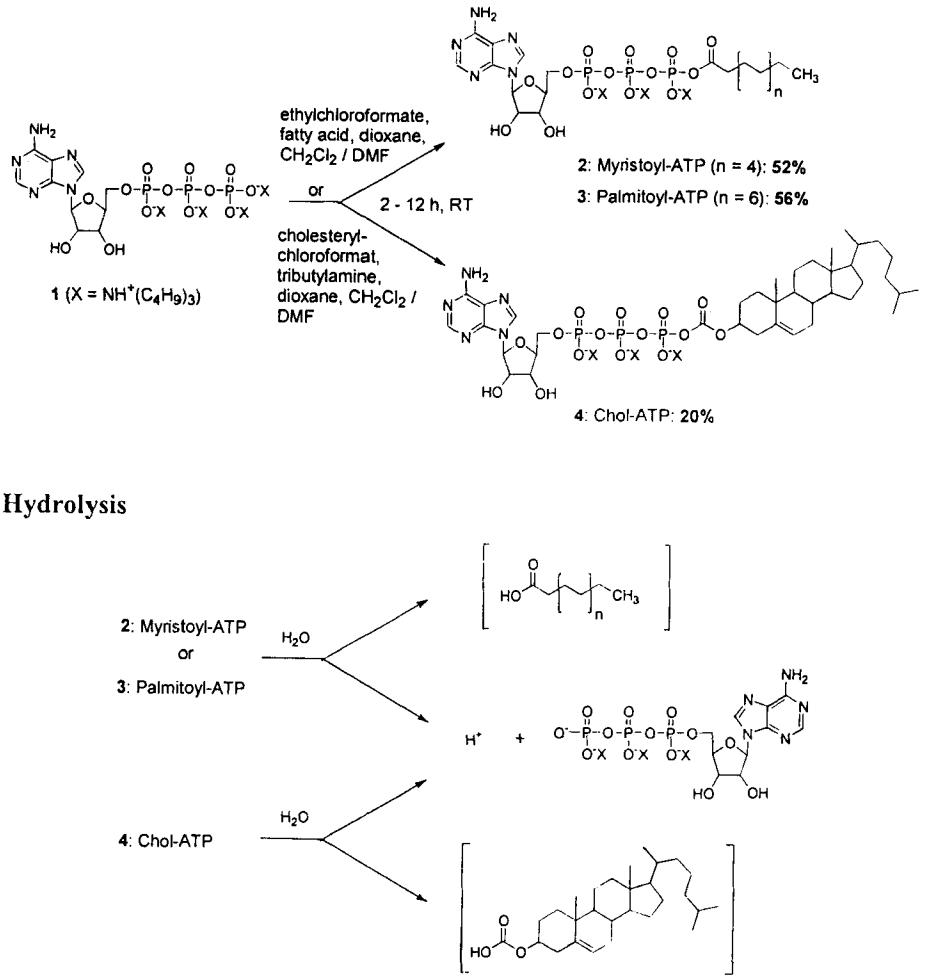

FIG. 1: Synthesis and hydrolysis of acyl-adenosine 5′-triphosphate derivatives.

liposomes prepared in a phosphate-buffered medium showed a large linebroadening of the intravesicular ATP resonances which will hinder the observation of ATP internalized in liposomes; after an external pH jump, a second phosphate peak appeared owing to the pH gradient established, attesting that liposomes were not leaky (Figure 2). The incubation of Chol-ATP with liposomes containing no Chol-ATP was monitored by ^{31}P-NMR (Figure 3). After a long incubation time, the pH jump was

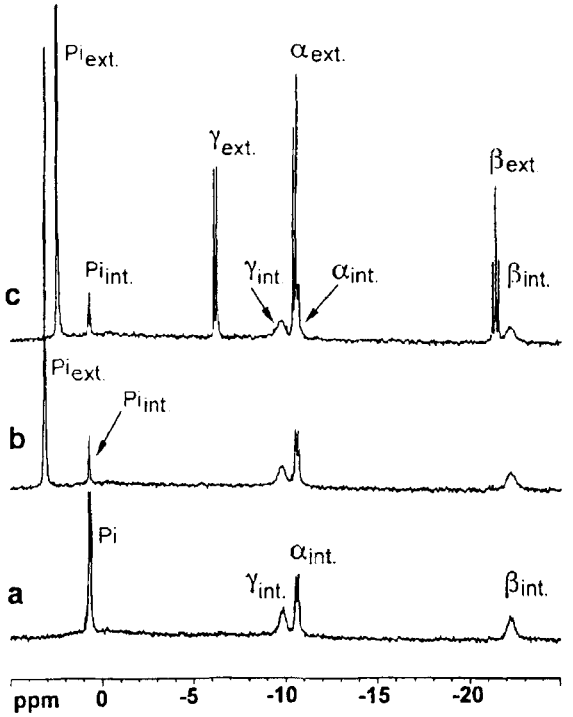

FIG. 2: 121.49 MHz ^{31}P-NMR spectra of ATP entrapped in liposomes in a phosphate-buffered solution at 25 °C: (a) equilibrated at pH 5; (b) after addition of aqueous NaOH, external pH set at pH 8 – no phosphorus lines of external ATP were detected; (c) after addition of 3 mM ATP extravesicular. int. = intravesicular; ext. = extravesiculat; Pi = inorganic phosphate.

performed and a weak signal of the γ-phosphorus of entrapped ATP centered at −8.2 ppm appeared, in agreement with its predicted chemical shift calculated via the pH derived from the chemical shift of internal inorganic phosphate resonance. By addition of the detergent triton X100, the intra- and extravesicular ATP resonances coalesced. The surface of extravesicular ATP increased in 10 %, indicating the amount of entrapped ATP from Chol-ATP.

We have demonstrated that ATP bearing a lipophilic moiety at the γ-phosphate group can be transported through liposomes. This is the first successful example of the passive transfer of a nucleoside 5′-triphosphate across membranes. It is now of interest to evaluate these model drug carriers in living cells with various therapeutic nucleotides.

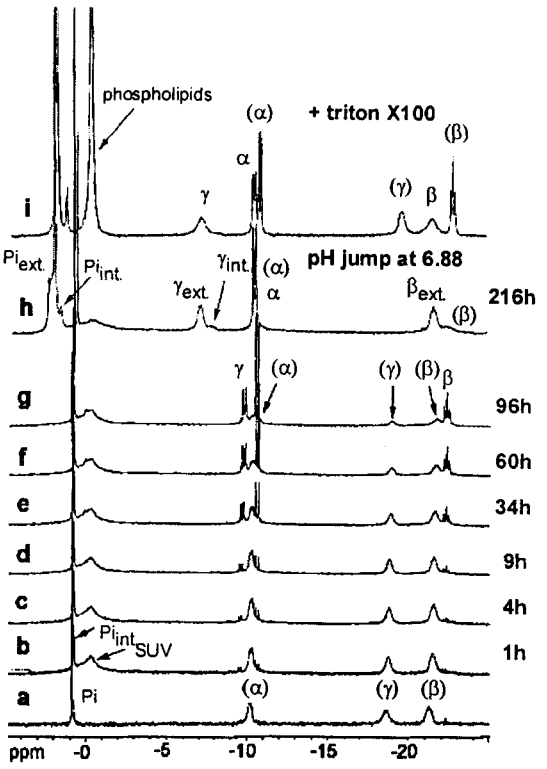

FIG. 3: 121.49 MHz ^{31}P-NMR spectra of Chol-ATP incubating in a phosphate-buffered solution containing liposomes (32 mg/ml of lipids) recorded (a - g) at different incubation times at pH 5, (h) after 216 h incubation time and the addition of aqueous NaOH, pH 6.88, and (i) after addition of triton X100. Chol-ATP signals are quoted in parentheses; int. = intravesicular; ext. = extravesicular.

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4.) Unpublished results.

5.) Chol-ATP: ^{1}H-NMR (D_2O) showed the characteristic signals of adenosine; protons of the cholesteryl moiety were observed between 2.05 and 0.57 ppm. ^{31}P-NMR (D_2O, proton decoupled, pH 7.1) δ (ppm) = -10.35 (d, α-P, $J_{P\text{-}P}$ = 19.8 Hz), -18.82 (broad, β-P), -21.51 (broad, γ-P). Mass spectrum (electrospray): calcd for $C_{38}H_{60}N_5O_{15}P_3(NBu_3)_3$ M = 919.33; found M = 919.4.

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