On the Interaction of Naphthalene Derivatives with Nucleotides Studied by Fluorescence Spectroscopy

J. Fick, R. Lawaczeck, F.W. Schneider

Institut für Physikalische Chemie, Universität Würzburg,
Marcusstr.9/11, D-8700 Würzburg

(γ-AmNS)NTP molecules (nucleoside-5'-triphosphoro-γ-1-(5-sulfonic acid) naphthylamidates) are well suited substrates for RNA-polymerases and phosphodiesterase. In both cases, the liberation of the pyrophosphate-moieties of the 1-amino-naphthalene-5-sulfonate, (AmNS)PP_i, leads to an increase of the fluorescence quantum yield. This is especially pronounced in the case of uracil as nucleobase. We have investigated the fluorescence properties of (γ-AmNS)UTP, (γ-AmNS)ATP, (AmNS)PP_i and uncomplexed AmNS because, in combination with a phosphodiesterase, these molecules are excellent probes to study the fusion of phospholipid vesicles and other transport phenomena across vesicular bilayers.

Yarbrough and Bock (1) have reported that the quenching of the naphthalene fluorescence by nucleotides (intra- and intermolecularly) is a consequence of the stacking interaction between these molecules. In the case of ATP molecules an energy-transfer was suggested. However, both an energy-transfer and the stacking interactions cannot explain the dramatic differences in the fluorescence spectra between (γ-AmNS)UTP and (γ-AmNS)ATP.

We show that these differences can be explained on the basis of the interaction of the pyrimidine-(N or O)-proton, especially in the enol-form, with the adjacent naphthalene-sulfonate molecule. Space filling molecular models allow a hydrogen-bond between the pyrimidine-enol-proton and an oxygen of the sulfonic acid group. This interaction stabilizing the folded form seems to be absent in ATP-derivatives. Consequently the naphthalene fluorescence is quenched more strongly by UTP than by ATP as observed. The pH-dependence of (γ-AmNS)UTP clearly demonstrates the participation of the enolic proton in the folded form. In the deprotonized form (pK ~ 9.5) the molecule opens, looses the formerly favoured overlapping of the two ring-systems and an increase of the fluorescence intensity results. Both the life-times and the quantum yield are now comparable with the values of the free (AmNS)PP_i molecule. This interpretation is supported by the solvent-isotope-effect (SIE) (D_2O vs. H_2O) of the fluorescence quantum yield. The SIE is hardly observed in the folded form (pH < pK of the enol-proton) but pronounced in the open form (pH > pK of the enol-proton). These results are further confirmed by titration experiments of (AmNS)PP_i with nucleotides, where the quenching by UMP is reduced at pH values above the pK of the pyrimidine-N-(or enolic)-proton.

(1) Yarbrough, L.R., and Bock, J.L., J.Biol.Chem. 255, 9907 (1980)

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