Cenk A Andac, J Chromatogr Sep Tech 2017, 8:4(Suppl)
DOI: 10.4172/2157-7064-C1-028

Synthesis, HPLC purification, NMR characterization and AMBER molecular dynamics structure of the anticodon stem-loop region of a t-RNA with a modified 3-methyl uridine (3MU) residue at position 33

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A specific H-bond between U33 and the phosphate group between C36 and G34 in the anticodon loop region of t-RNA renders a very compact anticodon loop structure, hence called a closed-loop structure. It is known that the closed loop structure is in exchange with an open-loop structure in which the aforementioned specific H-bond in the anticodon loop region is broken. In order to shed light on to the dynamics of the open-loop structure of the transfer RNA, a 17-mer RNA hairpin molecule, representing the open-loop structure anticodon region of a transfer RNA, with the sequence 5' GGGAGUXAGCGGCUCCC 3' (X=3-N-methyl uridine) was synthesized using the dimethoxytrityl, tert-butyldimethylsilyl and 2-cyanoethyl-diisopropylphosphoamidite. The crude product was then purified by reversed-phase HPLC using an 8 mm C-18 Radial-Pak column (by Waters Assoc., Inc) on a Beckman (Fullerton, CA, USA) System Gold HPLC instrument with ultra-violet detection at 254 nm. Optimal conditions for the separation of the 17-mer RNA were provided by using an isocratic elution system based on 0.05 M ammonium acetate (NH4OAc) solution in RNase-free dd.H2O (buffered at pH=7.0). The retention time was 9 min and the elution speed was 0.7 ml/min. The purified RNA hairpin was further studied by NMR and it was found out that two major conformationally different structures of the RNA exist in slow exchange with concentrations ~51% and ~43% on NMR time scale. AMBER molecular dynamics and cluster analysis studies indicate reveal two open-loop structures of the RNA hairpin.

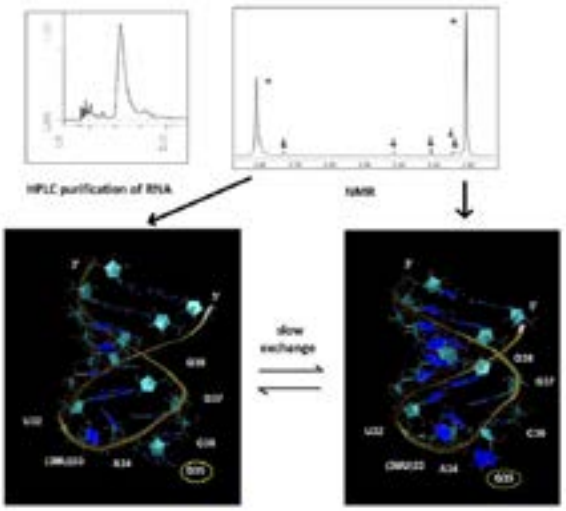

Figure 1: HPLC purification, NMR identification and AMBER structures of 17-mer RNA hairpin.

Biography

Cenk A Andac works as an Assistant Professor in the School of Pharmacy at Istinye University. He has completed his Master's degree and PhD work from the Faculty of Pharmacy at the University of Wisconsin-Madison, WI, USA (UW-Pharmacy, USA). He has been involved in teaching drug actions and delivery, and pharmaceutical biochemistry and biotechnology courses at UW-Pharmacy, USA for four and half years. He has also taught Medical Pharmacology courses as an Assistant Professor for three years in Medical School of Turkey. His current researches are development of novel anticancer agents inhibiting G-coupled receptors in cancer stem cells; development of novel aminoglycoside antibiotics; determination of 3D structures of biological and synthetic compounds by NMR techniques; computer-assisted drug development by AMBER, CHARMM and quantum mechanics; and pharmacokinetics and pharmacodynamics properties of drug-receptor interactions. He currently holds a patent for a potentially active anti-cancer agent against breast cancer.

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