

Biology 5357

Chemistry & Physics of Biological Molecules

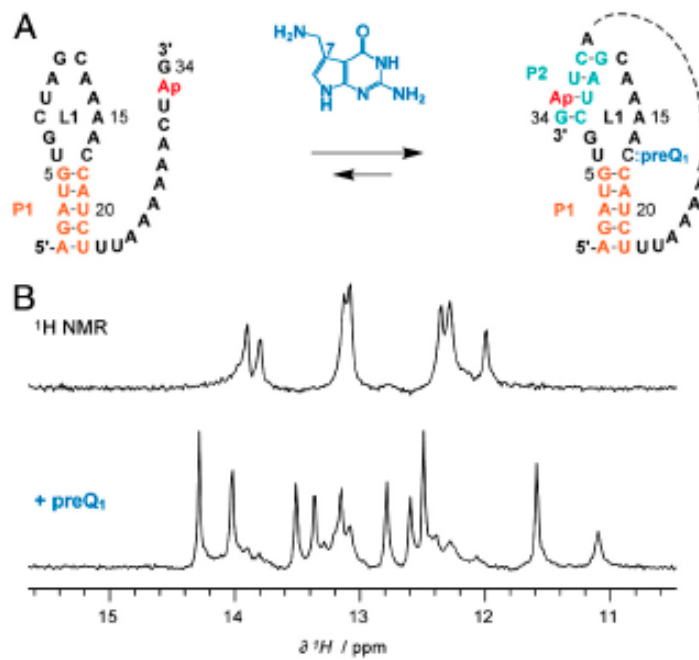
Examination #2

Nucleic Acids
Membranes

December 16, 2010

1. The pre-Q riboswitch has the smallest known aptamer domain. Here, a schematic of the structural change that occurs upon preQ₁ binding is shown. It involves formation of a pseudoknot, in which a new stem (P2) is formed when the 3' tail base-pairs with the loop nucleotides. PreQ₁ forms a hydrogen-bonded interaction with C17. (Ap is an adenine analog that isn't important here).

Also shown is the imino proton spectrum of the aptamer with and without preQ₁. [RNA] = 0.2 mM, [preQ₁] = 0.4 mM, [Mg²⁺] = 2 mM, in 25 mM sodium cacodylate at pH 6.5 and 298 K.



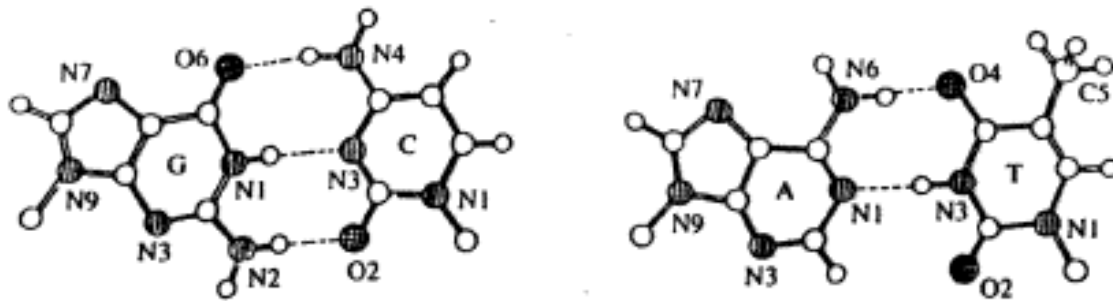
A. (5 points) You remember that when a base pair is stable, its imino proton(s) are observed. How do you interpret the two imino proton spectra shown?

B. (10 points) The +preQ₁ spectrum includes both preQ₁ and Mg²⁺. Predict how the aptamer structure might or might not change in the presence of Mg²⁺ but without preQ₁. Explain your reasoning.

C. (10 points) If Mg²⁺ is required for preQ₁ to bind, what might you conclude about the aptamer structure?

D. (extra credit) Draw a plausible base pairing scheme between preQ₁ and rC17.

2. A. (5 points) Identify the major and minor grooves of the base pairs assuming B-form DNA. Indicate the imino protons.



B. (5 points) Which groove would you expect a helix-turn-helix protein to use? Explain your answer.

C. (5 points) Write the equilibrium expression for a protein binding to a nucleic acid in a solution containing sodium glutamate and $MgCl_2$.

3. (20 points) Write the discussion for the figure below, explaining the concepts and pathways.

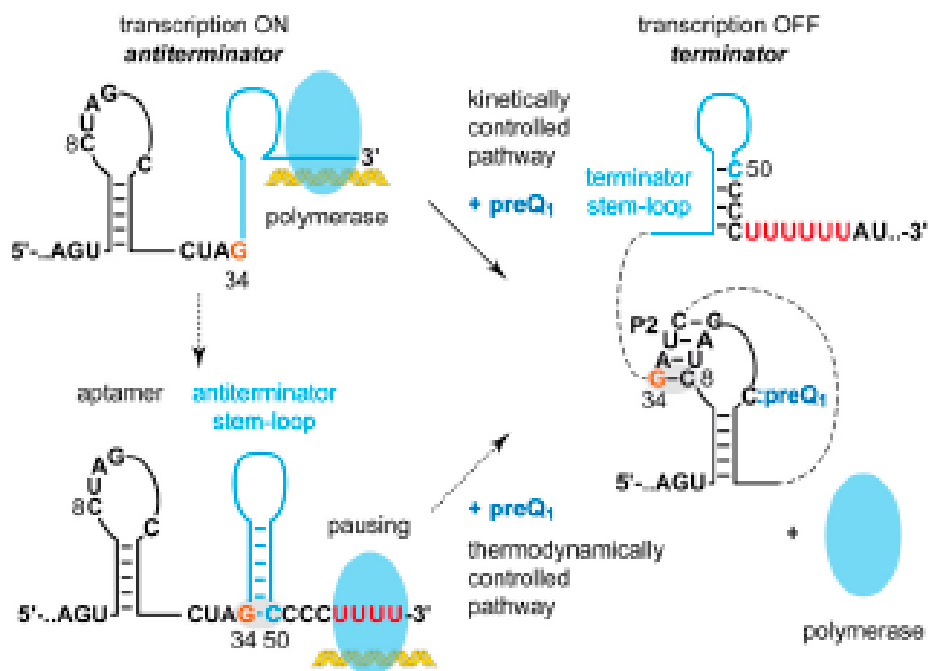


Fig. 6. Proposed model for the ligand-dependent antiterminator/terminator interplay in vivo (for discussion, see main text).

4. (10 points) Pure hydrated lipids were sonicated extensively to prepare liposomes. When the mean diameter of the resulting particles was determined the size depended upon the lipid composition with DSPC (C_{18}), DPPC (C_{16}) or DMPC (C_{14}) having diameters of 28, 24 and 22 nm respectively. The inclusion of 30 M% of DPPE (dipalmitoyl phosphotidyl ethanolamine) increased the mean diameter of the preparations to 50 ± 10 nm. Discuss these observations and postulate a basis for the measured sizes. These preparations were used in the study of a phospholipase that removed the sn-2 acyl chain from membrane phospholipids. The enzyme activity was $DMPC > DPPC > DSPC$, but the mixed lipid preparations all had the same activity. Define the characteristics (composition, size) of lipid preparations likely to assist in the study of the chain length specificity of the phospholipase. Discuss how easy or difficult you think that it would be to produce appropriate lipid preparations.

5. (10 points) In cells, both phosphotidylcholine and phosphotidyl ethanolamine are common lipid types that are observed to be present. Discuss how this difference in head group identity is important in cellular membranes. You may use the intracellular distribution to support the importance of the chemical difference, or the chemical difference resulting from the two head groups to infer an intracellular distribution.

6. (10 points) The diffusion of lipids in membranes reflects the membranes structure and stability. It has been postulated that some lipids form complexes in the membranes that produce local phase changes in the membrane, “rafts”. Assuming that the formation of these complexes is diffusion controlled their existence has been studied by measuring the diffusion constant for DOPC and sphingomyelin in lipid bilayers.

M% Cholesterol	D_{DOPC}	$D_{DOPC:SM}$
0	6.3E-8	2.0E-8
20	5.8E-8	1.1E-9
30	4.0E-8	9.0E-9
60	2.5E-8	12.0E-9

From these data determine which lipids are required to form the “raft” domains. In these studies the “rafts” were studied by two methods, fluorescence microscopy of giant liposomes and atomic force microscopy (AFM) on supported bilayers. While the fluorescence studies indicate that

the “rafts” were present at 20 and 30 M% cholesterol, the AFM indicated that membrane thickening “rafts” were present in all membranes containing cholesterol. Analyze these results in terms of lipid composition and membrane structure. Suggest additional studies that can clarify the phenomena that are responsible for the apparently contradictory results.

7. (10 points) Discuss the lipid chemistry that is important to the formation of bilayer structures. Please include in your discussion the interactions and binding forces that stabilize or disrupt the bilayer structure. Use in your discussion a characterization of the distribution of the chemical components of lipid molecules across the membrane thickness.