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Role of Mg^{2+} ions in flavin recognition by RNA aptamer

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ABSTRACT

The role of Mg^{2+} ion in flavin (flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)) recognition by RNA aptamer has been explored through steady state and time-resolved fluorescence, circular dichroism (CD), thermal melting (TM) and isothermal titration calorimetry (ITC) studies. A strong quenching of flavin emission is detected due to stacking interaction with the nucleobases in the mismatched region of aptamer, and it enhances manifold with increasing Mg^{2+} concentrations. A comparatively lower binding affinity toward FAD compared to FMN is attributed to the presence of intramolecular 'stack' conformer of FAD, which cannot participate in the intermolecular stacking interactions with the nucleobases. CD and TM studies predict that flavin detection causes structural reformation of RNA aptamer. ITC results indicate that flavin detection is thermodynamically feasible and highly enthalpy driven.

1. Introduction

Aptamers are defined as small sequence of nucleotides which are engineered through repeated rounds of *in vitro* systematic evolution of ligands by exponential enrichment (SELEX) and can sense specific molecular targets with high selectivity [1–4]. RNA aptamers are characterized by unpaired or mismatched bases which help to fold the RNA in distinct secondary and tertiary structures containing loops and bulges. These loops and bulges are suitable for selective interactions with small molecules, proteins, nucleic acids, cells, tissues and organisms [5–11]. Although the importance of RNA aptamer as small molecular sensor has been portrayed in a number of reports [12], a little has been focused on flavoprotein cofactor binding aptamers. Flavins are the most ubiquitous cofactor [13–16] in nature is known to catalyze a huge number of redox [13–16] reactions. From recent past flavin binding RNA aptamers had been highlighted as point of interest. Burgstaller et al. first isolated aptamer sequences, which were able to recognize flavin cofactors [17,18]. NMR results concluded that isoalloxazine ring of flavin mononucleotide (FMN, Scheme 1) can intercalate between G-G mismatch and G-U-A base triple, which results the selective affinity for flavins [19]. Moreover, this recognition specificity is associated with hydrogen bonding of the uracil like edge of isoalloxazine to Hoogsteen edge of adenine at the interaction site. This structural stabilization had been employed to the design allosteric ribozyme whose self-cleavage and self-ligation reactions were enhanced nearby 300-fold in presence of FMN [20,21]. Later Lauhon et al. isolated riboflavin and nicotinamide detecting RNA aptamer which upon binding with riboflavin resulted a two-tiered G-quartet structure [22]. Although selective to redox co-factors, aptamers never found to differentiate between oxidized and reduced form of 5-deazariboflavin derivative, which is a structural analog of riboflavin [22]. SELEX method was also used to identify RNA aptamers that recognizes flavin adenine dinucleotide (FAD, Scheme 1) but unable to distinguish FAD from $FADH_2$ [23]. Through experimental and computational methodologies Anderson et al. detected 14-mer RNA aptamer which was selective to flavins (riboflavin, FMN and FAD) and showed better interaction with FMN compared to others [24]. The electron density on the phosphate group was speculated to partially delocalize on the flavin ring, which strengthened hydrogen bonding and π-stacking interactions between isoalloxazine and nucleobases [24]. Notably, literatures lack any detailing about higher selectivity toward FMN as well as structural information about FAD-aptamer complexation. The major complexity of FAD compared to FMN is the existence of FAD in variable conformations (namely, 'stack', 'partially stack' and 'unstack') in water, as it constituted by a fluorophore (isoalloxazine) and a quencher (adenine) [25–27]. Stack conformer of FAD is more stable than unstack the conformer due to the π–π stacking interaction between flavin and adenine moiety. This stacking interaction results a quenched lifetime of ~9 ps due to the excited state intramolecular electron transfer from adenine to flavin moiety [25–27]. In this context, it seems interesting to explore particularly which conformer of FAD is appropriate for the interaction with aptamer. Since the dinucleotide cofactors have special evolutionary importance, it is worth to have a better insight about their interactions with RNA aptamer (Scheme 1). We have

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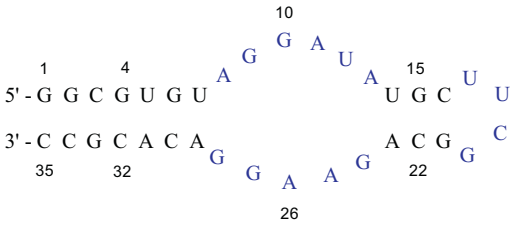
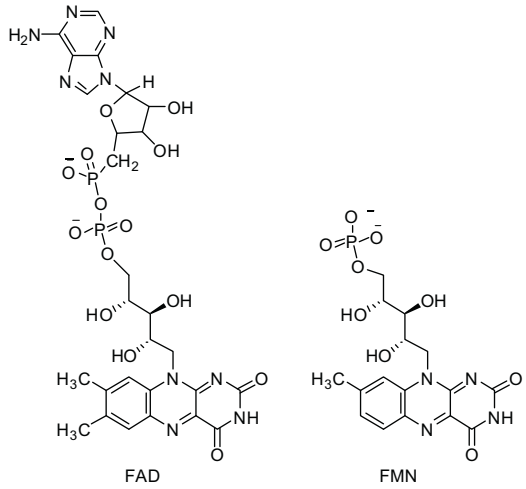


RNA Aptamer



Scheme 1. Schematic representation of 35-mer self loop forming RNA aptamer, FAD and FMN.

utilized several biophysical techniques like steady state, time-resolved fluorescence, circular dichroism (CD) and isothermal titration calorimetry (ITC) to explore flavin-RNA aptamer binding interaction. Although it is well established that thermodynamic parameters, like, enthalpy and entropy changes are the governing factors for small molecule detection, previous reports paid least attention to the thermodynamics insight of flavin recognition by RNA aptamer. Hence, a detailed investigation about thermodynamics of flavin-aptamer interaction has been performed to explore the selectivity and feasibility of flavin sensing. The choice of the RNA aptamer (Scheme 1) is motivated by the recent NMR crystal structure of FMN-bound RNA aptamer reported by Fan et al. [19].

It is well known that few mono and bivalent metal ions at high concentrations stabilize RNA tertiary structure, though the exact mechanism is still under mist. Researchers found K^+ and Mg^{2+} offers most effective stability to RNA structures. Draper and co workers established that a folded RNA structure interacts in a better way to Mg^{2+} ions, as the phosphate charges of RNA are accumulated to a smaller volume, which results a strong electrostatic interaction with the metal ion [28,29]. Moreover, a water solvated bivalent Mg^{2+} state is speculated to interact through co-ordination and hydrogen bonding interactions, offering a better stability to RNA [30]. A recent report by Fiore et al. shows the overall interaction between RNA and Mg^{2+} is exothermic and entropically costly [31]. In this work we intend to explore the effect of Mg^{2+} ion concentration over flavin detection by RNA aptamer.

In a nutshell, the present work is executed with three distinct targets, which are (a) exploring the binding interactions flavins (specially FAD and FMN, as they have vast structural diversity) by RNA aptamer and uncovering how the conformational flexibility of FAD affects the recognition, (b) a detailed thermodynamic insight of flavin recognition by RNA aptamer from steady state fluorescence and isothermal titration calorimetry studies, and (c) the effect of Mg^{2+} ion concentrations over flavin recognition. We believe that the biophysical insights of flavin-aptamer interaction may improve the basic understanding of small molecule recognition by RNA aptamer.

2. Experimental section

2.1. Materials and methods

FAD (HPLC grade, purity ⩾ 96%) was purchased from Sigma Aldrich and FMN was bought from Fluka (HPLC grade, purity ~90%). Both the flavins were used without further purification. Na_2HPO_4 and NaH_2PO_4 (Molecular biology grade) were procured from Sisco Research Laboratories (SRL-India). NaCl (BioXtra purity ⩾ 99.5%) and EDTA (BioUlra purity ⩾ 99%) were purchased from Sigma Aldrich. RNA aptamer (100 nano-moles synthesis scale, HPLC purified) was brought from integrated DNA technology (IDT). Phosphate buffer (Na_2HPO_4 + NaH_2PO_4, 10 mM) of pH 7.0 (containing 150 mM NaCl, 0.1 mM EDTA) was used for all sample preparations, dilutions and experiments. The Mg^{2+} ion concentration was maintained by addition of concentrated stock of $MgCl_2$ (biograde, Sigma–Aldrich, purity ⩾ 97%). All the samples and buffer preparation were done in autoclaved Milli-Q water (18.2 μΩ cm^{-1}).

For all the spectroscopic studies, concentrations of FAD and FMN were kept at ~5 μM to avoid molecular aggregation. RNA concentration was varied from 1 μM to 50 μM for steady state and time-resolved fluorescence measurements. The molar extinction coefficient (347,200 M^{-1} cm^{-1}, from IDT) at 260 nm was used for preparation of RNA solution. Before using RNA, it was annealed up to 90 °C in phosphate buffer saline (PBS) with respective Mg^{2+} ion concentration and kept overnight at 4 °C to have a stable tertiary structure of the RNA. All the spectroscopic and calorimetric data collections were done at 298 K, unless otherwise mentioned.

2.2. Instrumentations

Solution pH was measured by pH-1500 (Eutech Instruments, USA) and verified by silicon micro sensor pocket sized pH meter (ISFETCOM. Co. Ltd., Japan). Absorption spectra were recorded in Evolution 300 UV–Visible spectrophotometer (Thermo Fisher Scientific, USA). Thermal melting (TM) studies were performed in CARY-300 Bio UV–Vis spectrophotometer (Agilent U.S.A.). RNA melting studies in absence and in presence of flavin were done by heating the sample from 20 °C to 90 °C followed by slow cooling up to room temperature. The concentration of flavin (10 μM) and RNA aptamer (2 μM) were kept fixed in TM studies.

Steady state fluorescence spectra were collected in Fluorolog-3 (Horiba JobinYvon, U.S.A.). Fluorescence lifetimes were collected from time-correlated single photon counting (TCSPC) set-up from IBH Horiba JobinYvon (U.S.A.) using 444 nm diode laser. The detail description of the instrument is mentioned elsewhere [32,33]. Analysis of lifetime data was done by IBH DAS6 software. The lifetime data were fitted with a minimum number of exponential. Quality of each fitting was judged by χ^2 values and the visual inspection of the residuals. The value of $\chi^2 \approx 1$ was considered as best fit for the plots.

Circular dichroism (CD) spectra were recorded on a J-815 CD spectro-polarimeter (JASCO, USA). Each CD profile is an average of 5 scans of the same sample collected at a scan speed of 20 nm/min. During CD measurement, RNA concentration was kept fixed and the concentrations of flavin was increased steadily. For CD studies a fixed concentration of RNA aptamer (~2 μM) was titrated with increasing flavin concentrations (up to ~10 μM for FAD and FMN). CD spectrum of an identical concentration of blank flavin (~10 μM for FAD and FMN) was collected and subtracted from CD spectrum of flavin containing RNA aptamer.