Supporting online material

Structural basis of thiamine pyrophosphate analogues binding to the eukaryotic riboswitch

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Materials and Methods

RNA synthesis, Crystallization and structure determination.

RNAs were prepared by *in vitro* transcription of linearized plasmid DNA containing the *Arabidopsis thaliana* riboswitch encoding sequence with the same modification as previously reported.^{1,2} Transcription and purification of the riboswitch RNA was carried out as described previously,² except that after ethanol precipitation, the riboswitch RNA was refolded in the presence of either TPP, OTPP or PTPP in a 10 fold molar excess. Crystals were obtained under conditions similar to those described previously for the TPP-bound riboswitch.² After growth, crystals were transferred to a stabilization solution containing 30% 1,6-hexanediol, 0.5 mM spermine, 10 mM MgSO₄, 40 mM sodium cacodylate, (pH 6.8), and 1.5 mM of TPP, OTPP or PTPP for 5 minutes prior to flash freezing in liquid nitrogen.

Diffraction data were collected at beamline X06SA of the Swiss Light Source (Villigen, Switzerland) and processed with XDS³ for OTPP and PTPP or HKL2000⁴ for TPP. Subsequent rounds of rigid body and simulated annealing with positional and B-factor refinement were done in CNS⁵ for the OTPP/RNA structure and with the PHENIX software⁶ for the TPP/RNA and PTPP/RNA complexes. Statistics for final model structures are shown in Table 1. Figures were prepared using PyMOL (Delano, 2002).

Oxythiamine and pyrithiamine pyrophosphate synthesis.

PTPP has been synthesized according to Sudarsan *et al.*, 2005.⁷ Briefly, ortho-phosphoric acid (0.5 g, Fluka #79622) was liquefied over flame and cooled to room temperature.

Pyrithiamine hydrobromide (5 mg, Sigma #P0256) was then added and the mixture was stirred for 15 min in an oil bath heated to 110 °C. After cooling, the reaction was quenched by adding 2 ml of water and magnesium oxide powder until the pH value reached 7.0. This step allows the removal of the unreacted inorganic phosphate for further purification using a C18 column (Vydac 218TP 54 or 1022) with TriEthylAmmonium solution at 100 mM (pH 7.0). Pyrithiamine Mono, Di and Tri-phosphate could easily be separated by the HPLC chromatography. Fractions containing the PTPP were collected and concentrated.

An identical procedure was used for the synthesis of OTPP starting from oxythiamine chloride hydrochloride (Sigma #O4000).

References

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Table S1. Crystallographic Statistics

	TPP bound AT riboswitch	PTPP bound AT riboswitch	OTPP bound AT riboswitch
Data collection			
X-ray source	SLS X06SA	SLS X06SA	SLS X06SA
Space group	P2 ₁ 2 ₁ 2	P2 ₁ 2 ₁ 2	P2 ₁ 2 ₁ 2
Cell dimensions			
a, b, c (Å)	76.0, 111.0, 55.8	74.8, 110.8, 55.8	75.5, 111.2, 55.4
α , β , γ	90, 90, 90	90, 90, 90	90, 90, 90
Wavelength (Å)	1.0	1.0	1.0
Resolution (Å)	15-2.25 (2.31-2.25)	50-1.97 (2.02-1.97)	15.0-2.5 (2.55-2.50)
R _{merge} (%) [*]	7.5 (97.2)	4.8 (40.7)	15.3 (53.3)
$\langle I/\sigma(I) \rangle$ [*]	23.45 (2.0)	33.5 (5.87)	14.63 (2.85)
Completeness (%) [*]	100.0 (100.0)	98.8 (92.7)	97.2 (97.1)
Redundancy	7.1 (7.2)	7.1 (5.3)	7.3 (7.5)
Refinement			
Resolution (Å)	15-2.25	15.0-2.00	15.0-2.50
No. reflections	22939	33,016	16,268
R _{work} /R _{free} (%)	20.9 / 25.7	20.7 / 25.1	23.3 / 26.7
R.m.s. deviations			
Bond lengths (Å)	0.017	0.003	0.006
Bond angles (°)	3.16	0.857	1.100
σ_A coordinate error (Å)	0.35	0.28	0.37

^{*} Highest resolution shell in ().

Figure S1. Hydrogen bond arrangement between the pyrimidine ring of OTPP or TPP and the Guanosine 28 of the TPP-sensing riboswitch. The left panel shows the distances for the hydrogen bonds observed in the OTPP-bound TPP-specific riboswitch structure. The right panel shows that almost identical distances are observed in the native TPP-bound riboswitch complex. This suggests that the interaction is mediated by two hydrogen bonds in both cases, which would be the case if the OTPP is stabilized in its enol form.

Figure S2. Stereo view of the pyrophosphate interaction in the TPP-bound *A. thaliana* riboswitch structure at 2.25 Å resolution. The elongated pyrophosphate group is directly interacting with the RNA nucleotide C65 and G66 (with green backbone and atom-type coloring). Several additional contacts are mediated by the two magnesium ions (Mg_1 and Mg_2 depicted as cyan colored spheres) and their coordinated water molecules (small red spheres).