

Conformational Change of *Arabidopsis thaliana* Thioredoxin Reductase after Binding of Pyridine Nucleotide and Thioredoxin

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We have found that the binding of NADP⁺ ($K_d = 0.86 \pm 0.11 \mu\text{M}$) enhanced the FAD fluorescence of *Arabidopsis thaliana* NADPH:thioredoxin reductase (TR, EC 1.6.4.5) by 2 times, whereas the binding of 3-aminopyridine adenine dinucleotide phosphate (AADP⁺) ($K_d < 0.1 \mu\text{M}$) quenched the fluorescence by 20%. Thioredoxin (TRX) also enhanced the FAD fluorescence by 35%. The K_d of TR-NADP⁺ and TR-AADP⁺ complexes did not change in the presence of 45 μM TRX. Our findings imply that the binding of NADP⁺ and AADP⁺ at the NADP(H)-binding site of *A. thaliana* TR, and/or the binding of TRX in the vicinity of the catalytic disulfide increase the content of fluorescent FR conformer (NADP(H)-binding site adjacent to flavin). The different effects of NADP⁺ and AADP⁺ on FAD fluorescence intensity may be explained by the superposition of two opposite factors: i) increased content of fluorescent FR conformer upon binding of NADP⁺ or AADP⁺; ii) quenching of FAD fluorescence by electron-donating 3-aminopyridinium ring of AADP⁺.