

Loop-Tryptophan Human PNP Reveals Submillisecond Protein Dynamics

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Human PNP is a homotrimer, containing three non-conserved tryptophan residues at positions 16, 94 and 178 which are all remote from the catalytic site. The catalytic site of PNP is located near the subunit-subunit interface where residue F159 is a catalytic site residue donated from an adjacent subunit. It is the only residue from the neighboring subunit which participates in the catalytic site. QM/MM calculations of human PNP have shown that F159 is the center of the most mobile region of the protein. Calculations also reveal correlated motions between residues 57-65 of subunit-A (the phosphate-loop) and residues 155-160 of subunit-C (the F159-loop). These loops interact through the subunit interface¹. Interestingly, F159 is the key residue in a cluster of hydrophobic residues that shield substrate/TS analogues in the catalytic pocket from bulk solvent. Trp-free non-fluorogenic human PNP (Leuko-PNP) was engineered by replacing the three Trp residues of native PNP with Tyr, which allowed collection of ligand (guanine) fluorescence spectra, free of native PNP chromogenic effects². From this construct, a single Trp residue was engineered in the proposed dynamic loop (F159W-Leuko-PNP) as a probe for the changes of protein dynamic properties on substrate (inosine/hypoxanthine) binding and catalysis. Relatively weak binding of substrate/product/TS-inhibitors (as indicated by $K_m/K_d/K_i^*$ values) to F159W-Leuko-PNP compared to native- and Leuko-PNP is consistent with higher chemical rates (as indicated by $k_{(chem)f} / k_{(chem)r}$) for F159W-Leuko-PNP compared to native- and Leuko-PNP. The rate limiting step for PNP is known to be release of purine^{3,4}. The fluorometric properties of F159W-Leuko-PNP compared to native PNP (~10 nm red-shift) suggest a solvent exposed Trp residue on the free enzyme. Upon ligand binding (hypoxanthine) to the enzyme active site, the 3-fold quenching in fluorescence intensity confirms the dynamic role of F159 in the conformational packing of the catalytic pocket hydrophobic-cluster. The thermodynamic parameters determined for PNPs suggest enhanced activation enthalpy (ΔH^\ddagger) and entropy (ΔS^\ddagger) of the reverse chemical reaction for F159W-Leuko-PNP compared native PNP. This enhancement significantly shifts the kinetic equilibrium (K_{eq}) of the chemical reaction catalyzed by F159W-Leuko-PNP at high temperature, enabling the collection of T-jump relaxation kinetics at the nano- to the micro-second time scale. The T-jump relaxation kinetics of F159W-Leuko-PNP in equilibrium with either substrate or product in the presence of phosphate indicate the conformational formation of at least two ternary complex intermediates in the nano- to micro-second time scale. The intermediates confirm the dynamic role of the F159 loop in the active site packing upon substrate/ligand binding and catalysis. F159W-Leuko-PNP provides a novel, single point chromatic protein platform to investigate the change in the dynamic properties of PNP upon ligand binding and catalysis.

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