ADP ribose pyrophosphatases (ADPRase) hydrolyze ADP ribose (ADPR) into AMP and ribose-5'-phosphate with the help of Mn2+. We are trying to explain how ADPRases split H2O to generate the nucleophilic OH- and exclude the unnecessary proton left behind from the catalytic core consisting of Nudix motif GX5E(1)X7RE(2)UXE(3)E(4)XGU. In this report, we focus on Glu residue at the position 3 (Glu3rd) whose role has not been uncovered despite a lot of previous crystallographic efforts and kinetic studies. In order to judge whether Glu3rd takes part in the proton transfer process or not, we performed two kinds of experiments using the crystals of ADPRase from Thermus thermophilus HB8, which diffracted X-rays up to 0.9 Å resolutions. The 11 crystals of 18 were used for the investigation of the time-dependent structural changes based on the in-situ observation of ADPR hydrolysis at constant pH. We soaked Mn2+ into ADPRase-ADPR (ES) binary complex to start the reaction, and cryo-trapped ES-Mn ternary reaction intermediates. All data sets were refined with SHELXL and the C-O bond lengths of carboxylates were calculated by the full-matrix inversion following the removal of structural restraints on Glu residues. Carefully observing how the C-O bond lengths of Glu3rd behave along the reaction time, we found that Glu3rd contributes to activate the nucleophilic H2O and to exclude the proton left behind. When the reaction reached the climax of ADPR hydrolysis, its two C-O bond lengths were 1.30(2) and 1.23(2) Å, indicating the resultant structure after the proton extraction from the nucleophilic H2O. The remaining 7 crystals were used for the pH titration of ES binary complex to investigate its acid dissociation property. We achieved the various crystalline pHs by soaking buffer solution without any reaction triggers. Based on the pH-dependent changes of C-O bond lengths, we revealed the pKa value of Glu3rd around 4.6, which makes the catalyst workable even in the weak acidic condition.