This work is dedicated to thorough structural analysis of plant bifunctional nuclease TBN1, the representative of plant nuclease I group. TBN1 along with homologous nucleases from this family plays an important role in plant cell life cycle and also shows considerable anticancerogenic effects. Two variants of TBN1 (wild type and N211D mutant) were studied. Properties of both variants in different solutions were analyzed. Both were successfully crystallized. Structures of both types of TBN1 were solved using X-ray diffraction. The phase problem was solved by Multi-wavelength anomalous dispersion using Zn2+ ions natively present in TBN1. Structural properties of TBN1 such as fold, active site composition, effect of glycosylation and surface electrostatic potential distribution were analyzed. Reaction mechanism of TBN1 was proposed on the basis of structural properties and comparisons with similar structures.