Abstract

Family 4 glycoside hydrolases (GH4) represent an unusual group of glucosidases with a requirement for NAD^+, divalent metal cations and reducing conditions. The family is also unique in its inclusion of both α- and β-specific enzymes. The α-glucosidase A, AglA, from *Thermotoga maritima* is a typical GH4 enzyme, requiring NAD^+, Mn^{2+} and strongly reducing conditions for activity. This work presents the crystal structure of the apo-protein refined to 1.8 Å and of AglA complexed with NAD^+ and maltose refined to 1.9 Å resolution. They reveal that the NAD^+ molecule is bound to a typical Rossman-fold NAD-binding site, and that the nicotinamide moiety is localised close to the maltose substrate. Within the active site the conserved Cys174 and surrounding conserved histidines are positioned in a way suggesting a role in the hydrolysis reaction. Previous biochemical studies on AglA have shown that the purified protein has only a low level of activity which is quickly lost, but is partially re-attainable for a brief time through treatment with high concentrations of reductants. The electron density maps indicate that Cys174 is oxidized to a sulfinic acid. Most likely, the strongly reducing conditions are necessary to reduce this oxidised cysteine side chain. Notably, the canonical set of catalytic acidic residues common to other glucosidases is not present in the active site, suggesting an unusual mechanism of action for a glycoside-hydrolysing enzyme. Additionally, AglA displays no structural similarity to other characterized glycosyl hydrolases, but high structural homology to NAD-dependent dehydrogenases. From the results of this study it is proposed that family 4 represents a new structural clan of glycosyl hydrolases.