Characterization of (β/α)_4-half-barrels of a (β/α)_8-barrel β-glycosidase

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The (β/α)_8-barrel or TIM barrel is one of the most common folds found in enzymes. The (β/α)_8-barrels provide an excellent model to study the evolution of enzymes. Evidence suggests that a large fraction of the (β/α)_8-barrel enzymes evolved divergently by gene duplication and fusion of an ancestral (β/α)_4-barrel (half-barrel). To approach this evolutive process, we used as a model the β-glycosidase Sfβgly from Spodoptera frugiperda, which is folded as a (β/α)_8-barrel as all members of the Glycoside Hydrolases Family 1. The cDNA coding (β/α)_4-half-barrels corresponding to the Sfβgly halves were cloned and expressed in E. coli ArcticExpress(DE3) and purified by Ni^{2+} affinity chromatography. The (β/α)_4-half-barrels were able to hydrolyse methylumbelliferonyl β-glucoside. In the present study we demonstrated that (β/α)_4-half-barrels are undergoing an activation process when incubated for 48h at 30°C. The structure and association states of (β/α)_4-half-barrels have been investigated by fluorescence quenching, dynamic light scattering (DLS) and circular dichroism (CD). The CD spectra of the (β/α)_4-half-barrels are indicative of native secondary structure. Additionally CD measurements of (β/α)_4-half-barrel corresponding to the N-terminal portion of Sfβgly showed the absence of conformational change in the structure by the end of the 48h incubation period. The DLS showed that the (β/α)_4-half-barrels exist predominantly as monomers to 0h and 24h of incubation and correspond to dimers in the end of the 48h incubation period. Finally our results of fluorescence quenching are consistent with the data of DLS suggesting the association of (β/α)_4-half-barrel. Therefore data suggest that the activation process of the (β/α)_4-half-barrels is related to the formation of dimers.

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