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Title:	Creation of active TIM barrel enzymes through genetic fusion of half-barrel domain constructs derived from two distantly related glycosyl hydrolases
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Keywords:	Beta/alpha barrels Enzymes Chimera enzyme Engineering
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Citation:	FEBS Journal,283(23),pp. 4340-4356.
Abstract:	Diverse unrelated enzymes that adopt the beta/alpha (or TIM) barrel topology display similar arrangements of beta/alpha units placed in a radial eight-fold symmetry around the barrel's axis. The TIM barrel was originally thought to be a single structural domain; however, it is now thought that TIM barrels arose from duplication and fusion of smaller half-barrels consisting of four beta/alpha units. We describe here the design, expression and purification, as well as characterization of folding, activity and stability, of chimeras of two TIM barrel glycosyl hydrolases, made by fusing different half-barrel domains derived from an endoglucanase from <i>Clostridium cellulolyticum</i> , CelCCA and a beta-glucosidase from <i>Pyrococcus furiosus</i> , CelB. We show that after refolding following purification from inclusion bodies, the two half-barrel fusion chimeras (CelCCACelB and CelBCelCCA) display catalytic activity although they assemble into large soluble oligomeric aggregated species containing chains of mixed beta and alpha structure. CelBCelCCA displays hyperthermophile-like structural stability as well as significant stability to chemical denaturation (C_m of 2.6 M guanidinium hydrochloride), whereas CelCCACelB displays mesophile-like stability (T_m of $\sim 71^\circ\text{C}$). The endoglucanase activities of both chimeras are an order of magnitude lower than those of CelB or CelCCA, whereas the beta-glucosidase activity of CelBCelCCA is about two orders of magnitude lower than that of CelB. The chimera CelCCACelB shows no beta-glucosidase activity. Our results demonstrate that half-barrel domains from unrelated sources can fold, assemble and function, with scope for improvement.
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