Abstract
Cyclodextrins (CD) are cyclic oligosaccharides consisting of 6-8 glucose units (alpha,- beta- und gamma-CD), which are synthesized by glycosyl transferases from starch and other polysaccharides. For the production of cyclodextrins, which can complex guest molecules in their interior, thermostable CGTases are required. Along these lines, the CGTase from the thermoalkaliphile bacterium “Anaerobranca gottschalkii” was produced and characterised. Moreover, it was attempted to increase its stability by directed evolution.
The cgtase gene was isolated from genomic DNA by PCR, and heterologously expressed in “Escherichia coli”. Using different chromatographic procedures, the CGTase was enriched from the soluble fraction of the cell extract to about 95 % purity. The characterisation by hydrodynamic and spectroscopic methods showed that the enzyme is a monomer with native secondary elements and a well defined tertiary structure. The stability of the CGTase was investigated by differential scanning calorimetry and irreversible thermal inactivation. It was shown that the enzyme is stabilized extrinsically by its substrate starch and Ca(2+)-ions. A closer analysis of the results suggests that the inactivation of the CGTase does not require the previous denaturation of the protein and, as a consequence, might be due to the chemical modification of the active site. Steady-state enzyme kinetic measurements of the catalytic activity at various enzyme and substrate concentrations, temperatures and pH values showed that alpha-CD is the main initial reaction product, independent of the applied conditions. After prolonged incubation, however, comparable amounts of alpha- and beta-CD are found, which are much larger than the produced amounts of gamma-CD.
In order to thermally stabilise the CGTase, two gene banks were produced by error prone PCR. In each of the two banks, a mutagenised half of the cgtase gene was fused to a native half. The gene banks were searched for stabilised CGTases, using a newly established screening assay. Several CGTases were isolated and purified. Their characterisation showed, however, that all of them were less stable than wild type CGTase, for reasons unclear so far.