Conformational destabilization of Bacillus licheniformis α-amylase induced by lysine modification and calcium depletion

Abstract

Bacillus licheniformis α-amylase (BLA) was chemically modified using 100-fold molar excess of succinic anhydride over protein or 0.66 M potassium cyanate to obtain 42 % succinylated and 81 % carbamylated BLAs. Size and charge homogeneity of modified preparations was established by Sephacryl S-200 HR gel chromatography and polyacrylamide gel electrophoresis. Conformational alteration in these preparations was evident by the larger Stokes radii (3.40 nm for carbamylated and 3.34 nm for succinylated BLAs) compared to 2.43 nm obtained for native BLA. Urea denaturation results using mean residue ellipticity (MRE) as a probe also showed conformational destabilization based on the early start of transition as well as ΔGDH2O values obtained for both modified derivatives and Ca-depleted BLA. Decrease in ΔGDH2O value from 5,930 cal/mol (for native BLA) to 3,957 cal/mol (for succinylated BLA), 3,336 cal/mol (for carbamylated BLA) and 3,430 cal/mol for Ca-depleted BLA suggested reduced conformational stability upon modification of amino groups of BLA or depletion of calcium. Since both succinylation and carbamylation reactions abolish the positive charge on amino groups (both α- and ε- amino), the decrease in conformational stability can be ascribed to the disruption of salt bridges present in the protein which might have released the intrinsic calcium from its binding site.

Keyword: BLA; Calcium; Conformation; Lysine; Salt bridges; Stability; Urea