Reductive alkylation of lipase: experimental and molecular modeling approaches

ABSTRACT

Candida rugosa lipase was modified via reductive alkylation to increase its hydrophobicity to work better in organic solvents. The free amino group of lysines was alkylated using propionaldehyde with different degrees of modification obtained (49 and 86%). Far-ultraviolet circular dichroism (CD) spectroscopy of the lipase in aqueous solvent showed that such chemical modifications at the enzyme surface caused a loss in secondary and tertiary structure that is attributed to the enzyme unfolding. Using molecular modeling, we propose that in an aqueous environment the loss in protein structure of the modified lipase is owing to disruption of stabilizing salt bridges, particularly of surface lysines. Indeed, molecular modeling and simulation of a salt bridge formed by Lys-75 to Asp-79, in a nonpolar environment, suggests the adoption of a more flexible alkylated lysine that may explain higher lipase activity in organic solvents on alkylation.

Keyword: Candida rugosa; Circular dichroism; Enzyme modification; Lipase; Molecular modeling