Synopsis of Original Research Paper

Fundamental study of the function on endonuclease (DNase I) in human sweat

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Human and porcine recombinant deoxyribonucleases I (DNases I) were expressed in COS-7 cells, and purified by a single-step procedure. Since affinities for concanavalin A (Con A) and wheatgerm agglutinin (WGA) were strong in these recombinant DNases I, purification using Con A-WGA mixture-agarose column was performed. By this method, the enzymes in culture medium could quickly be isolated to apparent homogeneity in approximately ten minutes. From 1 ml of culture medium, about 20~30 μg of purified DNase I with a specific activity ranging from 22000 to 41000 units/mg were obtained. The purified DNases I were subjected to enzymatic deglycosylation by either Peptide N-glycosidase F (PNGase F) or endoglycosidase H (Endo H). The recombinant enzyme was cleaved by PNGase F, not by Endo H, indicating that the recombinant enzymes are modified by N-linked complex-type carbohydrate moieties. In the human recombinant DNase I, activity was decreased by PNGase F-treatment, while that of the porcine DNase I remained unaffected. The thermal stability of the human enzyme was extremely susceptible to heat following PNGase F-treatment, as was the porcine enzyme to a lesser extent. This study suggests that N-linked complex-type carbohydrate moieties may contribute to the enzymatic activity and/or thermal stability of recombinant DNases I. Moreover, we developed a method for the simultaneous genotyping of SNP (A2317G) and 56-bp VNTR polymorphism within DNase I gene, and using this method, we determined the allele frequencies of both polymorphisms in Ovambo, Turk, Mongolian, Korean and Japanese populations and revealed wide differences between these ethnic groups.