PURIFICATION AND CHARACTERIZATION OF HEMAGGLUTININ BY *CLOSTRIDIUM BOTULINUM* TYPE C STRAIN STOCKHOLM

Norihiko SUZUKI

Department of Biochemistry Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060, Japan

Clostridium botulinum type C hemagglutinin (HA) was purified approximately 2,000-fold from the culture supernatant in an overall yield of 32% by ammonium sulfate precipitation, DEAE-cellulose chromatography and gel-filtration on Sephacryl S-400. The isolated HA was homogeneous in disc electrophoresis and the double gel diffusion test, and it had a specific activity of 1.6×10^4 units with rat red blood cells per mg protein. The molecular weight of HA was calculated as 230,000 from sedimentation equilibrium analysis by ultracentrifugation. The HA dissociated into five components, 120kDa, 115kDa, 55kDa, 35kDa and 27kDa, in polyacrylamide gel electrophoresis with sodium dodecyl sulfate, and the molar ratios of the components were 1 : 1 : 2 : 3 : : 1 from high to low molecular weight. From these results, HA was supported to be composed of an equal amount of two kinds of proteins, one of which consists of four peptide of 120kDa, 55kDa and 35kDa (their molar ratios, 1 : 1 : 1 : 1 : 1, respectively), and the other of three peptides of 115kDa, 55kDa and 35kDa (their molar ratios, 1 : 1 : 2, respectively). HA could agglutinate human, horse rat, chicken, sheep and rabbit erythrocytes and the intestinal cells of rat but not their erythrocytes treated by neuraminidase. HA agglutination activity to horse erythrocytes was inhibited by gangliosides (GM_4, GM_3, GD_{1a}, $GD_1{}^b$, $GT_1{}^b$), NeuGc and fetuin. These results suggest that HA binds to sialic acid on the surface of erythrocytes and intestinal cells, and may play a role in the intestinal absorption of the toxin.