The Influence of N-glycosylation and C-terminal Sequence on Secretion of HBV Large Surface Antigen from *S. cerevisiae*

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In *S. cerevisiae*, we synthesized and secreted L-HBVsAg(pre-S::S) and three mutants, i.e. pre-S°°::S (N15Q and N123Q), pre-S°°::S° (N15Q, N123Q, and N320Q), and pre-S°°::S°° (N15Q, N123Q, N233Q, and N320Q). All of the secreted pre-S::S was N-glycosylated. In the secretion of pre-S°°::S and pre-S°°::S°, besides the hyper-mannosylated form, another immunoreactive protein with lower molecular mass was observed, which seems to be unglycosylated form of pre-S°°::S and pre-S°°::S°. Only a part of the secreted pre-S°°::S or pre-S°°::S° molecules was N-glycosylated, and the site for the partial N-glycosylation seems to be N233 in S-antigen region. Compared to the N-glycosylated pre-S°°::S and pre-S°°::S°, pre-S°°::S°° was secreted with lower efficiency but showed immunoreactivity to anti-S antigen monoclonal Ab. Interestingly, unlike pre-S°°::S°° with authentic C-terminus, the recombinant pre-S°°::S°° with C-terminal myc or poly-histidine tag was almost all aggregated into insoluble proteins in the intracellular region. Conclusively, the C-terminal sequence and glycosylation in S-antigen region seem to be of crucial importance in determining the secretion efficiency of L-HBVsAg in *S. cerevisiae*.