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Expression, purification and immunological properties of glycosylated influenza H5N1 hemagglutinin produced in pichia pastoris

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Background: It is well established that the virus hemagglutinin is the main antigen, inducing the neutralizing antibodies. The HA gene mutates however fast making the production of a new vaccine very challenging since the vaccine production lasts several months. In the attempt towards developing influenza vaccine production that would be faster and safer we used surface antigen alone. In this report, we describe the expression of HA gene of H5N1 avian influenza virus in simple Pichia pastoris system. rHA was produced in a soluble form with high yield and was shown to be highly immunogenic in animals.

Methods: The A/swan/Poland/305-135V08/2006 (H5N1- subtype) hemagglutinin (HA) gene was cloned and expressed in yeast Pichia pastoris (P. pastoris). The HA cDNA lacking the C-terminal transmembrane anchor-coding sequence was fused to α-factor leader peptide and placed under control of the methanol-inducible P. pastoris alcohol oxidase 1 (AOX1) promoter. Two P. pastoris strains: SMD 1168 and KM 71 were used for protein expression. rHA fusion protein with His-6 affinity tag was secreted into the culture medium and was purified to homogeneity in one step using Ni-NTA agarose. Glycosylation sites of rHA were determined using LC-MS-MS/MS (liquid chromatography coupled to tandem mass spectrometry). The immunological properties of rHA antigen were tested in vivo. Mice were immunized intradermally with three doses of rHA (1, 5 and 25 μg) given at 3-weekly intervals. The control group received at the same intervals adjuvant only. Chicken were immunized subcutaneously twice with 25 μg of rHA given at 4 weeks interval. After chicken and mice immunization sera samples were tested by ELISA.

Results: Recombinant HA antigen was secreted into the culture medium reaching the approximately 15 mg/ L (KM 71 strain). rHA was purified to homogeneity in one step affinity chromatography. SDS-PAGE and MS/MS analysis indicated that the protein is cleaved into HA1 and HA2 domains linked with disulphide bond. Analysis of the N-linked glycans revealed that the overexpressed HA is fully glycosylated at the same sites as the native HA in the vaccine strain. The immunological activity of hemagglutinin protein was tested in chicken and mice, where rHA elicited high immune response.

Conclusions: The data presented here demonstrate that influenza antigen produced in P. pastoris with good efficiency is highly immunogenic and might be consider as a candidate for subunit vaccine.

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