（第３号様式）(Form No. 3)

学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Structural and functional analyses of egg white protein ovalbumin-related protein X in chicken embryo
（鶏卵白タンパク質ovalbumin-related protein Xの胚発生における構造と機能の解析）

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There exist 148 kinds of proteins in chicken egg white. Egg white proteins play a role in serving amino acids for development of chick embryo. In addition, some egg white proteins have their own specific functions. Ovotransferrin (OTF), and lysozyme (LYZ) exert antimicrobial action to protect egg yolk from microbial infection. Ovomucoid and cystatin have protease inhibitory activity. Furthermore, for ovalbumin (OVA), OTF, and LYZ, the proteins were shown to translocate from egg white to embryo during incubation of fertilized egg. This finding suggests that those egg white proteins have implications for developing embryo. However, to date, no study has sought that an egg white protein is involved in embryo development.

Ovalbumin-related protein X (OVAX) is a 50kDa chicken egg white protein. Recently, OVAX was reported to interact with heparin, a negatively charged glycosaminoglycan. Heparin and its analog heparan sulfate (HS) play crucial roles in embryogenesis via binding to growth factors. These facts raise the possibility that OVAX participates in embryo development through binding to heparin and HS. In this study, structural and functional properties of OVAX in embryonated egg were investigated to elucidate the involvement of OVAX in embryo development.

Firstly, migration and localization of egg white OVAX in embryonated egg were explored using an anti-OVAX antibody. Western blotting of egg yolks at different stages showed that egg white OVAX migrated into egg yolk during incubation of fertilized egg. Immunohistochemical analysis of embryo resided in 10day-incubated eggs showed that OVAX existed in almost all tissues of the embryo. These suggest that OVAX is incorporated from egg white into the embryo through egg yolk.

Secondly, heparin-binding affinity of OVAX was investigated by the monitoring the changes of heparin-binding affinity of the protein in egg white during incubation of egg at 37°C. Heparin sepharose chromatography and isothermal titrating calorimetry using fondaparinux (a low molecular weight heparin) as a ligand showed that there exist four OVAX types that differ in heparin-binding affinity (in descending order, $OVAX_4$, $OVAX_3$, $OVAX_2$, and $OVAX_1$). Before incubation of egg, high heparin affinity-forms,

$OVAX_4$ and $OVAX_3$, were dominant OVAX forms in egg white. After incubation of egg for 10 days, $OVAX_2$ became the dominant OVAX in egg white. These results suggest that $OVAX_4$ is altered to a low heparin-affinity $OVAX_2$ via $OVAX_3$ during embryo development. Tryptophan fluorescence and circular dichroism analyses showed that the decrease in heparin-binding affinity is due to the change in the secondary structure of OVAX, accompanying the reduction in positive charge of the molecular surface. The spontaneous transformation of OVAX might be concerned with the control of functions of heparin and HS during embryogenesis.

HS regulates cartilage differentiation by interacting with a growth factor bone morphogenetic protein 2 (BMP2) that is a potent promoter of cartilage differentiation during embryo development. The participation of OVAX in the regulation of BMP2 activity during chondrogenesis was investigated. Analysis with micromass culture system, which supports chondrocyte differentiation, showed that $OVAX_4$ enhanced the chondrogenic activity of BMP2 by increasing in the level of BMP2-mediated smad phosphorylation. On the other hand, $OVAX_2$ did not affect the BMP2 activity. These results suggest that high heparin-binding affinity of the dominant OVAX form ($OVAX_4$) in the early stage of egg incubation is crucial for enhancing chondrogenic activity of BMP2.

In conclusion, chicken egg white protein OVAX was incorporated into embryo during embryo development, but its heparin-binding affinity decreased as embryo development proceeds. The high-heparin-affinity form $OVAX_4$ which was dominant in the early stage of embryo development enhanced the chondrogenic activity of growth factor BMP2, while the low-heparin affinity form $OVAX_2$ occurring in the later developmental stage did not enhance the BMP2 activity. These results suggest that the alternation in heparin-binding affinity of OVAX during embryogenesis modulates the BMP2 activity. These findings provide a new insight that egg white proteins are involved in organogenesis during embryonic development.