The Vitellin Protease in Developing Embryos of the Silkworm, *Bombyx mori*

Motoko IKEDA and Okitsugu YAMASHITA

Laboratory of Sericultural Science, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya 464-01, Japan

In the egg of the silkworm, *Bombyx mori*, yolk proteins are composed of three major proteins, vitellin (Vtn, 40%), egg-specific protein (ESP, 25%) and 30 kDa proteins (30k proteins, 35%) (Zhu *et al.*, 1986). Each yolk protein is selectively used at the different stage of embryogenesis (Indrasith *et al.*, 1987; Zhu *et al.*, 1986). ESP is markedly degraded at the late middle stage of embryogenesis and completely exhausted before larval hatching. Vtn degradation occurs at the late stage of embryogenesis when most ESP disappears, whereas 30k proteins remains unused throughout embryogenesis.

The degradation of ESP has been demonstrated to be catalyzed by a protease, ESP protease, which appears at the late middle stage of embryogenesis (Indrasith *et al.*, 1988). The ESP protease specifically hydrolyzes ESP, but does not hydrolyze Vtn and 30k proteins. It is thus expected that there are proteases responsible for Vtn and 30k protein degradation.

In the previous report, we suggested that two species of proteases, both of which simultaneously appeared at the late stage of embryogenesis, were responsible for Vtn degradation (Ikeda *et al.*, 1990; Yamashita and Ikeda 1991). We purified and characterized these proteases. The one had a molecular mass of 30 kDa (30k protease) and the other 24 kDa (24k protease). These two proteases showed similar catalytic properties and attacked not only Vtn but also ESP. However, it is likely that these two proteases participate in the hydrolysis of only Vtn, since ESP is exhausted almost completely before appearance of these proteases. We called these two proteases, 30k and 24k proteases, as Vtn protease.

Studies on primary structure and biosynthetic mechanism of the Vtn protease demonstrated the molecular relationship between the 30k and 24k proteases (Ikeda *et al.*, 1991b). The NH_2-terminal amino acid sequence of 24k protease coincided with the internal sequence of 30k protease, indicating the 24k protease to be provided by autocatalysis of the 30k protease molecule (Ikeda *et al.*, 1991b; Yamashita and Ikeda, 1991).

Physiological properties of the Vtn protease are not well understood. In this report, to give information concerning the physiological function of the Vtn protease, we surveyed the developmental profile and tissue localization of the protease by using the antiserum raised against the purified Vtn protease. The results showed that the Vtn protease was localized in the midgut cells of the embryo on day 8 and 9 (1 day before larval hatching). These results strongly suggest that the Vtn protease participates in the degradation of Vtn in embryo.

By western blot technique, the anti-30k protease antiserum, which was generated by immunizing a mouse with the purified 30k protease, reacted with the 24k protease as well as the 30k protease, and the anti-24k protease antiserum with the 30k protease as well as the 24k protease (Ikeda *et al.*, 1991a, b). Comparison between the anti-30k and anti-24k protease antisera the higher reactivity was found in the anti-24k protease antiserum. The anti-24k protease antiserum was used throughout this experiment.

Developmental changes of the Vtn protease protein were surveyed by western blot technique. No signal for the protease protein was detected in embryos from day 0 to 7. Two Vtn protease proteins were detected on day 8 and 9, and abruptly disappeared on day 10, that was the day of larval hatching (Ikeda *et al.*, 1991a). This profile is well correlated with the developmental changes of enzymatic activities assayed in egg homogenates (Ikeda *et al.*, 1990). Throughout the larval development, the Vtn protease protein was not detected in digestive juice, gut, blood and remaining carcass, indicating that the Vtn protease is completely missing in the larvae. In the pupae, no signal of the protease protein was found until day 4 of pupal development, whereas from day 5 to 7 two polypeptides with the molecular mass of 24 and 22 kDa were clearly stained with the antiserum. The presence of these two polypeptides were restricted in the midgut (Ikeda *et al.*, 1991a). In the

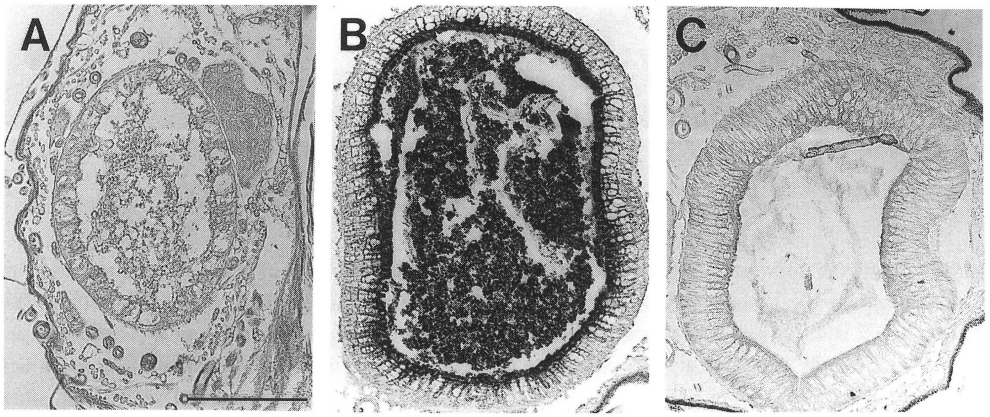

Fig. 1 Localization of the vitellin protease in developing embryos of silkworms. Histological sections were prepared from eggs of day 7 (A), day 9 (B) and day 10 (C), and stained with the anti-24k protease antiserum (Ikeda *et al.*, 1991a). The midgut of embryo on day 9 was stained with the antiserum, but not on day 7 and 10. Scale =100μm.

midgut homogenate from the pupae with the 24 and 22 kDa polypeptides, the Vtn protease activities were found (unpublished data). At present it is not known if the proteases found in the pupae are the products of gene encoding Vtn protease.

To see the tissue localization of Vtn protease in embryos, protease proteins were immunohistochemically stained in sections prepared from embryos at various stages. Positive staining was found in embryo on day 8 and 9, but did not found from day 0 to 7 and on day 10. By microscopic analysis, the midgut of embryo on day 8 and 9 was specifically stained with the antiserum (Fig. 1). The limited localization of the Vtn protease suggests that the enzyme is synthesized in the midgut cells and secreted into the lumen (Ikeda *et al.*, 1991a). The primary structure of Vtn protease supports that the protease is synthesized as a precursor containing a signal peptide (Ikeda *et al.*, 1991b).

The embryo of day 9 which exists the Vtn protease is characterized by the completion of midgut formation (Miya, 1976), and midgut cells come to absorb the nutrients stored in the lumen (Takeuchi, 1955). Immunohistochemical observation showed that the most Vtn was incorporated into the lumen before the completion of midgut formation (Izuhara and Yamashita, unpublished data). Taking into account the fact that the activity of Vtn protease, which is localized in the midgut, appears temporarily on day 8 and 9, it is probable that Vtn incorporated into the lumen is hydrolyzed by the Vtn protease secreted from the midgut cells (Ikeda *et al.*, 1991a).

Acknowledgments: We thank Drs Kobayashi and Yaginuma of our laboratory for their encouragement. The present study was partially supported by Grant-in-Aid for Encouragement of Young Scientists and Grant-in-Aid for Co-operative Research (A) (No. 02304020) from the Ministry of Education, Science and Culture, Japan.

References

Ikeda, M., T. Sasaki and O. Yamashita (1990) *Insect Biochem.*, **20**, 725-734.
Ikeda, M., T. Yaginuma and O. Yamashita (1991a) *J. Seric. Sci. Jpn.*, **60**, 178-185.
Ikeda, M., T. Yaginuma, M. Kobayashi and O. Yamashita (1991b) *Comp. Biochem. Physiol.*, **99B**, 405-411.
Indrasith, L. S., T. Furusawa, M. Shikata and O. Yamashita (1987) *Insect Biochem.*, **17**, 539-545.
Indrasith, L. S., T. Sasaki and O. Yamashita (1988) *J. Biol. Chem.*, **263**, 1045-1051.

Miya, K. (1976) *J. Fac. Agric. Iwate Univ.*, **13**, 95-122.
Takeuchi, K. (1955) *J. Seric. Sci. Jpn.*, **24**, 259-263.
Yamashita, O. and M. Ikeda (1991) *Proc. Arthropod. Embryol. Soc. Jpn.*, **(26)**, 23-25.
Zhu, J., L. S. Indrasith and O. Yamashita (1986) *Biochim. Biophys. Acta*, **882**, 427-436.