Degradation and Utilization of Yolk Proteins in the Egg of Silkworm, *Bombyx mori*

Okitsugu YAMASHITA and Motoko IKEDA

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

The yolk proteins are the maternally provided resources not only for the embryogenesis but also for the newly hatched larvae prior to the first meal (Kunkel and Nordin, 1985). Thus, it seems likely that the utilization of yolk proteins takes place coordinating with the developmental events of embryos and hatchlings. There are several studies on the biochemical mechanisms of yolk protein degradation during embryogenesis of several invertebrates (Yamashita and Indrasith, 1988). A particular feature of yolk protein degradation is selective and limited proteolysis which is catalyzed by the protease emerging during embryogenesis (Nordin *et al.*, 1990). In many cases, the general proteases are conceived to be responsible for yolk protein degradation, and to be stored as latent precursors in the egg until they are activated at the definite stage of embryogenesis (Faggoto, 1990; Kageyama and Takahashi, 1990; Medina *et al.*, 1988; Perona *et al.*, 1988). In contrast to this situation, silkworm eggs develop the unique system for degrading yolk proteins in the course of embryogenesis (Ikeda *et al.*, 1990; Indrasith *et al.*, 1988a). In this report, we summarize our recent studies on the proteases which are specialized for degradation of each yolk protein in silkworm eggs.

Protease for egg specific protein

In the eggs of the silkworm, *Bombyx mori*, yolk proteins are mainly composed of three proteins, vitellin (40%), 30 kDa protein (35%) and egg-specific protein (ESP; 25%) (Zhu *et al.*, 1986). ESP is selectively hydrolyzed at the middle to late stage of embryogenesis, and completely consumed before larval hatching (Indrasith *et al.*, 1987; Zhu *et al.*, 1986). ESP was purified from mature eggs and used as the substrate for detection of the protease which attacks ESP. A unique protease was identified in the egg extracts prepared at the late stage of embryogenesis, purified and characterized (Indrasith *et al.*, 1988a). The purified protease had a molecular mass of 28,000 Da and the optimum pH was 8 to 9. Substrate specificity to synthetic peptides and inhibitor spectrum suggest that this protease belongs to trypsin-like serine protease. The protease selectively hydrolyzed ESP, but not vitellin and 30 kDa proteins. Therefore, this protease is concluded to be responsible for the selective degradation of ESP in the egg, hence it was called ESP protease (Indrasith *et al.*, 1988a).

ESP was converted to three polypeptides by the action of ESP protease through two step hydrolysis; 8.7 kDa, 17.2 kDa and 36 kDa polypeptides (Indrasith *et al.*, 1988a). By the alignment of the NH_2-terminal amino acid sequence of these polypeptides to the primary structure of ESP which was deduced from nucleotide sequence of ESP cDNA, the cleavage sites of ESP by ESP protease, Lys^{114}-Asn^{115} and Arg^{210}-Asp^{211}, were deduced (Inagaki and Yamashita, 1989; Indrasith *et al.*, 1988a).

The activity of ESP protease became detectable at the middle stage of embryogenesis and increased until two days before larval hatching (Indrasith *et al.*, 1988a). This developmental change was closely correlated with translation activity of mRNA encoding ESP protease (Indrasith *et al.*, 1988b). Consequently, protease activity depends on the extent to which protease protein is newly synthesized, not on the activation of pre-existing inactive precursors.

Vitellin protease

The marked vitellin degradation occurred at the late stage of embryogenesis when ESP almost disappeared (Indrasith *et al.*, 1987; Zhu *et al.*, 1986). To search a protease which catalyzes degradation of vitellin, protease activity was analyzed through embryogenesis using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) including casein as a substrate (Ikeda *et al.*, 1990). Two protease bands were detected on the gel at the late stage of embryogenesis. Two proteases were separately purified from the egg and characterized (Ikeda *et al.*, 1990). One had a molecular mass of 30,000 Da (30k protease), and the other of 24,000 Da (24k

protease). Their NH_2-terminal amino acid sequences which were determined by the Edman method were completely different from one another. However, they showed similar enzymatic characters with each other. From their reactivity to protease inhibitors and synthetic substrates they are grouped into the trypsin-like serine protease. These proteases hydrolyzed ESP in addition to vitellin but not 30 kDa proteins. It was noteworthy that each protease preferentially hydrolyzed a heavy subunit (178 kDa polypeptide) of vitellin, but not a small subunit (42 kDa polypeptide). The several degradation products of vitellin by incubation with each protease were also identified in extracts of eggs at late stage of embryogenesis. Further, the appearance of these protease activities was closely correlated with degradation of the mass of vitellin not with ESP and 30 kDa proteins. Therefore, the 30k protease and the 24k protease are involved tightly in the vitellin degradation, so that we designated these proteases as the vitellin protease (Ikeda *et al.*, 1990).

The immunohistochemical observation showed that these proteases were only localized in the epithelial cells and contents of midgut at the late stages of embryogenesis (Ikeda *et al.*, 1991a). Correlating with the appearance of vitellin protease, vitellin is ingested into midgut of embryos (Izuhara and Yamashita, unpublished data). Thus, these enzymes are produced in the process of midgut cell differentiation for the digestion and absorption of vitellin.

cDNA library constructed against mRNA from the eggs exhibiting high protease activity was immunologically screened using an antiserum raised against the purified 24k protease and cloned, and cloned cDNA was sequenced (Ikeda *et al.*, 1991b). The amino acid sequence deduced from the nucleotide sequence was shown to be highly homologous to trypsin-like proteases from several animals. From the amino acid sequence it was implied that the vitellin protease is synthesized as a precursor form consisting of a signal peptide, an activation peptide and an active peptide (Fig. 1). The active peptide is decided to be the 30k protease, because the NH_2-terminal amino acid sequence of the 30k protease completely matched with that of the active peptide. Depending upon the post-translational processing, the precursor gives rise to the 30k protease by releasing the signal peptide and the activation peptide. The NH_2-terminal sequence of the 24k protease agreed with a sequence fragment which localized inside the sequence of the 30k protease, indicating that the 24k protease is provided by autocatalysis of the 30k protease through a single hydrolysis between Arg^{89} and Asn^{90} of the 30k protease.

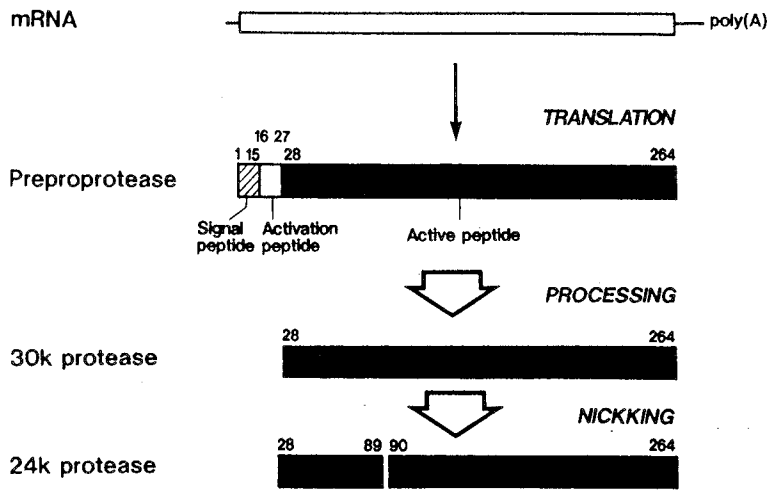

Fig. 1 Biosynthetic process of the vitellin protease. Vitellin-protease mRNA is translated to preproprotease which comprises 264 amino acids and consists of a signal peptide (1-15), an activation peptide (16-27) and an active peptide (28-264). By post-translational processing, the 30k protease is formed. The 30k protease is then nicked between Arg^{89} and Asp^{90} by autocatalysis, thereby the 24k protease is formed. The 24k protease is a dimer consisting of the 24 kDa polypeptide (90-264) and the polypeptide with a smaller molecular mass (28-89) (Ikeda *et al.*, 1991b).

Since the catalytic properties and specific activity of both proteases were similar, at present we can not expect a new function of 24k protease other than that of 30k protease (Ikeda *et al.*, 1990).

Northern blot analysis using the cDNA as a probe revealed that 1.3 kb mRNA encodes vitellin protease, and developmental change of the quantity of 1.3 kb mRNA was closely correlated with that of enzyme activity (Ikeda *et al.*, 1991b). Therefore, it is revealed that the 30k protease and the 24k protease are *de novo* synthesized at late stage of embryogenesis and show biological activity immediately after production (Ikeda *et al.*, 1991b).

Concluding remarks

The ESP protease and the vitellin protease are sequentially expressed from middle to late stage of embryogenesis, and are involved in the selective hydrolysis of ESP and vitellin, respectively. Since these proteases are not able to hydrolyze 30 kDa proteins which are the second major yolk proteins in silkworm eggs, other proteases should be required for the degradation of 30 kDa proteins. It remains to be clarified whether such other protease is newly induced in eggs which are ready to larval hatching. Nevertheless, our results on silkworm eggs provide the evidence that yolk proteins are sequentially utilized by the developing embryo through the expression of the genes encoding the specific protease. The programmed utilization of yolk proteins expand our understanding on the biochemical relationship between embryogenesis and yolk depletions.

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