Nucleotide sequence of the cDNA encoding silk gland elongation factor 1β′

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Eukaryotic elongation factor 1 (EF-1) catalyzes the GTP-dependent binding of aminoacyl-tRNA to 80S ribosomes. Silk gland EF-1 consists of four subunits designated as EF-1α, EF-1γ, EF-1β and EF-1β′, which correspond to EF-1α, EF-1γ, EF-1β and EF-1β′ in Artemia salina, respectively, from their order of molecular weight (1–3). EF-1α forms a ternary complex with aminoacyl-tRNA and GTP, while EF-1β′γ catalyzes the GDP/GTP exchange on EF-1α, and stimulates the binding of aminoacyl-tRNA to ribosomes.

Recently, it was demonstrated that phosphorylation of serine residue at position 89 in Artemia salina EF-1β by an endogenous casein kinase II affects the GDP/GTP exchange rate on EF-1α (4). We also found that silk gland EF-1β and EF-1β′ were phosphorylated in vivo and in vitro. To elucidate the structure of insect EF-1, we isolate and sequenced cDNA clone of silk gland EF-1β′.

The first 32 amino acids of purified silk gland EF-1β′ were sequenced with an Applied Biosystem Model 473A protein sequanator. Based on the sequence, the mixed oligonucleotide primers 5'-GCNGTGGNGAYGTAARACNGNCARGGG (SN-1), (N = A/G/C/T; R = A/G; Y=C/T), which corresponds to the N-terminal sequence 2–11(AVGDVKTAQG), was synthesized with an Applied Biosystem Model 381A DNA synthesizer. The other primer 5'-CNGTTCRCRTCCCTCC-ANGTCT (HM-1), which corresponds to the consensus sequence of human and Artemia salina EF-1β 147–153 (KPWDDDET), was also synthesized.

Poly(A)+ RNA was isolated from the posterior silk gland of Bombyx mori at 3rd days of the 5th instar and the first strand cDNA was synthesized using the cDNA synthesis kit (Amersham Corp.). Using the first strand cDNA as the template, the SN-1 and HM-1 as the primers, PCR was performed under the following conditions; 2 min at 93°C, 1 min at 94°C, 2 min at 55°C and 1 min at 72°C for 25 cycles followed by an extension for 5 min at 72°C. A product of 456 bp was subcloned into a T-vector (5), then used as the probe to screen a silk gland cDNA sequanator (Figure 1). Silk gland EF-1β′ shows 59% sequence identity with human EF-1β and 60% with Artemia salina EF-1β. The C-terminal half of silk gland EF-1β′ (residues 130–222) shows higher homology with those of human (73%) and Artemia salina EF-1β (75%). Silk gland EF-1β′ contains serine residue to be phosphorylated by casein kinase II (residues 99–109, DLFGSDDEED), which does not exist in rice and wheat EF-1′ (7, 8). It appeared that EF-1β′ is a highly conserved protein in evolution.

REFERENCES


Figure 1. Comparison of the amino acid sequences. The sequence presented are: Human 1β, EF-1β from human (8, 9); Artemia 1β, EF-1β from Artemia salina (10). Gaps introduced to facilitate alignment are presented with dashes. The N-terminal sequence obtained from the purified EF-1β′ is underlined.