Serological relationship between inclusion body proteins and a virus enhancing factor of an entomopoxvirus

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Abstract
An enhancing factor (EF) from *Pseudaleatoria separata* entomopoxvirus was serologically related to the fusolin but unrelated to the spheroidin. The spheroid contained a protein serologically related to the EF and the fusolin. The EF consisted of a protein and a polysaccharide.

Key words: Enhancing factor, fusolin, spheroidin, monoclonal antibody, *Pseudaleatoria separata* entomopoxvirus

INTRODUCTION

Entomopoxvirus (EPV) infection is characterized by the formation of two types of cytoplasmic inclusions, spindle and spheroid, in infected cells. The spindle consists of a crystalline protein called fusolin, while the spheroid occludes many virions within a matrix protein called spheroidin (Arif, 1995).

Xu and Hukuhara (1992) have shown that the spheroid of *Pseudaleatoria separata* EPV contains a virus enhancing factor (EF) which increases the infectivity of a nuclear polyhedrosis virus. Hukuhara et al. (1995) have demonstrated the presence of the EF in the spindle and in the occluded virion within the spheroid by means of immuno-electron microscopy. Hayakawa et al. (1996) have suggested the identity of the EF with the fusolin because of the similarity of an EF gene to several fusolin genes.

Xu and Hukuhara (1994) have suggested that the EF is a glycoprotein on the basis of its stainability with the periodic acid-Schiff reagent. Hayakawa et al. (1996) have shown that the amino-acid sequence of the EF, deduced from the gene sequence, has a possible site for N-linked glycosylation (Asn-X-Thr).

The present study was initiated to characterize the biochemical properties of the EF. We have demonstrated that it is a glycoprotein serologically related to the fusolin of PsEPV.

MATERIALS AND METHODS

The origin of PsEPV and the method of propagating the virus have been described previously (Hukuhara et al., 1990). Inclusion bodies of PsEPV were purified according to Xu and Hukuhara (1992) with a modification: the inclusion bodies were further purified by incorporating a step of density-gradient centrifugation in sucrose (65%, w/w). The EF was purified as previously described (Xu and Hukuhara, 1994).

The EF and EPV inclusion body proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) in 12% slab gels (Laemmli, 1970) under reduced conditions and stained with silver (Merril et al., 1981). The proteins separated by SDS-PAGE were transferred onto a polyvinylidene difluoride (PVDF) membrane at 100-200 mA for 2-3 h at 4°C (Towbin et al., 1979). Spheroidin and fusolin were recovered from 100- and 47-kDa bands in SDS-PAGE gel, respectively. Polyclonal antibodies were prepared in mice by intraperitoneal injection of these antigens. A mixture of 50 μg of each antigen and complete Freund's adjuvant was employed for the first booster. The sub-

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sequent immunizations were carried out once a week using 30, 30 or 20 µg of the antigen mixed with incomplete Freund's adjuvant. Anti-EF monoclonal antibodies (IgG type) were prepared from hybridoma clones, one of which was described previously (Hukuhara et al., 1995). Other clones were established in a similar manner.

For glycoprotein analysis, 19 µg of a protein sample was heated for 5 min at 100°C in 0.5% SDS and incubated overnight at 37°C in 0.1 ml of 0.2 M sodium phosphate buffer (pH 7.2) containing 24 U/ml N-glycosidase F (Boehringer), 0.1% SDS, 25 mM EDTA, 2% octylglucoside and 1% β-mercaptoethanol. Glycoproteins in PVDF membranes were detected with a G.P. Sensor Set (Honen, Tokyo). The sugar chains in the glycoproteins were oxidized with NaIO₄, biotinylated and combined with peroxidase-conjugated avidin, which was made visible with the use of a POD (peroxidase) Immunostain Set (Wako, Osaka).

For immunoblotting, the PVDF membrane was soaked overnight in 1% gelatin in PBS, rinsed three times with 0.05% Triton X-100 in PBS, and incubated for 1 h with an anti-EF monoclonal antibody (10 µg/ml) or an antispheroidin or -fusolin polyclonal antiserum. After three washings, the PVDF membrane was incubated for 1 h in 0.02% peroxidase-conjugated goat antibody against mouse IgG (TAGO) and, after at least five washings, analysed with the use of a POD Immunostain Set.

RESULTS AND DISCUSSION

SDS-PAGE of the EF and EPV inclusions yielded the following bands: major 38- and 34-kDa protein bands in an EF preparation, a major 100-kDa spheroidin band and minor 64-, 47-, 43-, 30-, 27-, 19-kDa bands in a spheroid preparation, and a major 47-kDa fusolin band in a spindle preparation (Fig. 1).

In immunoblotting experiments, anti-EF monoclonal antibodies from 4 hybridoma clones detected the major 38- and 34-kDa bands in the EF preparation (Fig. 2, left). An anti-fusolin serum revealed these two bands in the EF preparation and a 47-kDa band in the spheroid and spindle preparations (Fig. 3, right). An anti-spheroidin serum did not reveal these bands in the EF or spindle preparations (Fig. 3, left), but revealed 100-, 43-, 19-kDa bands in the spheroid preparation.

The results indicated that the EF was serologically related to the fusolin but unrelated to the spheroidin, and that the spheroid contained a 47-kDa protein serologically related to the EF and the fusolin. Presumably, the protein was derived from occluded virions, which had been shown to contain the EF antigen (Hukuhara et al., 1995).

The EF and fusolin bands and the 47-kDa

![Fig. 1. SDS-PAGE electropherogram of the EF and EPV inclusions. Lane 1: EF (2 µg); lane 2: spheroid (17.5 µg); lane 3: spindle (2 µg); lane 4: ovalbumin (1 µg); lane 5: bovine serum albumin (1 µg).]
Fig. 2. Immunoblotting of glycosidase-treated (T) or untreated (U) EF with monoclonal anti-EF sera (left) and the results of detection of sugar-carbohydrates (right).

Fig. 3. Immunoblotting of the EF and EPV inclusions with polyclonal anti-spheroidin (left) and anti-fusolin sera (right). Lane 1: EF (2 µg); lane 2: spheroid (17.5 µg); lane 3: spindle (2 µg).

band in the spheroid preparation exhibited positive reaction for sugar-carbohydrate (Fig. 4). When the EF preparation was treated with a glycosidase, the positive reaction of the two bands disappeared (Fig. 2, right). Immunoblotting of the same preparation with anti-EF monoclonal antibodies revealed two bands which were 2 kDa less in molecular mass than those detected in an untreated preparation (Fig. 2, left).

The results indicated that the EF consisted of a protein and an N-linked polysaccharide and that the anti-EF monoclonal antibodies recognized the protein moiety of the EF. Xu and Hukuhara (1994) concluded that the EF was derived through specific proteolytic cleavage from 55-kDa spheroid protein, which appears homologous to the 47-kDa protein detected in our study. The discrepancy in the molecular mass may be ascribed to different SDS-PAGE conditions that affect the degree of proteolysis and/or the mobility. The 34-kDa and smaller proteins in our EF preparation may also be the products of proteolysis.

The sequence analysis of the EF gene predicted a 2.4-kDa signal peptide and a 37.9-kDa truncated mature form of the EF (Hayakawa et al., 1996). The latter value corresponded well to the molecular mass of the EF as determined by SDS-PAGE (Xu and Hukuhara, 1992). However, we showed that the mass was
reduced by 2 kDa when the polysaccharide moiety was deleted. Further study is necessary to determine the effect of the polysaccharide moiety on the mobility of the EF in SDS-PAGE.

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REFERENCES


