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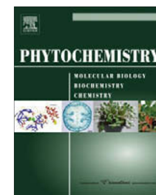

A unique latex protein, MLX56, defends mulberry trees from insects

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ABSTRACT

The mulberry (*Morus* spp.)-silkworm (*Bombyx mori*) relationship has been a well-known plant–herbivore interaction for thousands of years. Recently, we found that mulberry leaves defend against insect herbivory by latex ingredients. Here we report that a 56-kDa (394 amino acid) defense protein in mulberry latex designated mulatexin (MLX56) with an extensin domain, two hevein-like chitin-binding domains, and an inactive chitinase-like domain provides mulberry trees with strong insect resistance. MLX56 is toxic to lepidopteran caterpillars, including the cabbage armyworm, *Mamestra brassicae* and the Eri silkworm, *Samia ricini*, at 0.01% concentration in a wet diet, suggesting that MLX56 is applicable for plant protection. MLX56 is highly resistant to protease digestion, and has a strong chitin-binding activity. Interestingly, MLX56 showed no toxicity to *B. mori*, suggesting that the mulberry specialist has developed adaptation to the mulberry defense. Our results show that defensive proteins in plant latex play key roles in mulberry–insect interactions, and probably also in other plant–insect interactions. Our results further suggest that plant latexes analogous to animal venom contain a treasury of applicable defense proteins and chemicals that has evolved through inter-specific interactions.

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1. Introduction

Mulberry trees (genus *Morus*) are widely distributed in Asia, Europe, North and South America, and Africa, and are economically important plants that have been used for sericulture for thousands of years. As a model system of plant–herbivore relationships, intensive studies to detect feeding stimulants from mulberry leaves have been done, and Hamamura's study on feeding stimulants for *Bombyx mori* published half a century ago (Hamamura, 1959) is regarded as a classic in studies of plant–insects relationships. However, little attention has been paid to the anti-herbivore defense mechanisms and insect-resistant ingredients of the mulberry trees, because the silkworm, *B. mori*, grows very well on its leaves. Recently we found that mulberry leaves are toxic and well-defended against generalist lepidopteran caterpillars not specialized in mulberry trees, such as the Eri silkworm, *Samia ricini* (Saturniidae) and the cabbage armyworm, *Mamestra brassicae* (Noctuidae), a serious polyphagous pest in the Palearctic region. We determined that the high density of insect-resistant components contained in mulberry latex (Fig. 1A, a sap exuded from damaged leaf vein) defends mulberry trees against insect herbivores (Konno et al., 2006). The latex contained a high concentration of sugar-mimic alkaloids, e.g., 1,4-dideoxy-1,4-imino-D-arabinitol and 1-deoxynojirimycin, and was toxic to *S. ricini* but not to the silkworm, *B mori*. Studies by us and others further established the toxic mechanisms of sugar-mimic alkaloids, their target enzymes (i.e., sucrase and trehalase) and physiological consequences in unadapted generalist insects, and the enzymatic adaptation in a mulberry specialist, *B. mori* (Hirayama et al., 2007; Daimon et al., 2008), to the sugar-mimic alkaloids. However, the sugar-mimic alkaloids accounted for only about half the total insect toxicity in mulberry latex, and latex further contained an equally important insect-toxic high-molecular-weight factor(s), presumably a defense protein(s) that exhibit(s) strong growth-inhibitory activity (Konno et al., 2006). In this study, we purified and characterized defense protein designated mulatexin (MLX56) with unique molecular structures and a strong toxicity to pest insects in low concentrations from mulberry latex.

2. Results

2.1. Purification of the anti-insect defense protein from the latex of mulberry trees

In order to purify and identify the insect-toxic proteins contained in mulberry latex, six crude fractions were made (Fig. 1B and C) by extracting proteins from the protein bands excised from native PAGE gels (Fig. 1B), and were subjected to initial bioassays. The bioassays using neonate larvae of *S. ricini* established that the

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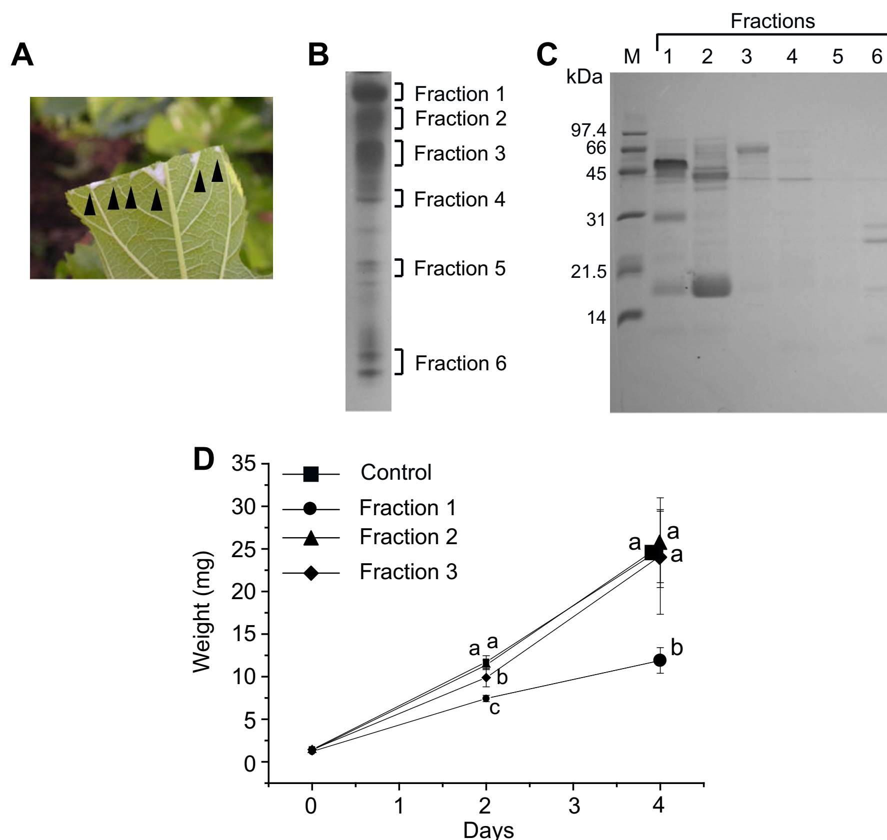

Fig. 1. Fractionation of proteins from the insect-toxic latex of mulberry trees. (A) Mulberry latex exuded from leaf laticifer. (B) Native PAGE profiles of the latex proteins of the mulberry tree. Fractions 1–6 were collected by cutting bands from the gel and then by elution. (C) SDS–PAGE analysis of fractions of latex proteins. (D) Insect toxicity tests of fractionated proteins against first-instar larvae of *S. ricini*. Larval weights were measured on days 2 and 4. Error bars indicate ±SD ($n = 5$). Values not followed by the same letters at the same point are significantly different ($P < 0.01$; Tukey's test for multiple comparisons).

fraction 1 solution had remarkable growth-inhibitory activity, whereas the other fractions did not (Fig. 1D) (fractions 4–6, data not shown). The proteins (56, 30, and 18 kDa; Fig. 1C) of fraction 1 were subjected to DEAE-Sepharose column chromatography for further purification (Fig. 2A), and a 56-kDa protein was successfully purified as a defense protein (Fig. 2B, lane 2). The relative toxicity, calculated from the amount of protein in solution and the bioassay using the Eri silkworm, increased 7.5 times during the whole purification processes, which is consistent with the SDS–PAGE data showing that the 56-kDa protein is one of the major proteins in mulberry latex (Figs. 1B and 2B). The 56-kDa protein, hereafter designated MLX56 (mulatexin), was strongly stained by Periodic acid-Schiff reagent, indicating that MLX56 is a glycoprotein (Fig. 2B, lane 3). The concentration of MLX56 in latex was

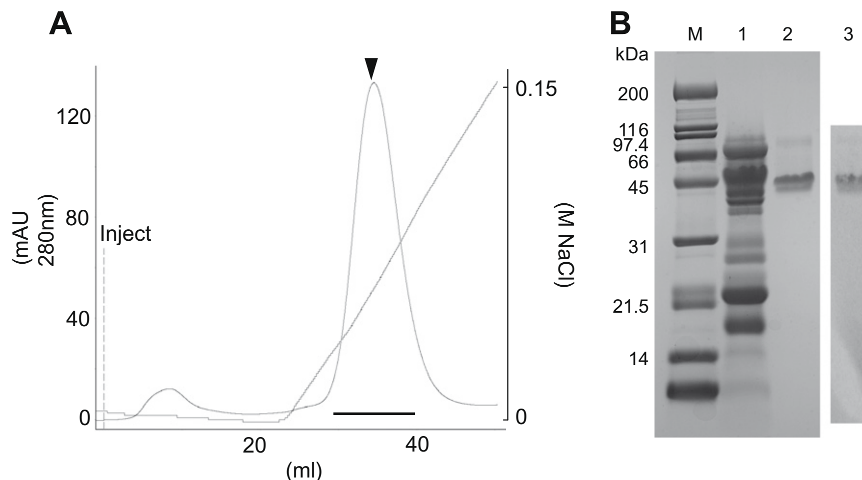

Fig. 2. Purification of the MLX56 protein from the insect-toxic fraction 1 proteins using DEAE-Sepharose column chromatography. (A) Chromatogram of fraction 1 proteins. The black bar indicates the fraction collected as purified MLX56 protein. (B) SDS–PAGE profiles of the purified MLX56 protein purified. Lane M, molecular marker; lane 1, total latex proteins; lane 2, MLX56 protein; lane 3, MLX56 stained by Periodic-Schiff reagent.

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