Major Biocontrol of Plant Tumors Targets tRNA Synthetase

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Infection of plants by pathogenic strains of *Agrobacterium tumefaciens* causes crown gall tumors with devastating economic consequences. The most successful bacterial biocontrol agent, nonpathogenic *A. radiobacter* strain K84, prevents disease by production of the "Trojan horse" toxin agrocin 84 (Fig. 1A) (*1*). Because it imitates a tumor-derived substrate [agrocinopine A (fig. S1)], agrocin 84 is specifically imported into *A. tumefaciens* strains that harbor certain types of tumor-inducing (Ti) plasmids. A toxic moiety is released from agrocin 84 (Fig. 1A) that inhibits the pathogen by an unknown mechanism (*2*). Agrocin 84 has a 9-(3′-deoxy-β-D-2,3-threopentafuranosyl) adenine nucleoside-like core linked to two substituents by phosphoramidate bonds (*1*). A 5′-phosphoramidate bond links the nucleoside-like core to a D-threo-2,3-dihydroxy-4-methylpentanamide, while a second phosphoramidate bond links a D-glucofuranosyloxyphosphoryl group to the adenine base and is the only known example of a 6N phosphoramidate bond found in nature (*3*). Although this moiety is required for the selective uptake of agrocin 84 into susceptible *A. tumefaciens* cells, it is not required for toxicity (*2*).

Plasmid pAgK84 in strain K84 contains the genes for agrocin 84 production and two immunity elements (*4*). The translation product of one of these immunity genes, *agnB2*, showed >40% sequence identity between its coding sequence and many leucyl-tRNA synthetases (LeuRSs). LeuRSs catalyze attachment of leucine to its cognate tRNAs in the first step of protein synthesis (aminoacylation). Aminoacylation assays showed the recombinant AgnB2 protein exhibits robust LeuRS activity (*5*). Importantly, the *agnB2* gene is not essential for growth (*6*). The structure of the toxic moiety of agrocin 84 is similar to that of leucyl-adenylate (Leu-AMP), a critical enzyme-bound reaction intermediate (Fig. 1A), having a relatively stable 5′-phosphoramidate bond instead of the labile phosphoanhydride linkage. Plausibly, the stable toxic moiety of agrocin 84 could impart its antibiotic effect on the bacteria by binding to the catalytic domain of the *A. tumefaciens* genomic-encoded LeuRS ($LeuRS_{At}$) as a Leu-AMP mimic.

Purified agrocin 84 showed pronounced inhibition of the agrocin-supersensitive strain NTL4(pTiC58Δ*accR*) in bioassays (fig. S2) (*5*). In contrast, the toxic moiety of agrocin 84 was inactive in this assay, because the sugar group needed for uptake was removed. The toxic moiety inhibited aminoacylation by purified $LeuRS_{At}$ [median inhibitory concentration (IC_{50}), <10 nM], whereas the complete agrocin 84 molecule did not (Fig. 1B). Neither *Escherichia coli* alanyl- nor isoleucyl-tRNA synthetases (IleRS) were inhibited (*7*). Cell-free extracts from NTL4(pTiC58Δ*accR*) and the resistant Ti-plasmidless strain NT1 were examined for LeuRS activity, after incubation of the cells in LB containing agrocin 84 (*5*). Extracts of the sensitive strain incubated with the antibiotic showed pronounced inhibition of $LeuRS_{At}$ activity compared to extracts from strain NT1, which cannot take up the antibiotic (Fig. 1C). In contrast, the activity of IleRS in either extract was not affected by growth with the antibiotic. Purified LeuRS encoded by the *agnB2* gene was far less sensitive to inhibition by the toxic moiety (IC_{50}, 9 μM) when compared to $LeuRS_{At}$ (IC_{50}, <10 nM) (Fig. 1B). This roughly 1000-fold difference in sensitivity of the two LeuRSs to the toxic moiety supports the hypothesis that the enzyme encoded by the *agnB2* gene is responsible for immunity to the inhibitor.

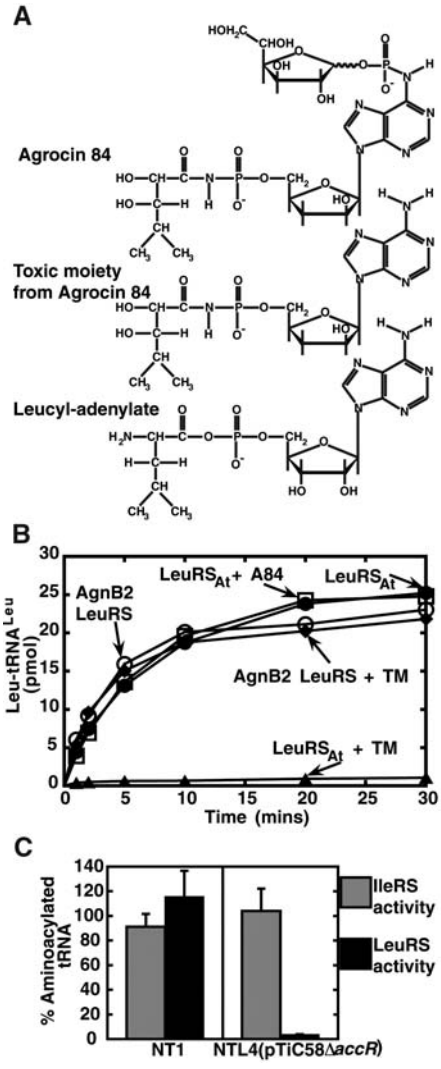

Fig. 1. (**A**) Structure of agrocin 84, the toxic moiety of agrocin 84, and Leu-AMP. (**B**) Effect of agrocin 84 (A84) and its toxic moiety (TM) on aminoacylation catalyzed by purified $LeuRS_{At}$. Aminoacylation by purified AgnB2 LeuRS in the presence or absence of the TM is also shown. (**C**) Comparison of inhibition of $LeuRS_{At}$ activity in cell-free extracts from resistant and susceptible *A. tumefaciens* strains grown with agrocin 84.

Biocontrol of crown gall tumors by agrocin 84 thus targets a tRNA synthetase in the pathogen. In turn, strain K84 carries a second, self-protective copy of the synthetase. In principle, this strategy from nature could be applied to other crop diseases by delivering pathogen-specific toxins with agents that protect the delivery vehicle.

References and Notes

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Supporting Online Material

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Materials and Methods
Fig. S1
References and Notes

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