Duplex structure of double-stranded RNA increases chemical stability of RNA interference biopesticides

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Abstract

RNA interference (RNAi) is a biological process in which double-stranded RNA (dsRNA) directs the degradation of homologous messenger RNA (mRNA), preventing the synthesis of essential proteins. In agriculture, several RNAi-based biotechnology products have been developed using dsRNA as active agents (i.e., dsRNA biopesticides) to protect crops from pests. The ongoing development of dsRNA biopesticides raises the importance of developing a fundamental understanding of the chemical stability of dsRNA molecules, which defines their ultimate potential to persist in biological and environmental systems in the absence of enzymes or microorganisms. In this study, we examined, for the first time, the specific physicochemical conditions that lead to dsRNA degradation by three mechanisms: alkaline hydrolysis, metal-catalyzed hydrolysis, and surface-catalyzed hydrolysis. We found that all three reactions were substantially slower for dsRNA relative to more conventionally studied single-stranded (ss)RNA. At environmentally relevant pH and metal concentrations, dissolved dsRNA was stable for timescales for days to months, indicating that solution-phase alkaline and metal-catalyzed hydrolysis are unlikely to contribute to dsRNA biopesticide degradation in soils or surface waters. However, surface-catalyzed hydrolysis led to the degradation of dsRNA adsorbed on environmentally relevant metal oxides on the timescale of hours. Overall, this finding suggests that dsRNA biopesticides in solution are unlikely to be degraded by abiotic hydrolysis in the environment, but dsRNA biopesticides adsorbed to particles may degrade by surface-catalyzed hydrolysis even in the absence of enzymes or microorganisms. Our results specifically challenge prior assumptions that ssRNA hydrolysis rates can be used to predict hydrolysis rates of dsRNA biopesticides and better define the environmental fate of these biopesticides.

Key words: RNA interference, biopesticides, plant-incorporated protectants, environmental fate