Regular article

日本食品化学学会誌、Vol. 32(2), 114-120 (2025)
Japanese Journal of Food Chemistry and Safety (JJFCS)

Heat-resistant RNAs in tea (*Camellia sinensis*) infusion

(Received November 6, 2024)
(Accepted April 13, 2025)

Manami Monobe, Kaori Ema, Hideki Horie

Iing Research Institute

Abstract

Some RNAs derived from plants involved in immune processes such as immune regulating functions and antiviral activities have heat-resistant properties, and tea (*Camellia sinensis*)-derived RNAs are one example. We examined the mechanism of heat stability of RNAs from tea infusions. Heat-resistant tea RNAs were found to be circular RNAs with guanine-cytosine contents of approximately 60%. The ratios of guanine/cytosine and adenine/uracil were 1.01 and 0.95, respectively. It is likely that these tea circular RNAs have higher-order structures such as a rod-like structures, because they were unstable to heat in the presence of urea.

Keywords : circular RNA, heat resistance, tea leaves, *Camellia sinensis*

I Introduction

RNA is more susceptible to hydrolysis than DNA due to the presence of a 2'-hydroxyl group (-OH) in its ribose sugar component. In a basic solution or high temperature environments, the 2'-hydroxyl group in the ribose sugar of RNA can interact with the phosphate group, which results in the breaking of the bonds through transesterification[1]. However, in recent years, heat-resistant RNAs with pharmacological activities, such as antiviral and immunostimulatory properties, have been found in plant decoctions[2-5]. Heat-resistant microRNA (MIR2911) from honeysuckle (*Lonicera japonica*) decoction showing the replication inhibitor activity against influenza H1N1 viruses and SARS-CoV-2[2, 3] and had high heat stability based on its specific sequence and high GC content[2]. Heat-resistant RNA from a *Glycyrrhizae* Radix decoction had immunostimulatory effects, such as the activation of nuclear factor kappa-light-chain-enhancer of activated B cells and the induction of tumor necrosis factor alpha (TNF-α) production on murine macrophages. Its length was 90 nucleotides, which is longer than microRNAs, and the mechanisms for heat stability remain to be clarified[4].

In the past, we also have discovered heat-resistant RNAs with immunostimulatory activity obtained from boiling water infusions of tea (*Camellia sinensis*)[5, 6]. These tea RNAs induced TNF-α production through Toll-like receptor 7 on human macrophage-like cells. The size of these RNAs was < 300 bp, as estimated by agarose gel electrophoresis. The tea RNAs were eluted at a level of approximately 1 mg from 2.5 g of dried tea leaves (one cup), and we noticed these RNAs were not easily hydrolyzed by autoclaving in this study. The tea RNAs seem extremely heat stable. However, the factors for their heat stability are still unknown.

It is known one of the heat-stable RNAs is a viroid RNA. Viroids are naked single-stranded covalently closed circular RNA molecules of 250–400 nucleotides and they are the smallest known plant pathogens[7-9]. Viroid RNAs have a high degree of self-complementarity and base-pairing resulting in the formation of twisted rod-like structures (Fig. 1a)[7-9]. Heat

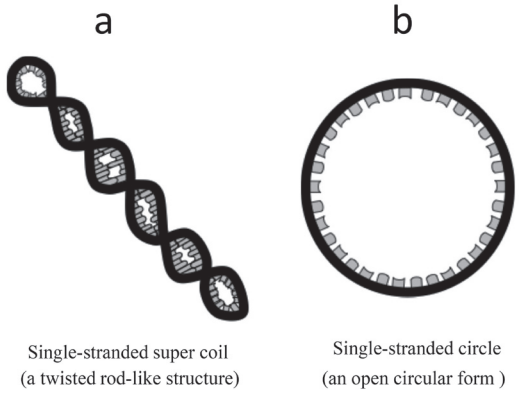

Fig. 1. Illustration of heat-stable tea RNA potential structures

Corresponding author: Manami Monobe, Iing Research Institute,
3451-71, Kanaya-Shishidoi, Shimada, Shizuoka 428-0039, Japan