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A comparison of the counteracting effects of glycine betaine and TMAO on the activity of RNase A in aqueous urea solution

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Abstract

Trimethylamine-*N*-oxide (TMAO) and glycine betaine are counteracting osmolytes found in cellular systems under osmotic stress, often in association with high urea concentrations. TMAO is a characteristic component of cartilaginous fish and marine molluscs, while glycine betaine is more widely distributed, occurring in plants, bacteria and the mammalian kidney. As part of a project to explain and understand the action of these methylamines, the RNase A-catalysed degradation of polyuridylic acid in the presence of urea and various osmolytes (0–1.0 M) was studied using ^{31}P Nuclear Magnetic Resonance spectroscopy. The decrease in reaction rate induced by urea could be fully recovered with 1 molar equivalent of trimethylamine-*N*-oxide or 1.4 molar equivalents of glycine betaine. These results indicate that the modification of RNase A activity induced by urea is not associated with gross irreversible structural changes and that both glycine betaine and trimethylamine-*N*-oxide have kinetically detectable counteracting effects.

Keywords: RNase A; Trimethylamine-*N*-oxide; Glycine betaine; Urea; Counteracting osmolytes

1. Introduction

Many unicellular organisms, and some cells of some multicellular organisms, are bathed in a fluid which may subject to large changes in composition and physical properties, such as fluctuating salinity, temperature variations, variable pH, desiccation or freezing. Because the permeability of cell membranes to water is generally greater than to other substances, water will move by osmosis into or out of the cells, in turn changing the concentration of intracellular solutes. The consequent change in activity of intracellular water influences both the structure of cell components and the rate of metabolic reactions. In their seminal paper, Yancey et al. (1982) drew attention to the fact that a diverse range of organisms from numerous phyla responded to changes in intracellular water activity by the accumulation or loss of small organic solutes that are compatible with, but not essential for, cellular functions (compatible solutes) (Yancey, 1994). These fall into four major categories; methylamines, urea, polyhydric alcohols, and certain amino acids. Wherever urea is found to be retained by living systems, it is accompanied by one or more methylamine, generally trimethylamine oxide, glycine betaine or glycerophosphoryl choline. For example, in chondrichthyan fishes, including skates and rays (Forster and Goldstein, 1976), and dogfish (Robertson, 1975), and the sarcopterygian coelacanth (Lutz and Robertson, 1971), the most abundant solute is urea, where its intracellular concentration is typically 0.3 to 0.6 M, a level that is well known to have a denaturing effect on proteins (Yancey and Somero, 1979, 1980). To offset the perturbing effect of urea on normal protein function, these organisms accumulate, in addition, methylamines, mainly trimethylamine-*N*-oxide (TMAO), and also glycine betaine and sarcosine (Yancey et al., 1982). These counteracting osmolytes appear to oppose the denaturing effects of urea, and permit normal

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cellular function by stabilizing proteins. TMAO is also thought to play a role in counteracting the effect of high hydrostatic pressure on protein structure and function in deep-sea fishes (Yancey et al., 2001; Treberg and Driedzic, 2002), and may contribute to buoyancy in fish (Withers et al., 1994). In the inner medulla of the mammalian kidney, cells are exposed to high and variable concentrations of both urea and sodium chloride as an essential component of the urinary concentrating mechanism. In contrast to the situation in the cartilaginous fishes and the coelacanth, the kidneys of a number of mammals, including rabbit, rat, possum (*Trichosurus vulpecula*) and man (Bagnasco et al., 1986; Bedford et al., 2002; Sizeland et al., 1993) contain large amounts of glycine betaine and glycerophosphorylcholine (GPC), instead of TMAO.

Although the function of these methylamines as osmolytes, countering the denaturing effects of urea, is well established (Garcia-Perez and Burg, 1991), the mechanism by which this is achieved is still unclear. We have previously reported that the hydrolysis of polyuridylic acid (poly(U)) catalysed by RNase A can be used as a model reaction to study the effect of TMAO on enzyme activity by utilising phosphorus-31 nuclear magnetic resonance (^{31}P NMR) spectroscopy (Palmer et al., 2000). The present study was initiated to investigate whether glycine betaine has a similar counteracting effect on urea-modified RNase A.

2. Materials and methods

2.1. Chemicals

Ribonuclease A, type XII-A from bovine pancreas (lot no 79H7619), polyuridylic acid (5′) potassium salt (lot no.

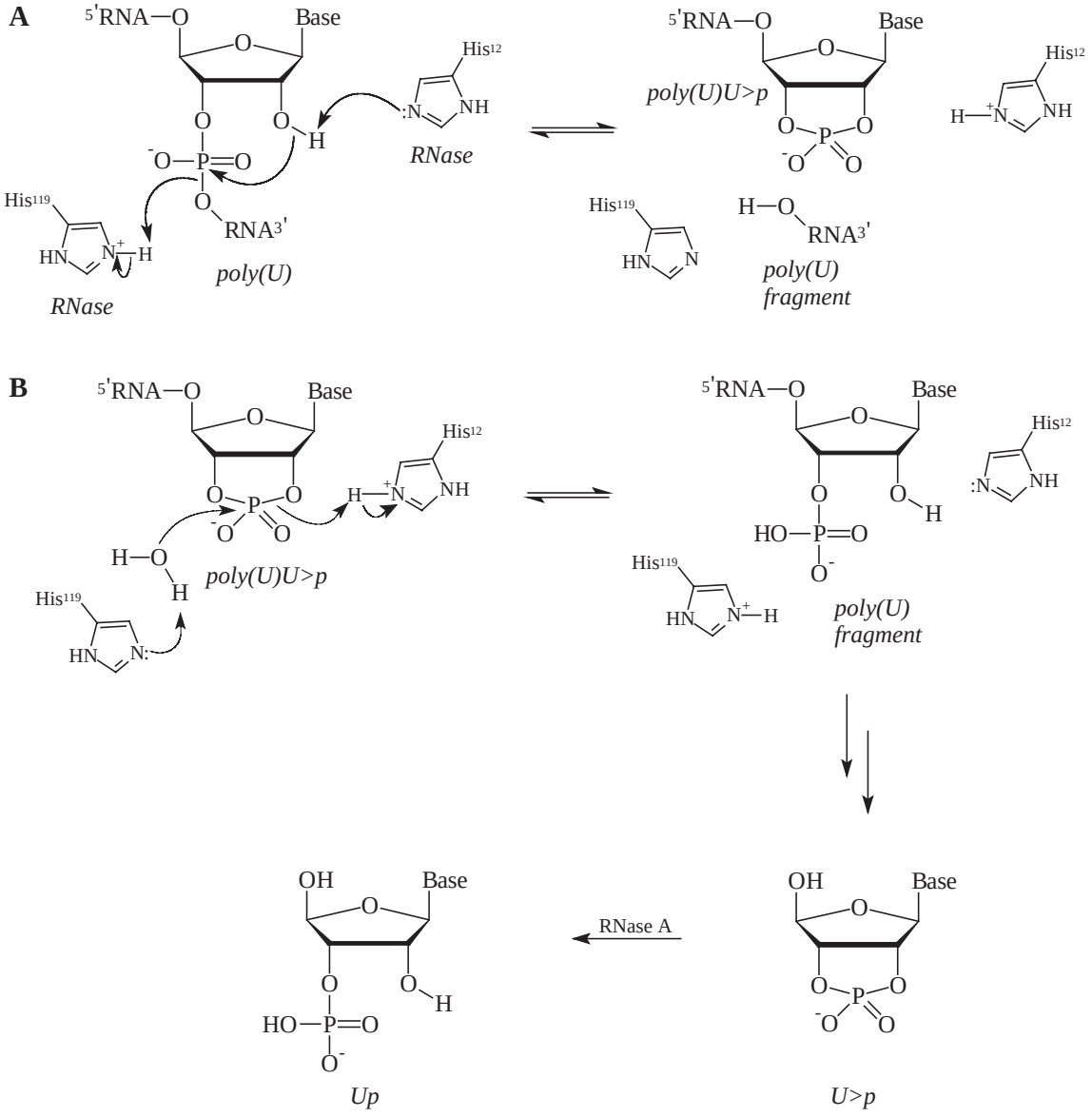

Fig. 1. Possible mechanism of RNase A-catalysed degradation of ribonucleic acid. (A) Transphosphorylation. (B) Hydrolysis.