NMR studies of the effect of GA-AG base pairs to the active conformation of hammerhead ribozyme

Mayumi Amano
Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565-0781, Japan

ABSTRACT

The effect of the conserved bases (GA-AG base pairs) in the core region to active conformation of hammerhead ribozyme was studied by NMR. This ribozyme has two conformers in the solution. One is the same as the crystal structure, which has sheared GA-AG base pairs and Y-shape conformation, while the other has Watson-Crick GA-AG base pairs and the extend conformation between the stems I and II.

On addition of magnesium ions, the peaks around GA-AG base pairs and cleavage site in only Y-shape conformation, shift or became broadening, following to produce the peaks of product by the cleavage reaction.

INTRODUCTION

Hammerhead ribozyme consists of three base paired stems flanking a central core of 15 conserved nucleotides. The conserved central bases are essential for ribozyme activity. The crystal structure of hammerhead ribozyme reveals Y-shape tertiary fold which is an enzymatic ground state, because a significant conformational rearrangement is required to reach an enzymatic transition state. However, the active conformation remains unresolved. To determine its structure in solution, Gel electrophoresis transient electric birefringence and fluorescence resonance energy transfer were used. All three of methods converged upon similar models at nearly the same time, concluding that the hammerhead is Y-shaped in solution under ionic conditions where it is active and sheared GA-AG base pairs are contained. In this present study, the effect of the conserved bases (AG-GA base pairs) in the core region in the solution structure and folding were studied by using H-NMR.

RESULTS AND DISCUSSION

This ribozyme construct employs 32-nt enzyme strand A and 11-nt substrate strand B. Figure 1A shows the imino proton spectrum of complex with strands A and B of hammerhead ribozyme in the absence of magnesium ions. The imino protons of A13U2.3, A11.2U10.2 and A15.3U16.3 resonate at lowest field and separate to two peaks, respectively. The imino proton of G1.2C2.2 resonates at 11.8 and 12.4 ppm. Compared with model oligomers A and B of stem 1, the peak at 11.8 ppm comes from the structure with the interaction between C17 and C3, while the peak at 12.4 ppm with that between C17 and G5. The peaks at 9.7 and 9.9 ppm come from G12A9 and G8A13, respectively, which contain less than one proton, judging from the chemical shift, coming from a sheared GA base pair. From these results, it is found that this ribozyme has two conformers in the solution (Figure 2). One conformer is similar to the crystal structure, which has sheared GA-AG base pairs, C17 and C3 interaction and the turn at U4, named Y-shape conformation. The other does not have sheared GA-AG base pairs but Watson-Crick GA-AG base pairs and extend conformation between stems I and II. Therefore, two conformation depend on the structure of the GA-AG base pairs.

On the addition of 6 magnesium ions, (Figure 1B) the peak at 13.3 ppm (A11.2U10.2) shifts at 13.5 ppm. The peak at 9.7 ppm (G12A9) shifts at 9.6 ppm. The peaks at 11.8 (G1.2C2.2) and 9.9 (G8A13) became broadening. In the crystal structure, a magnesium ion interacts with the proton of the A9 phosphate and N7 of G10.1. From NMR data, it is found that in the solution, a magnesium ion binds to the GA-AG base pairs, similarly to the crystal structure. The broadening of 11.8 ppm may reflect the binding of a magnesium ion to the cleavage site (C17) or rearrangement to active conformation. At this concentration of magnesium ions, cleavage reaction did not occur.
On the addition of 15 mM magnesium ions (Figure 1C), the peak at 11.8 ppm became very broad and disappeared. After 5 days at 20°C (Figure 1D), the major peak at 14.3 ppm decreased and minor peak at 14.2 ppm increased, and the peak at 11.8 ppm recovered. After 2 weeks (Figure 1E), the major peak at 14.3 ppm disappeared, while the minor peak at 14.2 ppm became one proton intensity. The new peaks at 13.8 (A15.3 U16.3), 12.9 and 12.8 ppm (G15.4C16.4 and G10.1C11.1) appeared. These peaks come from the product by the cleavage reaction. Therefore, cleavage reaction occurs in Y-shape conformation, but does not in the other conformation.

REFERENCES