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The Correlation of RNase A Enzymatic Activity with the Changes in the Distance between $N_{\varepsilon 2}$-His_{12} and $N_{\delta 1}$-His_{119} Upon Addition of Stabilizing and Destabilizing Salts

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The effect of stabilizing and destabilizing salts on the catalytic behavior of ribonuclease A (RNase A) was investigated at pH 7.5 and 25°C, using spectrophotometric, viscometric and molecular dynamic methods. The changes in the distance between $N_{\varepsilon 2}$ of His_{12} and $N_{\delta 1}$ of His_{119} at the catalytic center of RNase A upon the addition of sodium sulfate, sodium hydrogen sulfate and sodium thiocyanate were evaluated by molecular dynamic methods. The compactness and expansion in terms of Stokes radius of RNase A upon the addition of sulfate ions as kosmotropic salts, and thiocyanate ion as a chaotropic salt, were estimated by viscometric measurements. Enzyme activity was measured using cytidine 2′, 3′-cyclic monophosphate as a substrate. The results from the measurements of distances between $N_{\varepsilon 2}$ of His_{12} and $N_{\delta 1}$of His_{119} and Stokes radius suggest (i) that the presence of sulfate ions decreases the distance between the catalytic His residues and increases the globular compactness, and (ii) that there is an expansion of the enzyme surface as well as elongation of the catalytic center in the presence of thiocyanate ion. These findings are in agreement with activity measurements.

KEY WORDS: catalytic activity; distance calculation; folding/unfolding; molecular dynamics; RNase A; sulfate ions; thiocyanate ion.

1. INTRODUCTION

Bovine pancreatic ribonuclease A (RNase A) catalyzes the cleavage of polyribonuleotides through a transphorolytic step generating a terminal cyclic 2′, 3′-phosphate nucleotide, followed by a hydrolytic step in which the cyclic phosphate is hydrolyzed to a 3′ nucleotide. The kinetics of RNase A catalyzed hydrolysis of 2′, 3′-cyclic cytidine monophosphate (cCMP) has been extensively studied (Gharanfoli *et al.*, 2004; Moussaoui *et al.*, 1998; Pares *et al.*, 1978). There is much evidence that the main catalytic sites ($B_1R_1P_1$) in addition to the several subsites such as ($B_2R_2P_2$), are involved in the hydrolysis of the substrate (Barkakoti *et al.*, 1983; Katoh *et al.*, 1986; Wlodawer *et al.*, 1988; Wyckoff *et al.*, 1967). The stereochemistry studies of catalysis, low and high-resolution crystallographic analysis and NMR

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Abbreviation: cCMP, 2′, 3′- Cyclic Cytidine Monophosphate; 3′CMP, Cytidine 3′-Monophosphate; MD, Molecular Dynamic; PDB, Protein Data Bank; RNase A, Ribonuclease A; SDS, Sodium Dodecyl Sulfate.

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