FORMATION AND ENZYMIC PROPERTIES OF DIMERIC RNASE P*

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1. Introduction

The formation of enzymically active dimers of RNase A prompted Crestfield et al. [1] to propose a mechanism of dimerization of this enzyme which involved both amino and carboxy terminal residues. Recent investigations using a synthetic 70-residue analog of RNase S-Protein have shown that this analog, when mixed with the natural S-Protein, had high activity against cyclic cytidine-2′,3′-monophosphate despite the fact that both components were missing the first 20 to 25 residues of the N-terminus of native RNase A [2]. This study brought into question the necessity of an intact amino terminus for the formation of active dimers of RNase A. To investigate the importance of the amino and carboxy terminal residues in the aggregation of RNase A, studies were undertaken using RNase-S-Protein and RNase P. Although, under the conditions of Crestfield et al. [1], RNase-S-Protein could not be aggregated, RNase P formed dimers with increased activity when compared with the monomer.

2. Materials and methods

RNase A and tRNA (baker's yeast) were from Boehringer. RNase-S-Protein and cyclic cytidine-2′,3′-monophosphate (sodium salt) were from Sigma. Pepsin (2 × cryst., from pig, 2883 U/mg) was obtained from Serva. Sephadex G-10, G-25, G-75 (fine and superfine) and SE-Sephadex C-25 were from Pharmacia. Protein concentrations were determined from amino acid analyses of acid hydrolysates. Optical densities of column effluents were read at 280 nm or 570 nm. All enzymic reactions were followed in a Cary 15 spectrophotometer.

2.1. *Preparation and isolation of RNase P*

The procedure described by Anfinsen [3] and Lin et al. [4] was followed. The isolated RNase P fraction was dialyzed against deionized water for 7 h at 7°C and lyophilized. To avoid contamination by undigested native RNase A the material was rechromatographed three times. The specific activity toward cyclic cytidine-2′,3′-monophosphate was then 0.6%.

2.2. *Preparation of aggregates*

The RNase derivatives were treated as described by Fruchter and Crestfield [5]. The lyophilized products were applied on a Sephadex G-75 column (2 × 75 cm) and eluted with 0.2 M phosphate buffer, pH 6.47. In the case of S-Protein only one peak was obtained eluting at the same position as monomeric S-Protein. No aggregates had formed. Acetic acid-treated RNase P, however, was separated into two fractions eluting at 142 ml and 176 ml, respectively (fig.1).

The monomeric fraction (fig.1, fraction II) was repeatedly submitted to lyophilization from acidic solution followed by chromatography on Sephadex G-75. The dimeric fraction (fig.1, fraction I) was desalted by dialysis, lyophilized, and rechromatographed on Sephadex G-75. Its position agreed with that of native RNase A dimer.

2.3. *Assay of RNase A and RNase P*

Specific activities were determined by the methods of Crook et al. [6], del Rosario and Hammes [7], and

*Abbreviations: RNase P, des-(121–124)-RNase A; S-Protein, des-(1–20)-RNase A.

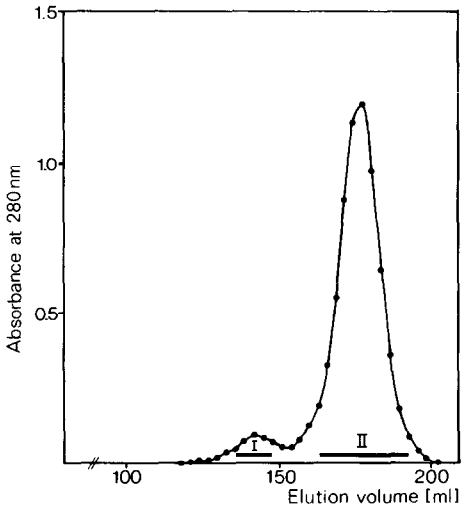

Fig.1. Separation of monomeric and dimeric RNase P on a Sephadex G-75 column (2 × 75 cm) equilibrated with 0.2 M phosphate buffer, pH 6.47. Amount of material applied on column, 50 mg. Flow rate, 21 ml/h. Mol. wts. of fraction I and II were determined by thin-layer gel filtration; fraction I, mol. wt. ~ 27 800, and fraction II, mol. wt. ~ 13 700.

Kunitz [8]. Cyclic cytidylate was dissolved in 0.1 M Tris–HCl/0.1 M NaCl, pH 7.0 (1.02×10^{-4} M). To 2 ml of this solution 0.154 mg RNase A or P was added in 20 μl of the same buffer. The increase of absorbance was recorded at 286 nm and activities were calculated from initial slopes.

RNA was dissolved in 0.1 M sodium acetate, pH 5.0, to a concentration of 0.5 mg/ml. To 2 ml of this solution 2.9 μg of RNase A and 22 μg of RNase P, respectively, were added in 20 μl of buffer each. The decrease of optical density was recorded at 300 nm and activities were calculated as described above (table 1).

2.4. *Reactivation of monomeric and dimeric RNase P*

Essentially the procedure of Lin et al. [9] was followed with the exception that RNA was used as substrate. Solutions of monomeric and dimeric RNase P (3.65×10^{-5} M) were mixed with the C-terminal undecapeptide and dodecapeptide of RNase A in 3.25 to 14-fold excess. The mixtures were incubated for 30 min at room temperature. Activities were then determined by the method of Kunitz [8] using RNase A as standard (table 2).

3. Discussion

Crestfield et al. [1] proposed a two-step mechanism for the formation of active dimers of RNase A. First, the noncovalent interactions of the N-terminus with the remainder of the molecule are interrupted by treatment with 50% acetic acid; then, during lyophilization, the N-terminal portion of a partially denatured RNase A monomer binds to the C-terminus of another molecule in such a way that a new active site is formed the essential residues of which are contributed by both components of the complex.

The fact that RNase-S-Protein showed no tendency to aggregate after treatment with 50% acetic acid followed by lyophilization, emphasized the importance of the N-terminus of RNase A for aggregation and supported the mechanism put forward by Crestfield et al. [1]. Aggregation of the synthetic 70-residue analog of RNase-S-Protein and formation of a complex of this analog with natural S-Protein [2] therefore must follow a completely different mechanism. Both reactions were observed in 0.05 M NH_4HCO_3, a basic rather than acidic medium, indicating also that RNase analogs with altered primary structures aggregate in a different manner.

Table 1
Activities of monomeric and dimeric RNases A and P using cyclic cytidine-2′,3′-monophosphate and RNA as substrates

	RNase A		RNase P	
Substrate	monomer	dimer	monomer	dimer
cyclic cytidylate	100%	105%	0.6%	1.8%
RNA	100%	41%	0.7%	1.7%

Table 2
Reactivation of monomeric and dimeric RNase P by mixture with synthetic C-terminal peptides of RNase A[a]

Enzyme	Activaties of RNase P–peptide complexes						
	Without peptide	Molar ratio RNaseP/ undecapeptide[b]			Molar ratio RNaseP/ dodecapeptide[c]		
		1:3.5	1:7	1:14	1:3.25	1:6.5	1:13
Monomeric RNase P	0.7%	17%	19%	32%	14%	17%	37%
Dimeric RNase P	1.7%	15%	15%	21%	12%	13%	26%

[a] 2ml of RNA solution (0.75 mg/ml) were mixed with 15 μl of protein–peptide complex containing 3.65×10^{-5} M protein.
[b] C-terminal undecapeptide of RNase A, (114–124): Pro–Tyr–Val–Pro–Val–His–Phe–Asp–Ala–Ser–Val [10].
[c] C-terminal dodecapeptide of RNase A, (113–124): Asn–Pro–Tyr–Val–Pro–Val–His–Phe–Asp–Ala–Ser–Val [10].

The formation of a dimer of RNase P under the conditions used by Crestfield et al. [1] showed that the presence of the last four C-terminal residues of RNase A was not essential for aggregation. The low yield of this dimer (3% as compared with 25% obtained from RNase A) and the complete absence of higher aggregates (fig.1), however, suggested a weaker association of the RNase P monomers. Most likely, removal of the C-terminal tetrapeptide of RNase A by pepsin caused a conformational change at this end of the molecule [4,11] which prevented optimal binding of the N-terminal portion of a second RNase P monomer. Direct participation of residues 121 to 124 in the aggregation process is unlikely because the X-ray structure of RNase [12] revealed that these residues are not in contact with the N-terminus of the molecule.

Dimeric RNase P showed a three-fold increase in activity against cyclic cytidylate and RNA as compared with the monomer (table 1). It is conceivable that through dimerization the C-terminus of RNase P was stabilized in a structure which resembled more closely that of the C-terminus of RNase A and that the active sites of the dimer could thus bind the substrate in a position more favourable for catalysis.

Like monomeric RNase P and RNases (1–114), (1–115), (1–118) and (1–119) [9,13], dimeric RNase P exhibited increased enzymic activity when RNase (114–124) or RNase (113–124) was added (table 2) to provide the missing C-terminal tetrapeptide of RNase A and in addition an overlap of 7 or 8 residues. From this it was concluded that dimerization of RNase P did not prevent the binding of the synthetic peptides at the C-terminus of each component of the complex. All activities, however, generated by mixtures of dimeric RNase P with increasing amounts of RNase (114–124) and RNase (113–124), were lower than those shown by monomeric RNase P in the presence of these peptides (table 2). Assuming the results listed in table 2 (ratios 1:14 and 1:13) were the maximum regenerable activities, this indicated that the binding of the C-terminal peptides to dimeric RNase P was weaker, probably for steric reasons.

The exact arrangement of the two subunits in dimeric ribonucleases must still await its elucidation by X-ray analysis.

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