Acta Biochimica Polonica
Vol. 40 No. 1 1993

The ribonuclease activity of the two synthetic polypeptides having zinc finger sequence**

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The zinc finger domain was first postulated to occur in the transcription factor IIIA (TFIIIA) from *Xenopus laevis*. TFIIIA is a 40 kDa protein required for transcription of 5S RNA genes by RNA polymerase III. It contains nine repeats (called "zinc finger") of 30 amino acids [1]. Each of the zinc fingers can potentially bind zinc ion through the two invariant cysteine (C_2) and histidine (H_2) residues. N.m.r. studies revealed that the TFIIIA-like zinc finger motif contains an antiparallel β-sheet and α-helix held together by the zinc ion and a set of hydrophobic residues [2, 3]. The two cysteines of the β-sheet region and the two histidines of the α-helix can bind only one zinc ion.

For some time we have been interested in the structure of plant 5S rRNA and its interaction with the transcription factor IIIA. Till now the TFIIIA-like transcription factor from plants has not been isolated and characterized, but some studies on the *Xenopus* TFIIIA-5S RNA complex revealed that the region of 5S RNA containing helices IV and V binds to the transcription factor IIIA [4 - 6]. On the other hand, it has been shown that the proteolytic fragments of *Xenopus* TFIIIA of 20 kDa and 30 kDa form a complex with the cognate 5S rRNA [7].

In this paper, we present data on the interaction between the plant 5S rRNA and synthetic polypeptides with amino-acid sequences identical to that of the second zinc finger of the *Xenopus laevis* TFIIIA, namely: PFPCKEEGCEKGFTSLENLTRHSLTHTGEK (ZF-1) and another: PFPCKEEGCEKEFTSLENLTRHSLTHTGEK (ZF-2), in which glutamic acid replaced glycine. We clearly show that, in addition to the weak binding properties of these two polypeptides, they also hydrolyse plant 5S rRNA. Analysis of the digestion patterns of 5S rRNA with ZF-1 and ZF-2 showed that the RNA cleavage sites occurred mainly after the cytosine residues. The RNase A-like type specificity of the ZF-1 and ZF-2 polypeptides was confirmed by digestion of *E. coli* tRNA with both zinc fingers. To our knowledge, this is the first report showing specific RNase activity of the synthetic peptides. These findings open the possibility for synthesis of peptides with desired properties.

Two polypeptides with the zinc finger sequences were prepared by chemical synthesis. We were particularly interested in the complex formation between 5S rRNA and the zinc finger domain. The experiments showed, in addition to the small rate of complex formation, distinct degradation of wheat germ 5S rRNA. Similar observations were reported in the literature on interaction of the entire TFIIIA with 5S RNA [8]. To gain more understanding this effect, we carried out a detailed analysis of the pattern of wheat germ 5S rRNA hydrolysis by the two zinc finger polypeptides. Knowing the primary structure of wheat germ 5S rRNA, we identified the bands corresponding to nucleotides

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**This work was supported in part by the Polish Scientific Research Committee (KBN). Research Grant no. DS/5-300-7-016-1

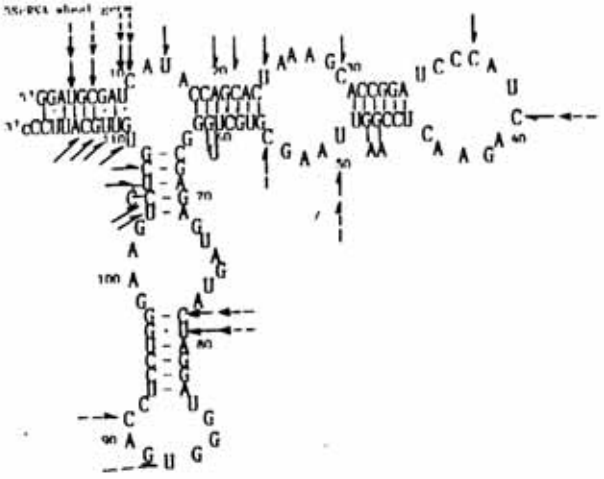

Fig. 1. Secondary structure of 5S rRNA from wheat germ.
Arrows show the digestion sites with synthetic polypeptides ——> ZF-1 and ZF-2 - - - - > ; ——> strong digestions; ——\ weak digestions; - - - - > strong digestions; - - - -\ weak digestions

U4, C6, U9, C10, U12, C15, C18, U21, C26, C36, C39, U53, U73, C78, U79, U103, C104, U106, C107, U109, U111, U112, C114 for wheat germ 5S rRNA hydrolysed by ZF-1 and U4, C6, U9, C10, C39, U53, C57, U73, C78, U79, U88, C91, for wheat germ 5S rRNA digested with ZF-2. These data, summarized in Fig.1, clearly show the hydrolytic activity of these two synthetic polypeptides. Hydrolysis of the RNA phosphodiester bonds occurs after the pyrimidine nucleotides; in addition, the hydrolysis sites are located mainly, although not exclusively, in the double-stranded regions of RNA (Fig.1). One can easily notice that the patterns of wheat germ 5S rRNA hydrolysis by the ZF-1 and ZF-2 are slightly different. ZF-2 (having Glu12) splits the loop D; but ZF-1 (with Gly12) cleaves the double-stranded fragments of the domain gamma and a part of the domain alpha. One can suggest that the differences in ribonuclease activity are due to the different amino-acid sequences of ZF-1 and ZF-2. Substitution of glycine (ZF-1) with glutamic acid (ZF-2) at position 12 causes probably some conformational flexibility restrictions or structural changes of the polypeptide which affect slightly the RNase activity. Interestingly, the distribution of the cleavage sites of 5S rRNA with ZF-1 resembles very much the hydrolysis pattern of plant 5S rRNA by pancreatic RNase [9], the enzyme which cuts pyrimidine nucleotides phosphodiester bonds. The polypeptides ZF-1 and ZF-2 contain (like RNase A) two histidine residues (positions 22 and 26) very close to each other, and this suggests that the mechanism of RNA hydrolysis is very similar, if not identical, to that of RNase A action [10, 11]. Comparison of the results of hydrolysis of 5S rRNA and tRNA

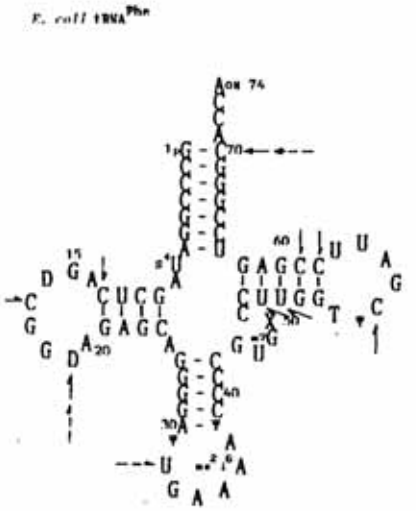

Fig. 2. The secondary structure of tRNA ^{Phe} from E. coli.
Arrows show the digestion sites with synthetic polypeptides ——> ZF-1 and ZF-2 - - - - > ; ——> strong digestions; ——\ weak digestions; - - - - > strong digestions; - - - -\ weak digestions

obtained under the same conditions with the synthetic peptides ZF-1 and ZF-2 (Figs. 1 and 2) leads to the conclusion that both peptides require a higher ordered structure of RNAs for specific recognition. It is well known that all tRNAs have very similar, if not identical, tertiary structure where dihydrouridine and ribothymidine loops form two tertiary base pairs G18–C57 and G19–ψ56. The region of tRNA involved in formation of tertiary interactions is not digested by ZF-1 and ZF-2 (Fig. 2). The same concerns plant 5S rRNA (Fig. 1). The nucleotides (35)CCCA(38) and (85)GGGU(88) of plant 5S rRNAs which have been suggested to form tertiary base pairs between the loops C and D, respectively [9] are not extensively digested. It is well known from crystallographic data that the tRNA structure has regular RNA A form and therefore comparison of the hydrolysis pattern of tRNA and 5S rRNA enabled us to suggest that the tertiary structure of the plant 5S rRNA could be achieved by interactions similar to those found in tRNA. The data presented here support the model of higher ordered structure of plant 5S rRNA proposed earlier by some of us [12].

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