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The tertiary structure of *Aspergillus saitoi* minor ribonuclease (Ms) predicted from the structure of RNase T_1

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Ribonuclease Ms from *Aspergillus saitoi* is a small acidic protein (11714 Da) containing 106 amino acids of known sequence. Unlike other enzymes belonging to the RNase T_1 family this ribonuclease is base-unspecific. Using interactive computer graphics and energy minimisation we predicted the structure of RNase Ms on the basis of sequence homology to RNase T_1 of known structure. In this report the predicted structure of this protein is presented and characterised.

Ribonuclease Ms; Structure prediction; Computer graphics; Energy minimization; (*Aspergillus saitoi*)

1. INTRODUCTION

Among ribonucleases (RNases) which are very diverse, cleaving RNA as single, double and hybrid double strands, there are a number of closely related, mutually homologous microbial RNases usually referred to as the RNase T_1 family [1]. Most of these RNases are guanine-specific. Exceptions are the purine-specific RNase U_2 (from *Ustilago sphaerogena*) and RNase Ms (from the mould *Aspergillus saitoi*, EC 3.1.4.23) which has been shown to cleave at all four nucleotides, with some preference for guanosine [2,3].

RNase Ms is a small acidic protein of 11714 Da. It contains 106 amino acids of known sequence [4]. Since the sequence homology of RNase Ms with RNase T_1 is ~60% and the three-dimensional structure of the RNase $T_1 \cdot 2'$-GMP complex has been determined by X-ray crystallographic methods to high resolution (1.9Å) [5], there is a good basis for modelling the structure of RNase Ms. Here, the predicted three-dimensional structure of RNase Ms is described.

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2. MATERIALS AND METHODS

Molecular modelling was performed on an Evans & Sutherland PS 300 vector graphics device. Energy minimization routines were run on a Cray X-MP/24 using the BIOSYM program package. Modelling of the structure of RNase Ms was performed in two steps. (i) Using the structure of RNase T_1 from the crystalline complex RNase $T_1 \cdot 2'$-GMP as a template, the structure of RNase Ms was modelled with the aid of interactive computer graphics. (ii) The predicted structure obtained in this first step was then subjected to energy minimization calculations.

According to the sequence alignment with RNase T_1, which is used here for numbering of amino acids, RNase Ms shows a deletion of two amino acids (after Ile 33), an insertion (after Ala 95) and the two disulfide bridges 2–10 and 6–103 are preserved (fig.1). An additional problem is posed by the *cis*-Pro 55 in RNase T_1, which is not present in RNase Ms, but there is a Pro in position 51. Since all these major changes occur in three loop regions, it was assumed that the core of the protein is similar in both structures. Consequently, these three loop regions, comprising residues 30–38, 45–60 and 92–100, were modelled independently of the RNase T_1 template.

Leaving the core of the template unchanged, the loops were placed with their terminal residues on corresponding positions of the template. Design principles for loops were the following: (i) maximizing hydrogen bonding, wherever possible; (ii) orienting hydrophilic residues towards the solvent accessible surface; and (iii) orienting hydrophobic residues such as to minimize their solvent accessibility. During the modelling of the loops,

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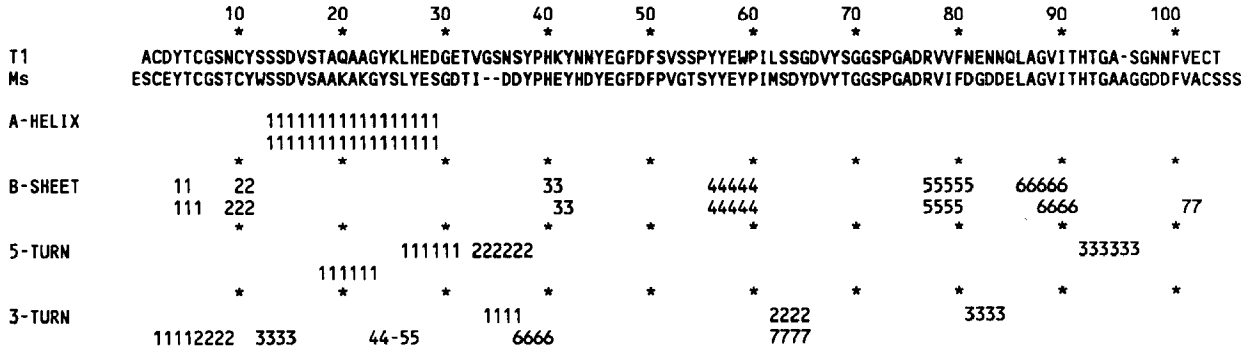

Fig.1. Sequence alignment of RNase T_1 and RNase Ms [1] with secondary structure elements according to [9].

care was taken to avoid clashes with core residues, and to keep the terminal residues of the loops within bond distance to their partners on the core. Furthermore main chain torsion angles in the loops were kept within allowed regions.

In order to assign the proper conformation, *trans* or *cis*, to Pro 51, the structure of RNase C_2, which belongs to the same enzyme family, was evaluated. In the loop 45–60, RNase C_2 shows high (80%) homology to RNase Ms and to RNase T_1. RNase C_2 has a Pro in the same position 51 as RNase Ms and no Pro in the corresponding position 55. Pro 51 in RNase C_2 is in the *trans* conformation, as established from the X-ray crystal structure [6], and consequently a *trans* conformation was chosen for Pro 51 in RNase Ms. Comparison of the tertiary structure of loop region 45–60 in RNase C_2 and RNase T_1 shows that the general spatial course of these loops is similar. The RNase T_1 *cis*-Pro 55 (which is Asn 55 in RNase C_2 and Ser 55 in RNase Ms) can be substituted smoothly if somewhat unusual, but sterically allowed torsion angles are permitted in the two adjacent Ser and Gly residues on the N-terminal side of Asn 55 (Ser 53 ϕ = 110.7, ψ = 147.1; Gly 54 ϕ = 69.4, ψ = 175.9).

After attaching the novel loop constructs to the core of the template, the amino acids in the core had to be exchanged. In the modelling of the core the following rules were applied. The new side chains were fitted into the core structure with torsion angles χ_n taken from the original side chains in RNase T_1 [7]. Whenever this caused steric clashes or no sufficient template was available (Trp 12, Lys 22, Met 62, Tyr 62), the proper rotations were made in order to relieve steric hindrance. Applying all of the previously mentioned criteria, the same was done with the C-terminal and N-terminal residues where no template was available.

Energy minimization of the modelled RNase Ms structure was conducted with the molecular dynamics and energy minimization program DISCOVER employing the Consistent Valence Force Field [8]. The pH of the system was set to 7.0. At this pH all aspartic acids, glutamic acids and the C-terminus are negatively charged; all lysines, arginines and the N-terminus are positively charged. A positive charge was also assigned to His 40 and His 92. A cut-off value of 10 Å was used for non-bonding interactions, employing a switching function above 8.5 Å. The list of neighbouring residues was regenerated every 20 iterations.

For comparative purposes the structure of RNase T_1, as obtained from the crystal structure of the complex with 2′-GMP [5], was also energy minimized.

3. RESULTS

The predicted three-dimensional structure of RNase Ms is shown in a stereo plot in fig.2a. Secondary structure elements of the predicted structure of RNase Ms and of the energy-minimized RNase T_1 are presented in fig.1. The assignment of the helical and β-pleated sheet regions and of different turns is based on the program by Kabsch and Sander [9].

In the N-terminal region of RNase Ms, strands 1 and 2 are part of the first antiparallel β-sheet. This is followed by an α-helix comprising Ser 13–Ser 29. A second antiparallel β-sheet is formed by strands 3–7. In the energy-minimized structure of RNase T_1 strands 1 and 2 are part of the first antiparallel β-sheet, and strands 3–6 of the second. 63 hydrogen bonds of type C=O---H-N were found in the backbone of the predicted structure of RNase Ms by the DSSP program, compared to 65 in the energy minimized RNase T_1. The total solvent-accessible surface in RNase Ms was calculated to be 5783 Å^2. The value in minimized RNase T_1 is 5027 Å^2.

The solvent accessibilities of the individual amino acids in the two β-sheets are generally low because the sheets are buried in the core of the enzyme. Sheet 2 provides a defined geometrical arrangement for Glu 58 and His 92 (fig.2b), the residues believed to be involved in the catalytic action of RNases belonging to this family [10]. It is noteworthy that in the catalytic region of RNase Ms only minor structural changes occurred during

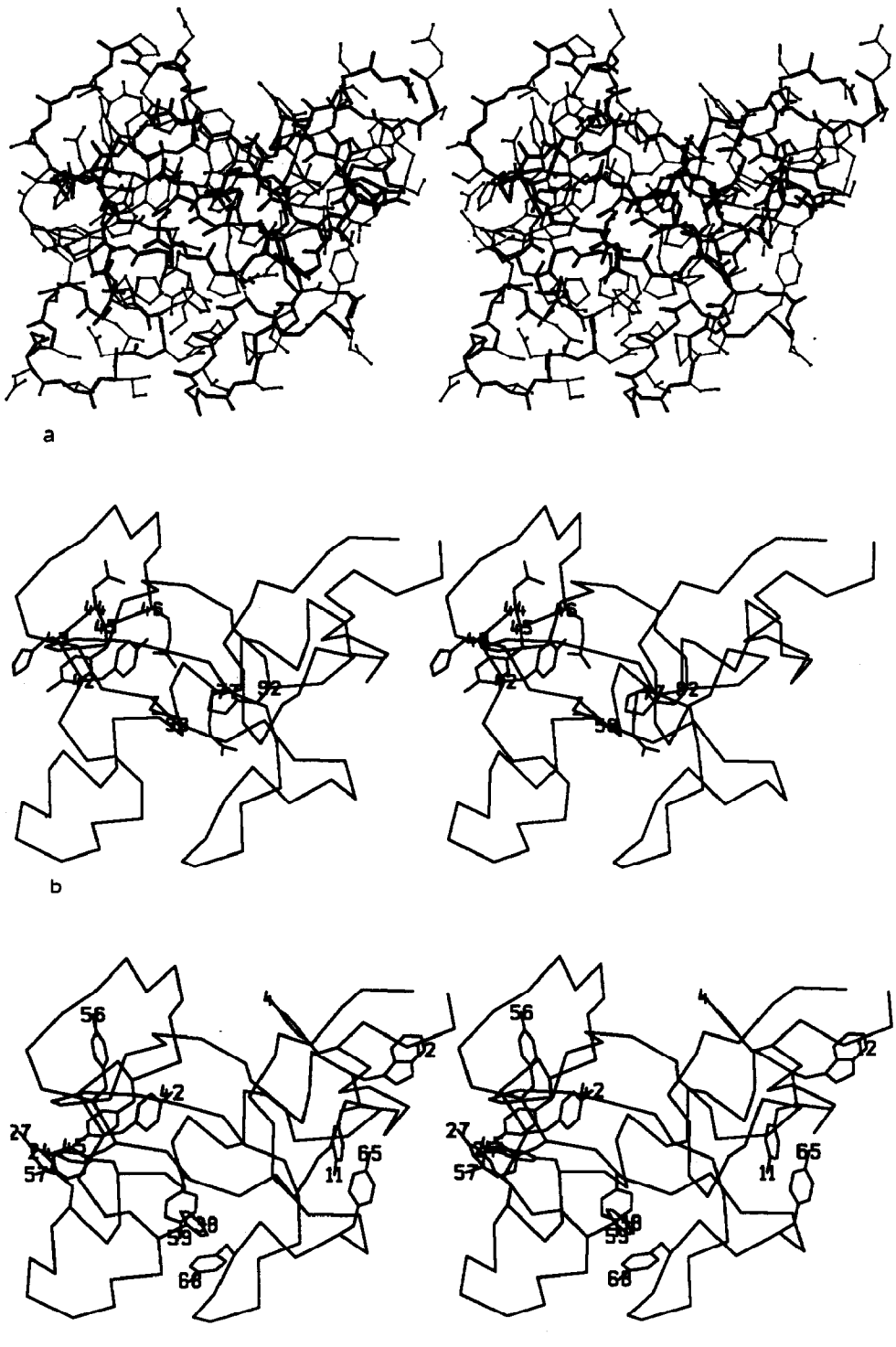

Fig.2. (a–c) Stereo plots drawn with SCHAKAL88 (by Egbert Keller, Institut für Kristallographie, Albert-Ludwigs-Universität, Freiburg). (a) The predicted structure of RNase Ms; (b) C_α trace with recognition site residues 42–46 and those involved in catalytic action; (c) location of Trp and Tyr residues in RNase Ms.

minimization. The distances between the Oε atoms of Glu 58 and Nε atoms of His 92 are virtually the same in RNase Ms and T_1 (5.3 Å). In the segment formed by residues 42–46, which are responsible for base recognition, conformational changes during energy minimization were more significant in RNase Ms than in RNase T_1. In the minimized structure of RNase Ms, the side chains of Tyr 42 and Tyr 45 are closer together than in RNase T_1 so that less space is available for the sandwich formation with guanine.

Arg 77, which probably plays a role in stabilising the transition state during catalysis, has a low solvent accessibility and is buried in the protein core similarly to RNase T_1. It is unusual in protein structures to find a charged residue like Arg in the interior, yet Arg 77 is conserved in all RNases of the T_1 family.

Amino acids suitable for experimental control are tryptophans and tyrosines. Trp 12, the only one present in RNase Ms, is located on the protein's surface (see fig.2c). Twelve tyrosines are present in RNase Ms. In the predicted structure, of the 8 solvent-accessible tyrosines, 3 are almost fully exposed. Totally or almost inaccessible to solvent are Tyr 11, 38, 59 and 68.

4. DISCUSSION

The overall energy minimized structures of RNase Ms and of RNase T_1 are very similar. No major structural changes occurred during minimization in the catalytic site, although the residue adjacent to Glu 58, Trp 59 in RNase T_1, is replaced by Tyr 59 in RNase Ms. This stresses the importance of the other conserved residues in the catalytic region which provide a defined geometrical arrangement for the residues involved in catalytic action. It is noteworthy that in the predicted structure of RNase Ms the only tryptophan Trp 12 is located on the surface of the protein, in contrast to RNase T_1, where the only Trp 59 is buried. The exposed location of Trp 12 is supported by experimental data on RNase Ms [11].

Another characteristic feature of RNase Ms is the twelve Tyr residues, which may be used in chemical modification experiments. Tyr 45 is part of the recognition site and one of the tyrosines with the highest accessibility in the predicted structure of RNase Ms. This agrees with an experiment in which modification of just one Tyr residue by diazonium 1(H)-tetrazole was enough to inactivate RNase Ms [12]. We assume that this inactivation is due to a modification of Tyr 45 in the recognition site, thereby reducing the capability of RNase Ms to bind nucleotides. If the solvent accessibilities of the tyrosines are compared with reactivity towards modifying agents, there is also overall agreement with our model, because six Tyr react with *N*-acetylimidazole under relatively mild conditions and two to three only with 450-fold molar excess [12].

Since RNase Ms was shown not to be specific for a particular base [2,3], the structural aspects which cause this lack of specificity are of interest. At present no structural reason for the recognition of other bases than guanine can be given, but further investigations in this direction are underway.

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