

Mechanical stability of compact modules of barnase

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Abstract Globular proteins are composed of structural elements such as secondary structures and modules. Modules are compact segments consisting of 10–40 contiguous amino acid residues and are often encoded by exons. Therefore, the view that the modular organization of proteins is a result of exon-shuffling or -fusion is given support. Secondary structures such as α -helix and β -sheet are stabilized by hydrogen bonds and are thus considered to be stable, structural elements of a globular domain. Since module boundaries are often located on α -helices or β -sheets, it is not obvious whether the modules are mechanically stable. We carried out molecular dynamics simulations on modules of barnase, a bacterial RNase from *Bacillus amyloliquefaciens*, for 1 ns in vacuo and 150 ps in water. Five of six modules (M1, 1–24; M2, 25–52; M3, 53–73; M4, 74–88; M5, 89–98) retained native-like conformations during these simulations. Only the C-terminal module (M6, 99–110) was deformed; it is less compact than the other modules. As the modules are mechanically stable they are suitable as parts combined into proteins. Together with RNase activity of the three isolated modules of barnase, M2, M3 and M6, our study supports the view that modules were indeed original building blocks of proteins.

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Key words: Module; Barnase; Molecular dynamics; Protein folding; Exon-shuffling; Molecular evolution

1. Introduction

Introns are non-coding DNA sequences that split various genes encoding proteins. In some genes, intron positions are closely correlated with structural elements of the gene-encoded proteins [1–11]. This means that such structural elements were the original building blocks and were recruited into proteins by exon-shuffling [12,13]. There are two contradicting hypotheses on the origin of introns. One is the ‘intron early’ [6,12,14] and the other is the ‘intron late’ hypothesis [15]. The finding of a close correlation of intron positions with protein structural units supports the intron early hypothesis.

Modules, candidates for such original building blocks of proteins, are compact substructures within a globular domain [2,3]. Correlation between module boundaries and intron positions was found in hemoglobin, lysozyme, ovomucoid 3rd domain and triosephosphate isomerase [1–8].

If modules are original building blocks and are recruited into globular proteins, they could be mechanically stable.

Such stability is required for useful parts to have their own conformation and/or function which is/are not influenced or deformed by surrounding partners. However, other investigators reported that no correlation was observed between introns and secondary structures [15,16]. As module boundaries are often located on secondary structures, the question of whether modules are mechanically stable elements like secondary structures must be clarified. Since module boundaries show a close correlation with intron positions ([10] and unpublished results), mechanical stability must be a crucial intrinsic character of modules, as original building blocks of proteins. We studied the molecular dynamics of barnase, a small enzyme.

Barnase, a bacterial RNase from *Bacillus amyloliquefaciens* [17], consists of 110 amino acid residues and has no disulfide bond. The three-dimensional structure (3D) of barnase has been determined by X-ray crystallography [18–20] and NMR spectroscopy [21]. Barnase was decomposed into six modules, M1–M6 [22]. Three of the six modules were found to have weak RNase activity [23], suggesting that they were primitive mini-enzymes, as independent segments.

The six compact modules are connected spatially to form the globular structure of barnase. In intact barnase, each module interacts with other modules and solvent. If these interactions are eliminated, the module may be deformed, or alternatively it may retain native-like conformations. This hypothetical state of the isolated module in vacuo can be simulated by molecular dynamics. Many mechanical properties of a protein molecule, such as conformational changes caused by ligand binding or by amino acid substitution, are characterized by dynamics in the time range of pico- to nanoseconds. We carried out molecular dynamics simulations of the six isolated modules for 1 ns in vacuo starting from their native conformations. If a module retains a conformation similar to its intact one during the simulation, the 3D structure of the module is mechanically stable. The mechanical stabilities of ‘pseudo modules’ were also studied by molecular dynamics, as a control study. Pseudo modules were defined as segments starting at the center of one module and ending at the center of the following one [22]. Five pseudo modules, P1–P5, were defined as: P1, 13–38; P2, 39–62; P3, 63–80; P4, 81–93; P5, 94–104. Molecular dynamics simulations were carried out for the pseudo modules isolated in vacuo for 50 ps. Our study was extended to simulation of modules and pseudo modules, with explicit inclusion of water molecules as solvent. We then compared the mechanical stability of modules in vacuo and in water.

2. Materials and methods

2.1. Modules and the secondary structures

Module boundaries were identified using a computerized method as

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Abbreviations: 2D/3D, two/three dimensional; MD, molecular dynamics; RMS, root mean square; RMSD, root mean square deviation

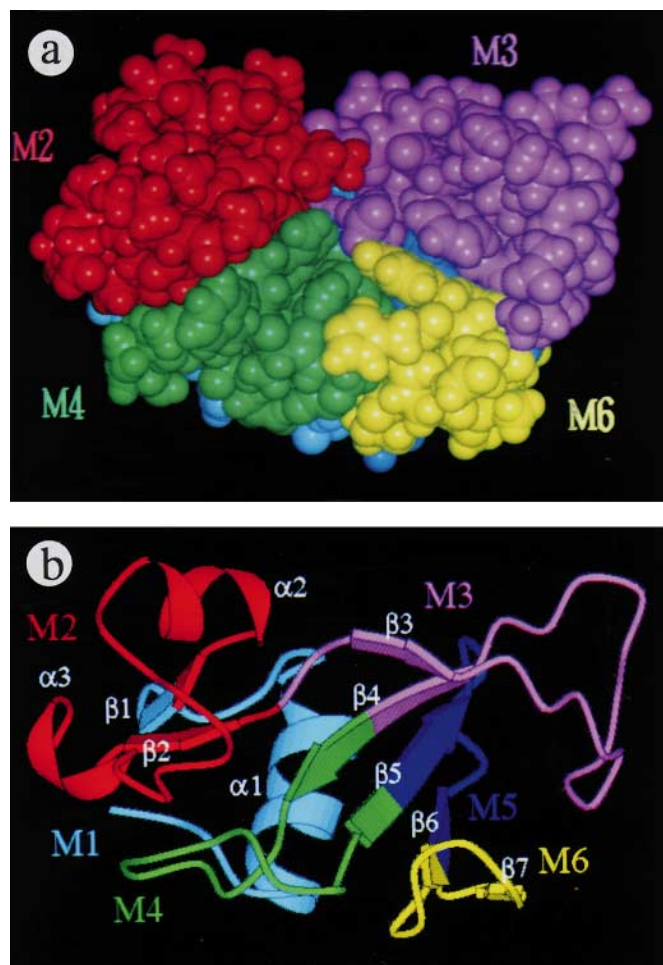


Fig. 1. Module structure of barnase. The 3D structure of barnase [18] is shown from the direction of the catalytic site. Modules M1–M6 are colored differently; M1 in sky blue, M2 red, M3 magenta, M4 green, M5 blue and M6 yellow. (a) Space-filling model. (b) Ribbon model of the mainchain drawn with the program MolScript [32]. Three α -helices, $\alpha 1$ – $\alpha 3$, and seven β -strands, $\beta 1$ – $\beta 7$, were identified by the algorithms of Kabsch and Sander [33].

24, 52, 73, 88 and 98 [22]. Thus, the six modules M1–M6 were identified as 1–24, 25–52, 53–73, 74–88, 89–98 and 99–110 (Fig. 1). Barnase has three α -helices, $\alpha 1$ – $\alpha 3$, (7–17, 27–32, 42–45), and seven β -strands, $\beta 1$ – $\beta 7$ (24–25, 50–51, 55–56, 71–75, 87–91, 96–99, 107–108) (Fig. 1b). The first and second β -strands form a parallel β -sheet and the other five form an anti-parallel β -sheet. Helix $\alpha 1$ belongs to module M1. Helices $\alpha 2$ and $\alpha 3$, and the parallel β -sheet are involved in module M2. Module boundaries between M1 and M2, between M3 and M4, between M4 and M5, and between M5 and M6 are located on parallel or anti-parallel β -sheets (Fig. 1b). The anti-parallel β -sheet is in the four consecutive modules M3–M6.

2.2. In vacuo simulation

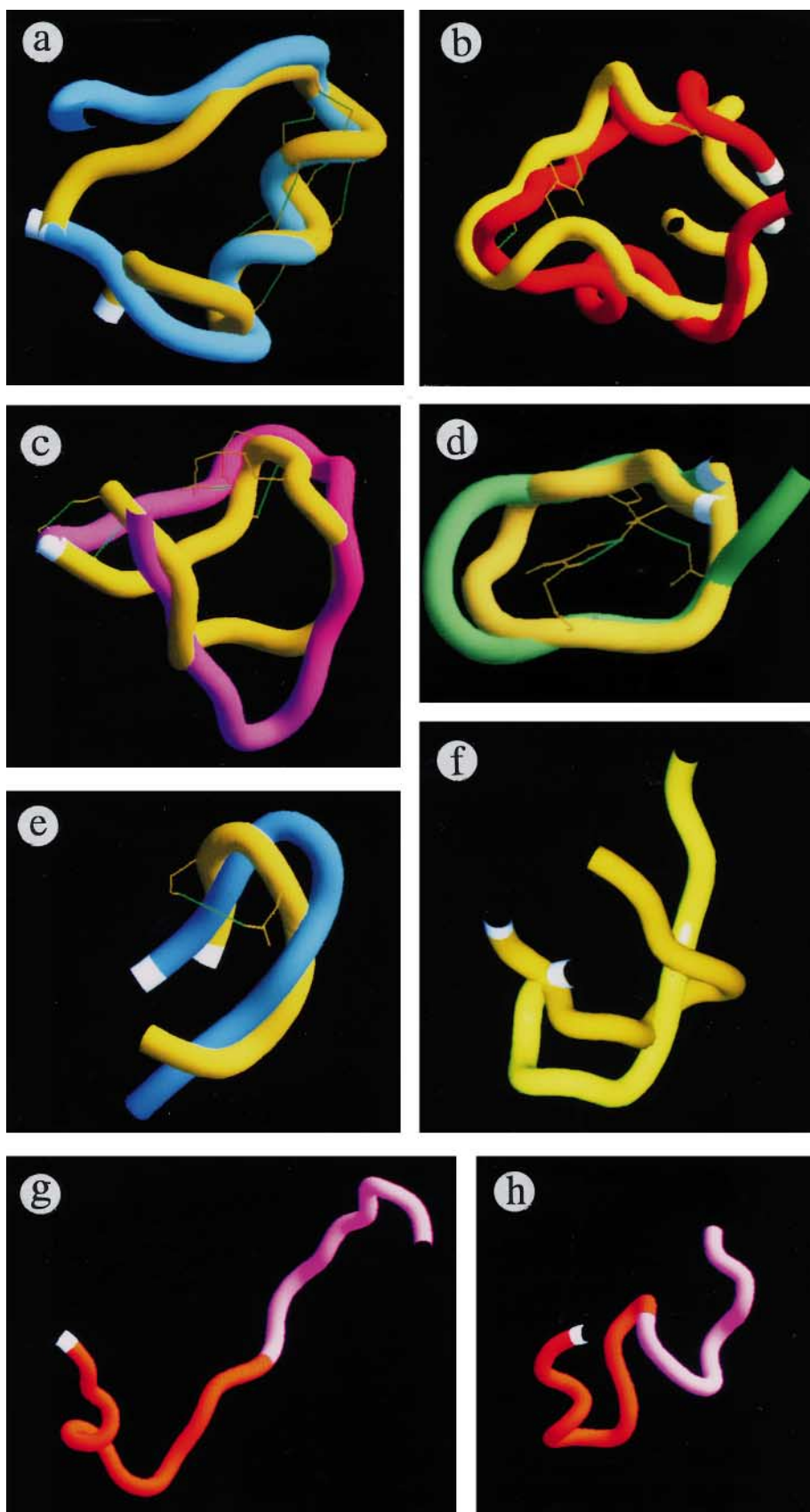
Conformations of fragments corresponding to modules or pseudo modules of barnase [18] were taken as the initial structures for simulations. To modules M1–M6, we assigned amino acid residues 1–24, 25–52, 53–73, 74–88, 89–98, and 99–110, respectively [22]. We used no overlapping residues. To pseudo modules P1–P5, we assigned residues 13–38, 39–62, 63–80, 81–93, and 94–104, respectively. These five pseu-

do modules correspond to pseudo modules m2–m6, respectively, defined by Noguti et al. [22]; the other pseudo modules m1 (3–12) and m7 (105–110) were not included in this study because they consist of only half of a module and thus cannot be compared with modules regarding conformational stability. Each polypeptide fragment was terminated at the peptide bond, without charged or capped ends. Lys, Arg, Asp and Glu were ionized. Each module or pseudo module was treated as being isolated in vacuo, except that a linear distance dependent dielectric function, $\epsilon = r$, was applied. Non-bonded interactions were included without cutoff. Each fragment was energy minimized until the root mean square (RMS) gradient of the potential was less than 0.01 kcal/mol per Å in the conjugate-gradient cycles. We found that the use of 0.1 kcal/mol per Å instead of 0.01 kcal/mol per Å as the criterion of convergence of energy minimization did not alter substantially the results of the following molecular dynamics simulations. Energy-minimized conformations were used as initial conformations of the molecular dynamics simulations. Molecular dynamics of individual modules and pseudo modules were carried out for 1 ns and 50 ps, respectively. The time steps were 0.25 fs. The

Fig. 2. Averaged simulation structures of modules and a pseudo module in vacuo and their corresponding X-ray crystal structures. Conformations were averaged over the trajectories in the last 500 ps of the 1 ns molecular dynamics of each module. The resultant average conformation was superimposed onto the corresponding part of the X-ray structure of barnase [18]. The mainchain folds are shown in tube models. X-ray structures of modules are in the same color as for Fig. 1. The N-terminus of each fragment is indicated in white. The simulated average conformation of a module is in dark yellow. Modules, M1–M6, are shown in (a–f), respectively. The conserved hydrogen bonds (see Section 2) are shown in green lines together with the related sidechain and mainchain. (g) X-ray structure of pseudo module P2, consisting of the C-terminal half of M2 (in red) and the N-terminal half of M3 (in magenta). (h) Average conformation of pseudo module P2, calculated from the last 25 ps trajectory of the 50 ps dynamics.

system temperature in the molecular dynamics was kept at 300 K by the heat-bath coupling method [24] with a coupling constant of 0.2 ps. Snapshot structures per ps in each simulation were stored as trajectory

and averaged over the last half-trajectory to obtain the averaged simulation structure. The AMBER (rev. 3a) energy parameters of united-atom model and programs [25] were used. We also carried out the



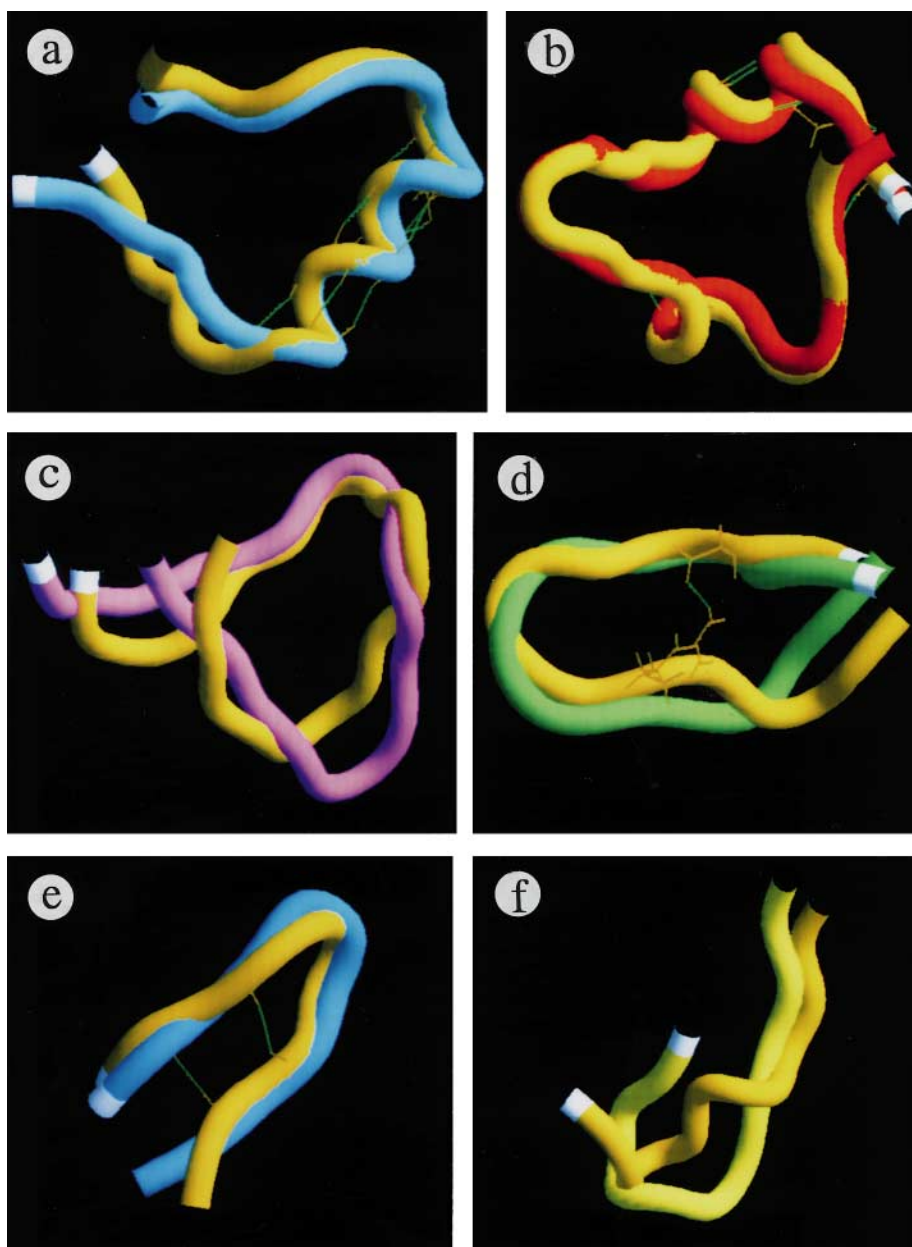


Fig. 3. Averaged simulation structures of modules in water and their corresponding X-ray crystal structures. Molecular dynamics simulations for 150 ps were performed for each module. Average conformation of each module in the last 50 ps trajectory is shown. The presentation by tubes, lines and coloring is the same as for Fig. 2.

same series of simulations using DREIDING energy parameters [26], the objective being to confirm the results obtained using AMBER energy parameters.

2.3. Solution simulation

Fragments corresponding to modules and pseudo modules were extracted from the X-ray crystal structure atomic coordinate of barnase [20] (BNL code: 1bgs) as initial structures for simulations. We used overlapping fragments consisting of residues 1–24, 24–52, 52–73, 73–88, 88–98, and 98–110, as modules M1–M6, respectively in the solution simulation. Overlapping of residues at the terminals was used in water simulation so as to be consistent with previous experimental studies [23,27]. Pseudo modules, P1–P5 consist of 12–38, 38–62, 62–80, 80–93, and 93–104, respectively. Both terminals of each polypeptide fragment were charged. Lys, Arg, Asp and Glu were ionized. Each fragment was energy minimized in vacuo with $\epsilon = r$ and no cut-off until the RMS gradient of potential was less than 0.1 kcal/mol per Å. The energy-minimized structures were spherically surrounded

by water molecules such that width of the water shell exceeded 12 Å. Radii of water spheres were 28 Å for modules and 29 to 36 Å for pseudo modules. Numbers of water molecules were about 3000 for modules and 3000–6000 for pseudo modules. Water molecules were restrained within the spheres by a soft half-harmonic potential with a force constant of 1.5, a dielectric constant of $\epsilon = 1$ and a cut-off of 12 Å were used for minimization. A dielectric constant $\epsilon = 1$, cut-off of 9 Å, and time steps of 1 fs were used for molecular dynamics. The temperature in the molecular dynamics was initially 0.1 K and was kept at 300 K by the heat-bath coupling method [24] with a coupling constant of 0.2 ps for solute and 0.4 ps for solvent. With solutes constrained, water molecules were energy minimized until the RMS gradient of potential was less than 1 kcal/mol per Å, and then subjected to 20 ps molecular dynamics simulation. All atoms of the systems were energy minimized until the RMS gradient of potential was less than 1 kcal/mol per Å. The resultant structures were used for the initial conformations of 150 ps molecular dynamics simulations of modules and pseudo modules in water. Snapshot structures per ps

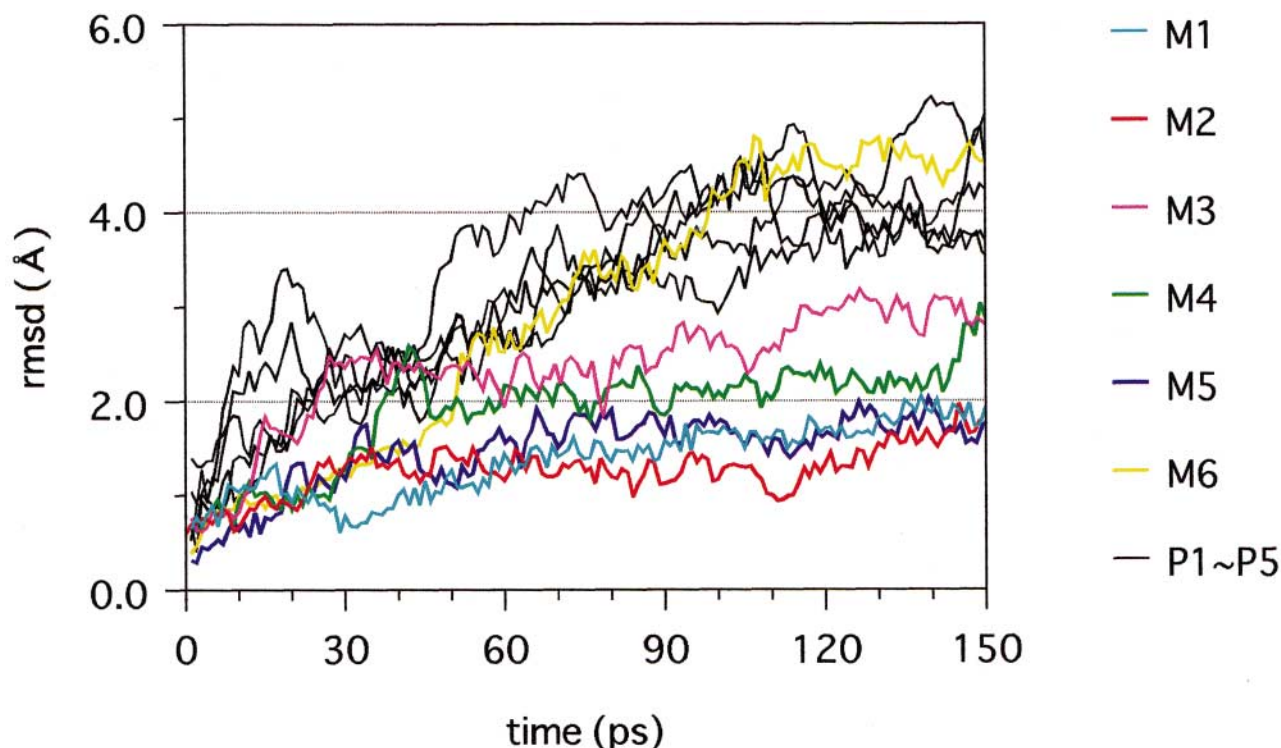


Fig. 4. Time courses of root mean square deviations (RMSD) of the C α atoms from the corresponding X-ray crystal structures in molecular dynamics simulation in water. The plot is drawn for each module (M1–M6) and pseudo module (P1–P5).

in each simulation were stored and averaged over the last 50 ps trajectory to obtain the averaged simulation structure. The AMBER (rev. 3a and 4) energy parameters of all-atom model and programs [28] were used.

2.4. Conserved hydrogen bonds

Hydrogen bond formation was defined under the following conditions; the distance between the hydrogen atom and the acceptor atom was less than 2.5 Å, and the angle between the chemical bond from the donor to the hydrogen atom and the hydrogen bond from the hydrogen atom to the acceptor exceeded 120°. For in vacuo simulation, we defined conserved hydrogen bonds during the simulation of each module as the hydrogen bonds formed in more than 30% of conformations in the last 500 ps trajectory of the simulation. For solution simulation, the conserved hydrogen bonds are defined as those formed in more than 80% of conformations in the last 50 ps trajectory.

3. Results

3.1. In vacuo

Five modules M1–M5, but not the C-terminal module M6, maintained native-like conformations through 1 ns simula-

tions in vacuo (Fig. 2a–e, Table 1). Relaxations of potential energy in the range of 40–115 kcal/mol were observed in the first 500 ps, and systematic relaxations were not observed in the last 500 ps, in all modules. This shows that the modules reached mechanically stable conformations in the first 500 ps. Root mean square deviations (RMSD) of the C α atom positions between X-ray structures of modules, M1–M5, and the average conformations in the last 500 ps trajectories of the simulations were in the range of 2.3–4.4 Å (Table 1). Main-chains in the average conformations of modules M1–M5 maintained compact conformations, similar to their native ones (Fig. 2a–e).

Hydrogen bonds important for specifying the native-like conformations of modules M1–M5 were conserved during the simulations (Fig. 2a–e). Particularly hydrogen bonds in turns and/or between the N- and C-termini of each module, which are important to maintain compactness, were conserved. Hydrogen bonds Lys³⁹ N–Val³⁶ O, Gly⁴⁰ N–Ala³⁷ O and Asn⁴¹ N^δ–Gly³⁴ O stabilizing the turn consisting of residues 36–40 in the center of M2 were observed in more than

Table 1
Root mean square deviations (RMSD) of the simulated conformations of modules and pseudo modules in vacuo from the X-ray structures

Modules	RMSD (Å)	Pseudo modules	RMSD (Å)
M1 (1–24)	2.3	P1 (13–38)	8.2
M2 (25–52)	4.4	P2 (39–62)	8.3
M3 (53–73)	3.4	P3 (63–80)	8.9
M4 (74–88)	3.0	P4 (81–93)	8.5
M5 (89–98)	2.6	P5 (94–104)	5.0
M6 (99–110)	4.7		

Conformations in the trajectories were averaged in the last 500 (25) picoseconds of the one nano (50 pico) second molecular dynamics of each module (pseudo module). N- and C-terminal residue numbers of modules (pseudo modules) are given in parentheses. The RMSD of C α atom positions of each module (pseudo module) was calculated between the resultant average conformation and the corresponding X-ray structure [18].

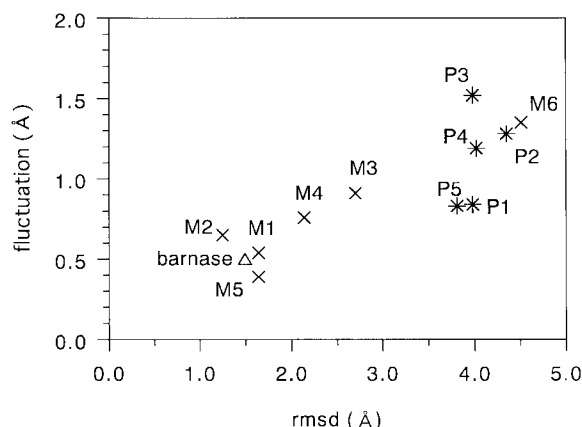


Fig. 5. Conformational fluctuation vs. RMSD from the X-ray structures for modules (M1–M6), pseudo modules (P1–P5) and barnase for the molecular dynamics simulations in water. Average and standard deviations of atomic coordinates were calculated over the conformations in the last 50 ps trajectory of each simulation. Mean of the standard deviations of the C α atoms around the averaged positions (fluctuation) is plotted for each module (×) and pseudo module (*) against root mean square deviation (RMSD) of the C α atoms of the averaged structures from the corresponding X-ray structures. For reference, plot of fluctuation vs. RMSD for the last 50 ps trajectory of 100 ps molecular dynamics simulation of barnase (Δ) is given.

88% of conformations in the last 500 ps trajectory of the simulation. The N- and C-termini of M3 form a part of the anti-parallel β -sheet in the native conformation. Two hydrogen bonds in this part of the β -sheet, Gly⁵³ N–Glu⁷³ O and Glu⁷³ N–Asp⁵⁴ O, were retained in about 40% of the simulated conformations. The hydrogen bond between sidechains of Glu⁶⁰ and Lys⁶² stabilizing a β -turn of M3 was conserved completely. The compact form of M4 was maintained by three conserved hydrogen bonds Asn⁷⁷ N ^{δ} –Asp⁸⁶ O ^{δ} , Arg⁸³ N ^{θ} –Asp⁷⁵ O ^{δ} 1 and Arg⁸³ N ^{ϵ} –Asp⁷⁵ O ^{δ} 2; they contributed to maintaining of the N- and C-terminal halves of M4 in close proximity. The β -turn consisting of residues 91–94 in the center of M5 was stabilized by the hydrogen bond Ser⁹¹ O ^{γ} –Asp⁹³ O ^{δ} , which was formed in 32% of the simulated conformations. To maintain the compact conformation of M1, helix α 1 must be retained. The hydrogen bonds in helix α 1 were formed in 35–99% of conformations in the last 500 ps trajectory of the simulation.

Secondary structures observed in the native conformations of the modules were not always maintained in the simulations. The helix α 1 in M1 and a part of the anti-parallel β -sheet in M3 were maintained as described above. Each of the hydrogen bonds Gln³¹ N–Lys²⁷ O and Leu³³ N–Ala³⁰ O of helix α 2 in M2 were retained 96% of the time course. Unexpectedly, helix α 3 in M2 melted. Hydrogen bonds of the parallel β -sheet in M2 and those of some parts of the anti-parallel β -sheet in M4 and M5 were not conserved.

Module boundaries tend to be situated in the interior of proteins. Therefore, the N- and C-terminal residues of the modules tend to be located in close proximity. However, the N-terminus of the first module and the C-terminus of the last module of the protein are not always buried in the interior, since protein termini are often exposed to solvent. Therefore, each of the termini of the first and last modules of a protein are positioned at a distance. Hence, some modules at the N-

or C-termini of proteins are less compact than the others. The C-terminal module M6, which is less compact in the native state than the other modules, folded into a substantially compact form in the simulation (Fig. 2f).

In contrast to modules, all pseudo modules did not maintain native conformations even during 50 ps. The native conformation of a pseudo module is of an extended form, as expected from its definition (Fig. 2g). Extended conformations of pseudo modules collapsed into compact forms in the simulations (Fig. 2h). RMSD of C α atom positions between their X-ray structures and the averaged conformations of the simulated trajectories were in the range of 5.0–8.5 Å (Table 1).

Qualitatively similar results were obtained from both series of molecular dynamics simulations, using the different energy parameters, AMBER and DREIDING. Thus, the results are not affected by particular energy parameters and/or initial conditions.

3.2. In water

As in the in vacuo simulation, modules M1–M5 maintained native-like conformations through solution simulations (Fig. 3a–e), while all pseudo modules and M6 (Fig. 3f) were deformed. The time courses of RMSD of the simulated structures of the modules and pseudo modules from their initial structures (their corresponding X-ray crystal structures) are shown in Fig. 4. The RMSD values for modules M1–M5 seem to become equilibrated in about 50 ps with asymptotic values of around 2.0 Å. For M3, the RMSD asymptotically approaches 3.0 Å after about 100 ps. The RMSD values for all pseudo modules and M6 seem to become equilibrated within about 100 ps with an asymptotic value of around 4.0 Å. Fig. 5 shows RMSD values of the averaged structures over the last 50 ps trajectories from the X-ray crystal structures in the horizontal axis, and the standard deviations (fluctuations) from the averaged atom positions in the vertical axis. There are essentially two clusters; one consisting of modules M1–M5 has smaller RMSD (1.2–2.7 Å) and fluctuation (0.4–0.9 Å), while the other consisting of module M6 and all pseudo modules P1–P5 has larger RMSD (3.8–4.5 Å) and fluctuation (0.8–1.5 Å). The RMSD and fluctuation values of the former cluster are comparable with those of barnase (RMSD, 1.5 Å; fluctuation, 0.5 Å). Therefore, the structural stability of M1–M5 in the 150 ps molecular dynamics is comparable with that of barnase.

The conserved hydrogen bonds of the modules during the last 50 ps of the solution simulations are shown in Fig. 3. Like the simulation in vacuo, hydrogen bonds in turns and/or between the N- and the C-termini of the modules, which are important for stabilizing compact conformations, modules, were conserved. The hydrogen bonds Ile²⁵ N–Ser⁵⁰ O connecting both ends of M2 and Val³⁶ N–Asn⁴¹ O ^{δ} around the central turn of M2 were retained for 90 and 74%, respectively, of the last 50 ps of the simulation. For M4, a long-range hydrogen bond Arg⁸³ N ^{θ} –Asp⁷⁵ O ^{δ} linking the N- and C-terminal halves of module M4 showed 86% retention. Two of four hydrogen bonds in the anti-parallel β -sheet in M5, Ser⁹¹ N–Leu⁹⁵ O and Tyr⁹⁷ N–Leu⁸⁹ O, were kept for 80 and 92%, respectively, of the simulation time, although the hydrogen bonds in the β -turn of M5 were not conserved. The hydrogen bonds in helix α 1 which is essential for maintaining the compact conformation of M1 were well conserved in simulation in water as well as in vacuo. M3 is an exception, behaving differ-

ently in water from in vacuo; no hydrogen bond was observed within M3 in the last 50 ps trajectory of simulation in water.

4. Discussion

Modules M1–M5 of barnase all retained their respective native-like conformations during the 1 ns in vacuo and during the 150 ps in water molecular dynamics simulations. Thus, they are mechanically stable both in vacuo and water. On the other hand, pseudo modules, which are extended segments, became deformed with simulations. The compactness of modules is thereby reflected in their conformational stability.

Secondary structures are stabilized mechanically by hydrogen bonds and are therefore regarded as structural elements of globular domains. On the other hand, protein modules are defined as compact structural elements of a globular domain. As in the case of barnase, where the boundaries of M1–M2, M3–M4, M4–M5 and M5–M6 are on the β -sheet, many module boundaries are located on α -helices or β -sheets [2–4,6]. For this reason, the question arose as to whether it was proper to consider modules as structural elements of proteins [29]. Our present study shows that modules are indeed mechanically stable structural elements.

The present study and the close correlation of module boundaries with intron positions in many proteins ([1–10] and unpublished data) suggest that modules behaved as building blocks to produce new proteins through exon shuffling or fusion, during evolutionary processes [4,12,13]. However, there is no evident correlation between the secondary structure and intron positions [15,16]. As a recruited block, it is essential for each module to be structurally stable so as to maintain a compact conformation in various combinations of modules. The mechanical stability of modules shown in our study supports our hypothesis of protein evolution, from structural and mechanical points of view.

In barnase, hydrogen bonds are localized mainly within modules [22]. In the present simulation about half the number of these hydrogen bonds were conserved (Figs. 2a–f and 3). Hydrogen bonds in turns and/or between the N- and C-termini of each module were conserved.

To determine the main contribution to mechanical stability of modules, we carried out another series of 1 ns molecular dynamics simulations of barnase modules in vacuo with modified potential functions in which ionic charge and hydrogen bond were eliminated [30]. Four of the six modules, M1, M2, M4 and M5, retained their native-like conformations, whereas M3 and M6 became deformed during the simulation. The observed mechanical stability of modules in the absence of ionic charge and hydrogen-bond interactions demonstrates that van der Waals and non-ionic partial charge interactions are sufficiently strong for most modules to be mechanically stable. Because these interactions are weak per atomic pair and only effective over a short distance, a sufficient number of atomic pairs must be in contact to stabilize the conformations by only these short-range interactions. In fact, the number of atomic pairs interacting in a module is not small because of its compact conformation. Thus, the compactness of modules at the atomic level is essential for the mechanical stability of modules.

The isolated modules of barnase maintained their native-like conformations in water for 150 ps, which indicates that

these modules are also mechanically stable in water, but it does not mean that these modules should form thermodynamically stable native-like conformations in solution, since the simulation time is too short to realize a state of thermodynamic equilibrium. Solution structures of isolated M2 and M3 of barnase were studied using 2D-NMR, and some native secondary structures were observed [27]. However, they did not maintain native-like 3D structures in solution; rather they seemed to have a mixture of conformations. Based on this observation, sufficiently long simulations should lead to large structural deviation of the modules. There should not be any clear difference in thermodynamic stability between modules and pseudo modules. However, we found that there were differences in mechanical stability between modules and pseudo modules. In other words, each native conformation of modules was at so deep a local energy minimum that the conformation was retained for at least 150 ps, even if the segment was dissected and isolated in water. In contrast, each native conformation of pseudo modules was at a shallow local minimum or was not at a local energy minimum so that the conformation was not retained in the 150 ps simulation.

The isolated modules in solution are not thermodynamically stable probably because their hydrophobic patch on the module surface involved in module-module interactions in intact barnase becomes exposed to solvent. Contributions of hydrophobic interactions to the thermodynamic stability of native conformations of barnase have been reported [31]. Hydrophobic cores are mainly formed between modules in the native conformation of barnase [22]. Protein structures and functions are the products of molecular evolution during the past 3.5 billion years. During early periods of evolution, independent primordial modules encoded by mini genes could exist in mechanically and thermodynamically stable conformations in solution. After being recruited into a globular domain, hydrophobic interactions between modules should have been elicited by amino acid replacements to stabilize the assembly. Thus, the thermodynamic conformational stability of modules isolated in solution would have been lost in the evolutionary process. The compactness and mechanical stability of the original building blocks may well be a remnant still existing as modules in contemporary proteins.

To determine whether the mechanical stability of modules is common to other proteins, we performed a similar molecular dynamics simulation in vacuo for four proteins, myohemerythrin, immunoglobulin heavy chain variable domain, flavodoxin and lysozyme. We selected each one as representative of α -, β -, α/β - and $\alpha+\beta$ -type proteins, respectively. As most of the modules were mechanically stable (unpublished data), mechanical stability of modules seems common to a variety of proteins. When designing new proteins, a combination of modules may prove to be a useful technique. The mechanical stability of modules provides insight into such building blocks.

We conclude, from the molecular dynamics simulation, that most modules of barnase are mechanically stable. This was unexpected since these structural units are characterized independently of the secondary structures. The mechanical stability of modules means that a module is a stable structural element of protein architecture. The present study provides a physico-chemical basis for the view that modules were original building blocks of evolving proteins, as well as the corre-

lation between modules and exons observed in various proteins.

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