Dynamic Properties of the N-Terminal Swapped Dimer of Ribonuclease A

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ABSTRACT Bovine pancreatic ribonuclease (RNase A) forms two 3-dimensional domain-swapped dimers with different quaternary structures. One dimer is characterized by the swapping of the C-terminal region (C-Dimer) and presents a rather loose structure. The other dimer (N-Dimer) exhibits a very compact structure with exchange of the N-terminal helix. Here we report the results of a molecular dynamics/essential dynamics (MD/ED) study carried out on the N-Dimer. This investigation, which represents the first MD/ED analysis on a three-dimensional domain-swapped enzyme, provides information on the dynamic properties of the active site residues as well as on the global motions of the dimer subunits. In particular, the analysis of the flexibility of the active site residues agrees well with recent crystallographic and site-directed mutagenesis studies on monomeric RNase A, thus indicating that domain swapping does not affect the dynamics of the active sites. A slight but significant rearrangement of N-Dimer quaternary structure, favored by the formation of additional hydrogen bonds at subunit interface, has been observed during the MD simulation. The analysis of collective movements reveals that each subunit of the dimer retains the functional breathing motion observed for RNase A. Interestingly, the breathing motion of the two subunits is dynamically coupled, as they open and close in phase. These correlated motions indicate the presence of active site intercommunications in this dimer. On these bases, we propose a speculative mechanism that may explain negative cooperativity in systems preserving structural symmetry during the allosteric transitions.

INTRODUCTION

Recent structural investigations have demonstrated that proteins frequently exchange domains with identical partners (three-dimensional domain swapping; Liu and Eisenberg, 2002). Specifically, in a three-dimensional domain-swapped dimer, a domain of one subunit replaces the identical domain of the other subunit, and vice versa. According to Eisenberg's definitions (Bennett et al., 1995; Schlunegger et al., 1997), the interface between domains that exists in both the monomer and the dimer is termed *closed interface* (C-interface) and the interface that is present only in the dimer is termed *open interface* (O-interface). Obviously, the segment that links the two domains (hinge loop) presents a different conformation for the monomer and the dimer.

Three-dimensional domain swapping has been invoked to explain several biological processes. In particular, it has been proposed that three-dimensional domain swapping plays a role in the evolution of monomeric proteins toward oligomeric states (Bennett et al., 1995; Schlunegger et al., 1997). Furthermore, it has been shown that three-dimensional domain swapping plays a role in important biological functions such as the modulation of enzymatic activities (Piccoli et al., 1992; Vitagliano et al., 1999) and immunosuppressive and antitumoral properties (Cafaro et al., 1995). Finally, it has been suggested that three-dimensional domain swapping can be a possible mechanism for protein aggregation, including the formation of amyloid fibrils (Liu et al., 1998, 2001; Sinha et al., 2001).

Pancreatic-like ribonucleases deserve a special position among three-dimensional domain-swapped proteins. Forty years ago Crestfield et al. (1962) inferred the occurrence of domain swapping from biochemical data on the two noncovalent dimers of bovine pancreatic ribonuclease (RNase A) obtained under lyophilizing conditions. Moreover, bovine seminal ribonuclease (BS-RNase), which is highly homologous to RNase A, presents the unique feature of forming two equilibrium species: the swapped (M×M) (Mazzarella et al., 1993) and the nonswapped (M=M) dimers (Piccoli et al., 1992). Pancreatic-like ribonucleases have also been used as model systems in the first energetic characterizations of the three-dimensional domain-swapping process (Mazzarella et al., 1995). Finally, very recent crystallographic studies on RNase A (Liu et al., 2001, 1998) and human pancreatic ribonuclease (H-RNase) (Canals et al., 2001) dimers have revealed unexpected novel quaternary structure assemblies. Interestingly, the two RNase A dimers present different swapping domains: one dimer (C-Dimer) swaps its C-terminal β-strands (Liu et al., 2001), whereas the other (N-Dimer) is formed by interchanging the N-terminal α-helices (Liu et al., 1998). In addition, they have different quaternary structures: the N-Dimer presents a very compact

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Abbreviations used: MD-NDimer, average structure of the N-terminal swapped dimer of bovine pancreatic ribonuclease derived from the molecular dynamics simulation; X-NDimer, x-ray structure of the N-terminal swapped dimer of bovine pancreatic ribonuclease.

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structure (Liu et al., 1998), whereas C-Dimer only shows a very loose O-interface (Liu et al., 2001) (see also Fig. 1, this article).

Although more than 30 domain-swapped protein structures have so far been reported (Liu and Eisenberg, 2002; Rousseau et al., 2003), our current knowledge of the dynamic properties of domain-swapped systems is limited. In this context, molecular dynamics (MD) studies of monomeric counterparts of domain-swapped enzymes have been carried out to gain insight into the structural mechanism of the swapping (Alonso et al., 2000; Ding et al., 2002). Information on the dynamic properties of three-dimensional swapping is even scarcer. Indeed, the first detailed MD analysis of a swapped dimer has been reported very recently (Sekijima et al., 2003).

To provide additional data in this field, we here report a molecular dynamics/essential dynamics (MD/ED) analysis of RNase A N-Dimer. This system is particularly indicated to analyze the effect of the three-dimensional domain swapping on the dynamics of the dimer since RNase A dynamics have been extensively characterized (see, for example, Nadig and Vishveshwara, 1997; Vitagliano et al., 2002; Merlino et al., 2002, 2003). We show that three-dimensional domain swapping has a marginal effect on the dynamics of the active site residues, although they belong to different subunits. Furthermore, each subunit retains the functional breathing motion (Vitagliano et al., 2002; Merlino et al., 2002, 2003) of RNase A. Notably, our data indicate that the motions of the two subunits are dynamically coupled. This coupling suggests the presence of a communication between the two active sites of the enzyme.

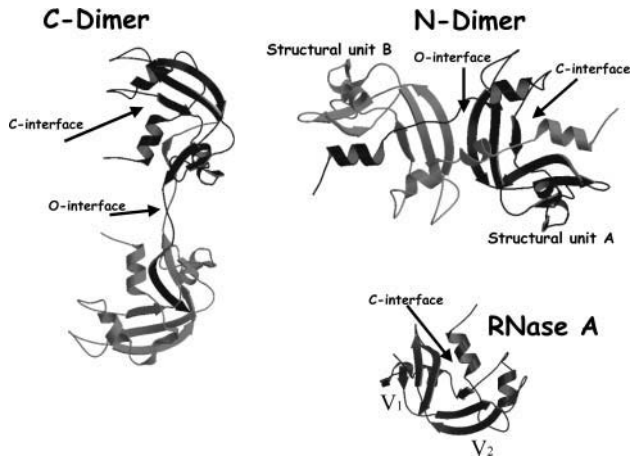

FIGURE 1 Ribbon diagrams of RNase A dimers. The structural unit formed by the body of the subunit A and the N-terminal α-helix of the subunit B has been termed *structural unit A*, whereas that formed by the body of the subunit B and the N-terminal α-helix of the subunit A has been termed *structural unit B*.

METHODS

System

The N-Dimer is constituted by two subunits each composed by three α-helices and a V-shaped β-sheet (Fig. 1). The β-sheet is constituted by two arms that will be hereafter denoted as V_1 and V_2. In particular, V_1 is made up of residues 61–63, 71–75, 105–111, and 116–124 and V_2 of residues 42–46, 82–87, and 96–101 (Wlodawer et al., 1983). The three helices are located in the N-terminal portion of the protein and encompass residues 3–13 (helix I), 25–35 (helix II), and 50–60 (helix III). The structure is characterized by the swapping of the N-terminal helices. The C-interface, therefore, consists of helix I of one subunit with the body (residues 25–124) of the other subunit.

Following the notation commonly used for three-dimensional domain-swapping proteins, the region (residues 16–22) connecting the body of each subunit with its N-terminal helix is denoted as hinge peptide. Since active site residues belong to both helix I and β-sheet regions, the N-Dimer contains two composite active sites. Therefore, in the N-Dimer two different structural units with functional capability, each composed by the body of one subunit and the N-terminal helix of the other, may be identified. The V_2 arms of the two subunits are involved in the interactions that characterize the O-interface. Notably, the union of the two arms produces a continuous six-stranded β-sheet. The O-interface is additionally stabilized by the interactions established by the hinge peptide of one subunit with residues of the other subunit (hinge-body interactions). It is worth noting that the hinge peptides adopts different conformations, either extended or α-helical, in the two subunits. The subunit whose hinge peptide adopts an extended conformation has been termed *A* throughout the article and the subunit with an α-helical hinge peptide has been termed *B*.

Simulation procedure

Molecular dynamics simulations were performed using the program GROMACS (van der Spoel et al., 1994). The structure of the N-Dimer (Protein Data Bank entry code 1a2w), refined at 2.1 Å resolution, was used as the starting model for the simulation (Liu et al., 1998). The dimer was immersed in a rectangular box of $52 \times 62 \times 49$ Å^3 containing 7126 water molecules. As for RNase A (Merlino et al., 2002) and human angiogenin (Merlino et al., 2003), the ionization state of charged residues was set to mimic a neutral pH environment. Lys and Arg residues were positively charged, Asp and Glu were negatively charged. All His residues were uncharged, following the indications of the atomic resolution studies carried out on RNase A (Berisio et al., 1999, 2002). In particular, His-12 and His-48 were protonated at $N^{\delta 1}$, whereas His-119 and His-105 were protonated at $N^{\varepsilon 2}$. As a result of these assumptions, the net charge of the dimer was +8. To make the overall system neutral, eight water molecules were replaced by chloride counterions. The simulation was, therefore, carried out on a system containing 2462 protein atoms, 7126 water molecules, and 8 chloride ions (23,848 atoms).

The MD simulations were carried out by using the same protocol applied for the simulation of RNase A (Merlino et al., 2002) and other members of the pancreatic-like superfamily (Merlino et al., 2003). To allow relaxation of solvent molecules, the energy of the system was preliminarily minimized by keeping the protein atoms fixed. The resulting system was submitted to 20 ps of molecular dynamics at 300 K. The energy of the system was then minimized, before starting constant temperature molecular dynamics at 300 K. The overall timescale of the simulation was 3 ns. All bond lengths were constrained using SHAKE algorithm (Ryckaert et al., 1977). Nonbonded cutoffs of 10 Å for Lennard-Jones and 13 Å for Coulomb potentials were used. A dielectric constant of $\varepsilon = 1$ and a simple-point-charge (Berendsen et al., 1981) water for the solvent molecules model were used in the simulation. An integration time step of 0.002 ps was used. The coordinates were saved every 0.2 ps.

An additional 3-ns simulation at 300 K using the particle-mesh Ewald method (Darden et al., 1993) to calculate electrostatic interactions for atoms at a distance >9 Å was also performed. As observed for other protein

systems (Ceruso et al., 2003; Merlino et al., 2003; Roccatano et al., 2003), the results were in good agreement with the cutoff method.

Analysis of the trajectory

The trajectory was checked to assess the quality of the simulation using GROMACS routines (van der Spoel et al., 1994) and in-house programs. To examine the collective motions in the MD simulations, the essential degrees of freedom were extracted according to the ED method (Amadei et al., 1993). This method is based on the construction of the covariance matrix of the coordinate fluctuations. The matrix was then diagonalized to obtain the eigenvectors and eigenvalues that provide information about collective motion in the protein. The eigenvectors are then sorted according to their eigenvalues in descending order. Usually, the first 10–20 eigenvectors suffice to describe most (80–90%) of the dynamic fluctuations in the protein. The covariance matrix of the positional fluctuations was constructed using only C^{α} atoms.

To determine the coupling between intrasubunit motion in the dimer, correlation matrices were built as previously reported (Ceruso et al., 1999a; Merlino et al., 2002). The matrices were constructed after an independent fit of each structural unit trajectory on the same reference, to eliminate the contribution of relative subunit motions. Cross-correlations between different subunits were calculated and normalized as $C_{ij} = \langle \Delta R_i \times \Delta R_j \rangle / (\langle \Delta R_i \times \Delta R_i \rangle \langle \Delta R_j \times \Delta R_j \rangle)^{1/2}$ with C_{ij} values that vary in the region $-1 < C_{ij} < 1$.

The similarity of the internal fluctuations between the structural units in the dimer was evaluated by comparing principal subspace (first 10 eigenvectors) of each structural unit trajectory by using the root mean-square inner product (RMSIP) between the first 10 eigenvectors as previously described (Amadei et al., 1999; Ceruso et al., 1999a,b, 2003; Merlino et al., 2003; Roccatano et al., 2003). The RMSIP is defined as

$$\sqrt{\frac{1}{10}\sum_{i=1}^{10}\sum_{j=1}^{10}(\eta_i^a \nu_j^b)^2},$$

where η_i^a and ν_j^b are the i^{th} and j^{th} eigenvectors of the two different sets, respectively.

Similarly, the consistency between the motions of each N-Dimer structural unit and RNase A was evaluated by estimating the overlap of the essential subspaces of each subunit by using RMSIP.

To identify hydrogen bonds, both donor-acceptor (D-A) distances (<3.5 Å) and D-H$\cdots$A angles (>110°) were checked.

RESULTS AND DISCUSSION

Structural stability

Several time-dependent properties of the N-Dimer were analyzed to ascertain the stability of the MD simulation. The root mean-square deviation (RMSD) of the coordinates during the simulation versus the starting x-ray structure (X-NDimer) (Liu et al., 1998) as function of time is reported in Fig. 2 *A*.

Some fluctuations are observed for the C^{α} atoms of the whole dimer during the simulation. However, the bodies (residues 25–124) of the two subunits appear to be rather equilibrated after 1200 ps. In this region, the RMSD for the dimer (~3.5 Å) is significantly larger than the RMSD observed for the bodies of the two subunits (~2.0 Å). This finding indicates that a rearrangement of the quaternary structure occurs during the simulation (see below). The time evolutions of the secondary structure elements (Fig. 2 *B*) and of the total number of hydrogen bonds (Fig. 2 *C*) indicate that each subunit maintains its correct globular folding.

To ensure that the structural and dynamic properties of N-Dimer derived from the simulation are free from initial nonequilibrium effects, the MD/ED analyses were performed in the portion of trajectory from 1200 to 3000 ps. In this region, the essential subspace converged as revealed by calculating the RMSIP value (see Methods) between the two halves of the trajectory (Table 1).

The average MD conformation of N-Dimer is slightly more compact than its x-ray structure

To characterize the domain reorientation in the N-Dimer during the simulation, an average structure (MD-NDimer) from the equilibrated portion of the trajectory was calculated. The C^{α} atom RMSD of the MD-NDimer compared to crystallographic structure (X-NDimer) is 3.3 Å. Separate superimpositions of the body of the A and B subunits yield lower RMSD (1.6 Å). Furthermore, when a single body of the MD-NDimer is superimposed onto the X-NDimer, the other body must be rotated ~27° to best fit X-NDimer. After this rotation, the RMSD of the second body reduces from 5.7 Å to 1.6 Å. The radius of gyration of the C^{α} atoms (Fig. 2 *D*) and the total accessible surface area (Fig. 2 *E*) suggest that MD-NDimer has a slightly more compact structure compared to X-NDimer.

Deviations

The RMSD per residue (Fig. 3) shows that deviations from the X-NDimer are very similar in the two subunits. As expected, larger values take place in the loop regions (residues 1–4, 36–40, 64–70, and 86–94), whereas very small deviations can be detected for the secondary structure segments. Significant differences between the two subunits are located in the hinge loop regions (residues 16–22). This is not surprising since the hinge peptide regions exhibit very different conformations in the two subunits of X-NDimer (Liu et al., 1998): in the subunit A, the hinge peptide adopts an extended conformation, whereas in the subunit B it is an α-helix.

Fluctuations

To describe the mobility about the mean conformation, C^{α} root mean-square fluctuations were evaluated for each residue of the polypeptide chain (Fig. 4, *A* and *B*). As observed for RNase A (Merlino et al., 2002), secondary structure regions show low mobility during the simulation, whereas pronounced fluctuations are observed for loop residues (1–4, 14–16, 36–39, 48–52, 66–71, 88–90, and 110–116). Fig. 4 also shows that fluctuations are similar in the two subunits.

The 16–22 loop shows very small deviations (Fig. 3) and fluctuations (Fig. 4 *B*) during the simulation in the subunit B, whereas it appears more mobile in the subunit A (Fig. 4 *A*).

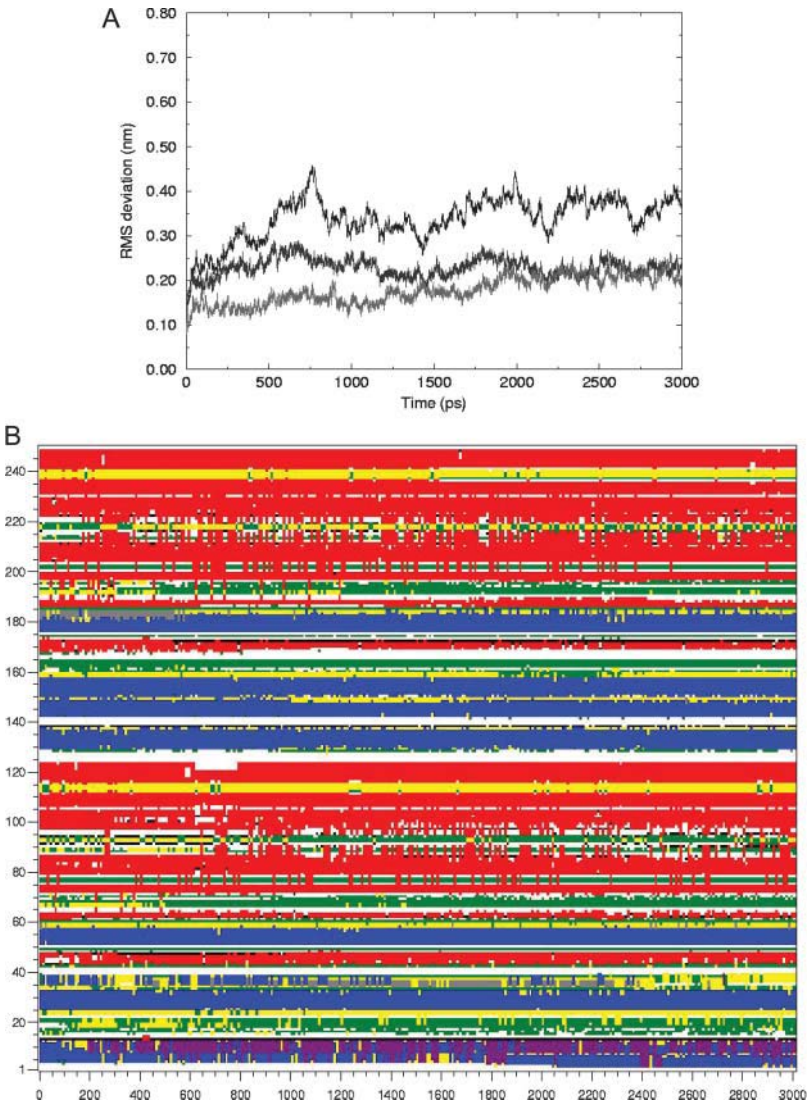

FIGURE 2 Time behavior of some geometrical properties during the simulation. (*A*) Root mean-square positional deviations from the x-ray starting structure computed on C^{α} atoms of the dimer (*black line*) and of the bodies of the two subunits (*dark shaded* and *shaded lines*). (*B*) Secondary structure of each residue. The α-helices are shown in blue, the 3_{10} helices in pink, the β-strands in red, the β-bridges in black, the bends in green, and the turns in yellow boxes. (*C*) Total number of hydrogen bonds. (*D*) Gyration radius. (*E*) Total solvent-accessible area.

This is in agreement with the experimental finding that subtilisin preferentially attacks the susceptible bond 21–22 of one of the two N-Dimer subunits (Nenci et al., 2001).

The root mean-square fluctuation derived from the simulation agrees well with the atomic mobility of X-NDimer as derived from B-factors (Fig. 4, *A* and *B*). The most significant differences are located in the region 60–70 of the subunit B (Fig. 4 *B*), but this is not surprising because the analysis of the crystal packing of X-NDimer reveals that this region is involved in close contacts with symmetry-related molecules.

O-interface motions: β-sheet-to-β-sheet interactions

The association of the two subunits in X-NDimer leads to the formation of a six-stranded β-sheet. The three strands of the two subunits are linked by two hydrogen bonds between residues belonging to the two subunits (Fig. 5 *A*). In particular, the N and O atoms of Gln-101 of the subunit A interact with the O and N atoms of Thr-99 of the subunit B, respectively. The contribution of these hydrogen bonds to the stabilization of the O-interface is corroborated by the time evolution of these interactions throughout the simulation. As shown in Fig. 5 *B*, these hydrogen bonds are well retained during the simulation (100% lifetime). The analysis of the trajectory reveals that additional hydrogen bonds may be formed at the O-interface. In particular, a rather stable hydrogen bond is formed by the O atom of Thr-99 of subunit A and the N atom of Gln-101 of the subunit B (100% lifetime) and by the O Asn-103 atom of the subunit A (Fig. 5 *C*) and the $O^{\gamma 1}$ atom of the Thr-87 of subunit B (~80% lifetime). In addition, a transient interaction (~20% lifetime) is observed between the N atom of subunit A Thr-99 and the oxygen atom of subunit B Gln-101.

Interestingly, the O-interface is also characterized by a hydrogen bond between the side chains of Gln-101 residues of the two subunits (Fig. 5 *C*). The conservation throughout the simulation of this hydrogen bond suggests

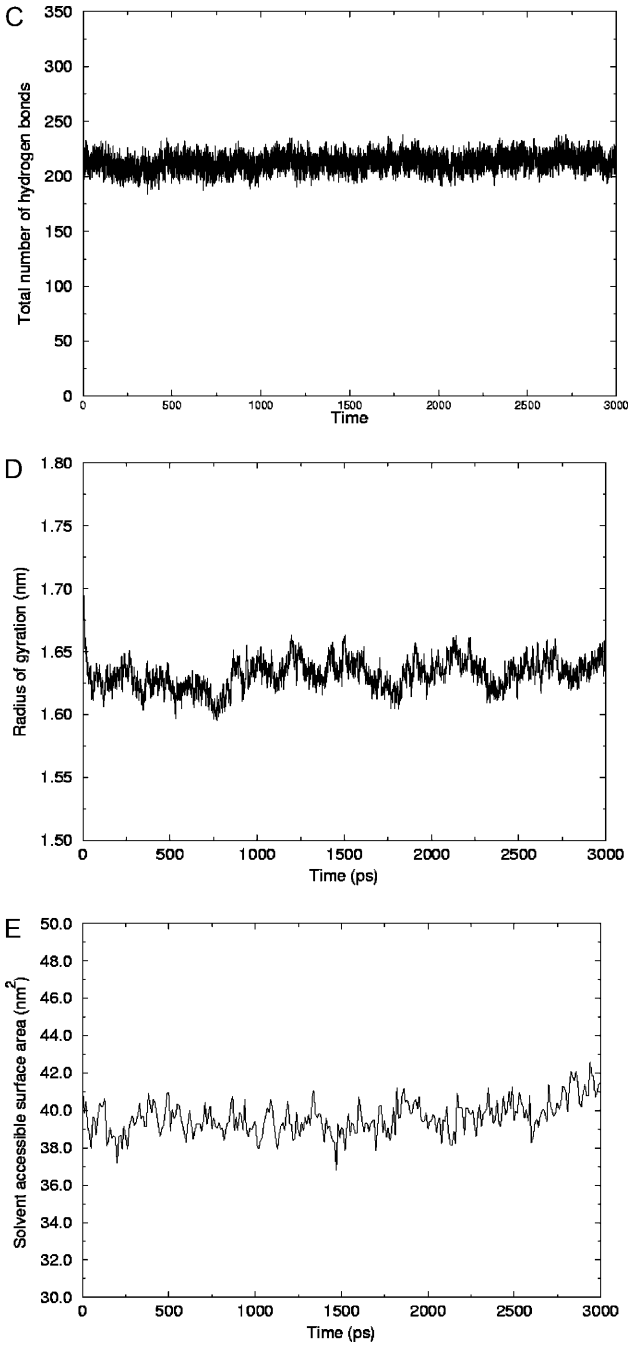

FIGURE 2 Continued

that this side chain-to-side chain interaction may contribute to the overall β-sheet stability, in agreement with the polar zipper hypothesis on the role played by Gln side chains in amyloid fiber formation proposed by Perutz (1999).

Taken together, these findings show that the MD-NDimer quaternary structure involves stronger interactions at the dimeric O-interface. These results suggest that crystal packing forces may prevent the optimization of O-interface interactions in X-NDimer.

TABLE 1 Root mean-square inner products for projection of the first 10 (C^α) eigenvectors of a set onto the first 10 of the other

	N-Dimer, structural unit A	N-Dimer, structural unit B	RNase A
N-Dimer, structural unit A	0.75*	0.70	0.68
N-Dimer, structural unit B		0.77*	0.74
RNase A			0.78*

*These values refer to the RMSIP obtained by comparing the two halves of the corresponding trajectory.

O-interface motions: hinge-body interface interactions

The hinge peptides of the A and B subunits of the X-NDimer form different intersubunit interactions with the rest of the protein. The x-ray structure has shown that the hinge peptide of the subunit A interacts with the V_2 arm of the subunit B through the hydrogen bonds that the O atom of Thr-17 establishes with $N^{\delta 1}$ atom of His-48 and that the O^{γ} atom of Ser-21 forms with $N^{\varepsilon 2}$ atom of Gln-101. By contrast, the hinge peptide of the subunit B is anchored to the V_2 arm of the subunit A by hydrogen bonds formed by the O atom of Ala-20 with the O^{γ} atom of Ser-80, by the O^{γ} atom of Ser-22 with $N^{\varepsilon 2}$ atom of Gln-101 and by the O^{γ} atom of Ser-23 with O of Ile-81. These interactions, though transient in some cases, are observed during the simulation (data not shown). Therefore, the MD analysis confirms that two hinge peptide regions have different conformations and different patterns of hydrogen bonds in the two subunits despite the identity of their sequences. Differences in the hinge peptide conformations have been also reported in other three-dimensional domain-swapped dimers (Mazzarella et al., 1993; Vitagliano et al., 1998; Canals et al., 2001).

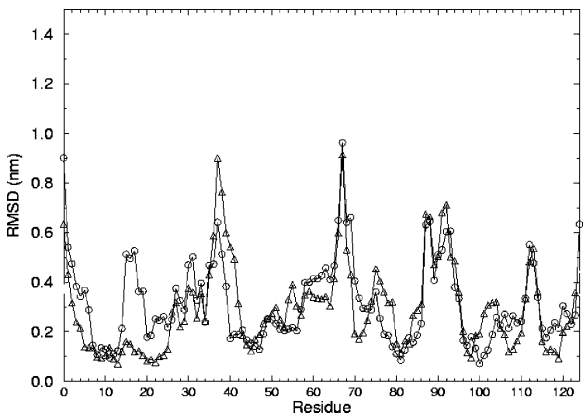

FIGURE 3 Structural differences/RMSD between the position of C^α atoms in the MD-NDimer and X-NDimer. The symbols ○ and △ refer to A and B subunits, respectively.

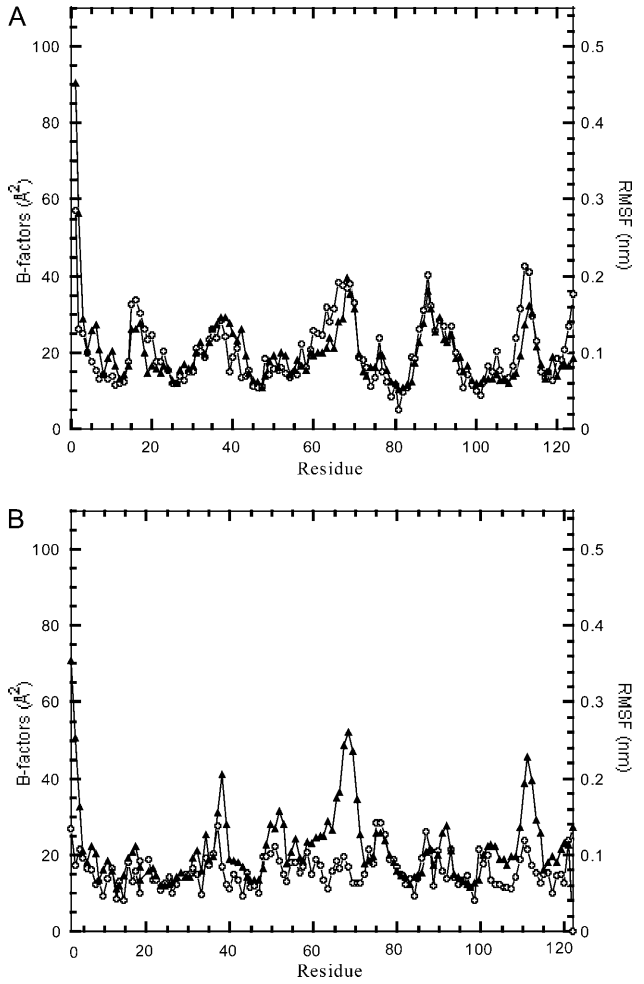

FIGURE 4 Comparison between the B-factor (○) and root mean-square fluctuations (▲) of the C^{α} atoms of N-Dimer. (*A*) Subunit A. (*B*) Subunit B.

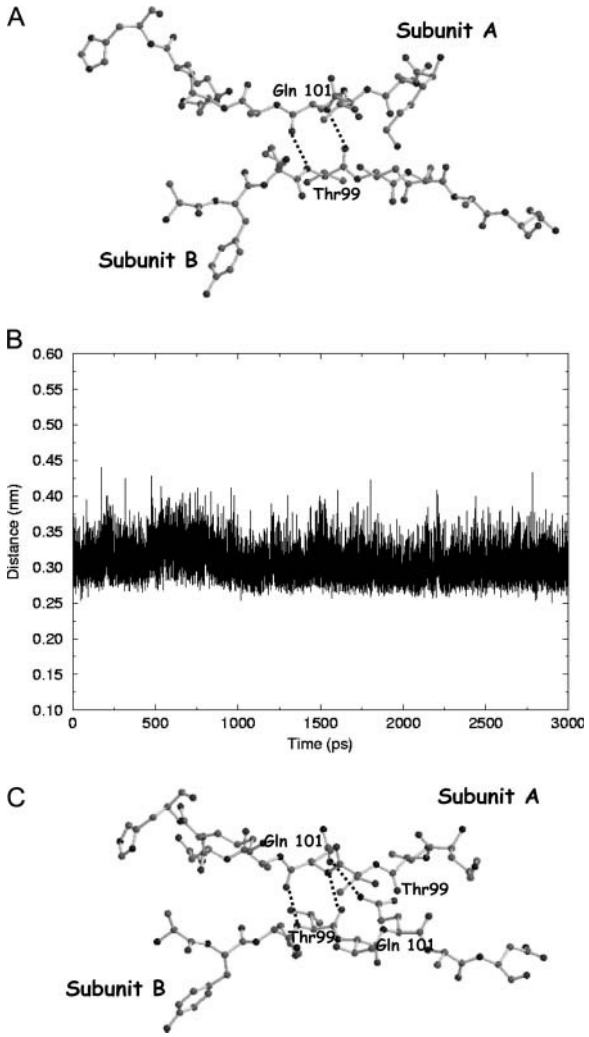

FIGURE 5 Hydrogen bonds at subunit interface. (*A*) In X-NDimer. (*B*) Distance between the N atom of Gln-101 and the O atom of Thr-99 as a function of time. (*C*) Hydrogen bonds in MD-NDimer.

C-interface motions: active site flexibility

In dimeric ribonucleases, the three-dimensional swapping fragments carry important catalytic residues. Therefore, the active sites of the dimers consist of residues from each monomer: they are composite active sites. To establish the effects of the three-dimensional domain swapping on the active site residue motions, the persistence of specific hydrogen bonds between catalytic important residues was monitored during the simulation.

The behavior of the hydrogen bonds formed by active site residues is in agreement with the experimental (Wlodawer et al., 1988; Fedorov et al., 1996; Gilliland, 1997; Berisio et al., 1999, 2002; Vitagliano et al., 2000) and theoretical (Brunger et al., 1985; Seshadri et al., 1994; Nadig and Vishveshwara, 1997; Merlino et al., 2002) data collected for RNase A. The time evolution of the distance between $N^{\delta 1}$ atom of His-12 and backbone O atom of Thr-45 (data not shown) shows that this hydrogen bond is very stable during the simulation in accordance with several structural data obtained for RNase A (Wlodawer et al., 1988; Santoro et al., 1993; Fedorov et al., 1996). In contrast, as in RNase A (Merlino et al., 2002), the hydrogen bonds between His-119 and Asp-121 and between Thr-45 and Asp-83 side chains were transient. In a subunit of the N-Dimer the hydrogen bond between His-119 and Asp-121 is lost when His-119 adopts the alternative inactive conformation (Zegers et al., 1994), well characterized by several crystallographic studies (Gilliland, 1997; Berisio et al., 1999). The hydrogen bond between the Asp-83 and the Thr-45 side chains is also transient, in agreement with the simulation of the monomeric form (Merlino et al., 2002). Altogether, these findings indicate that three-dimensional domain swapping does not affect the dynamics of the composite active sites.

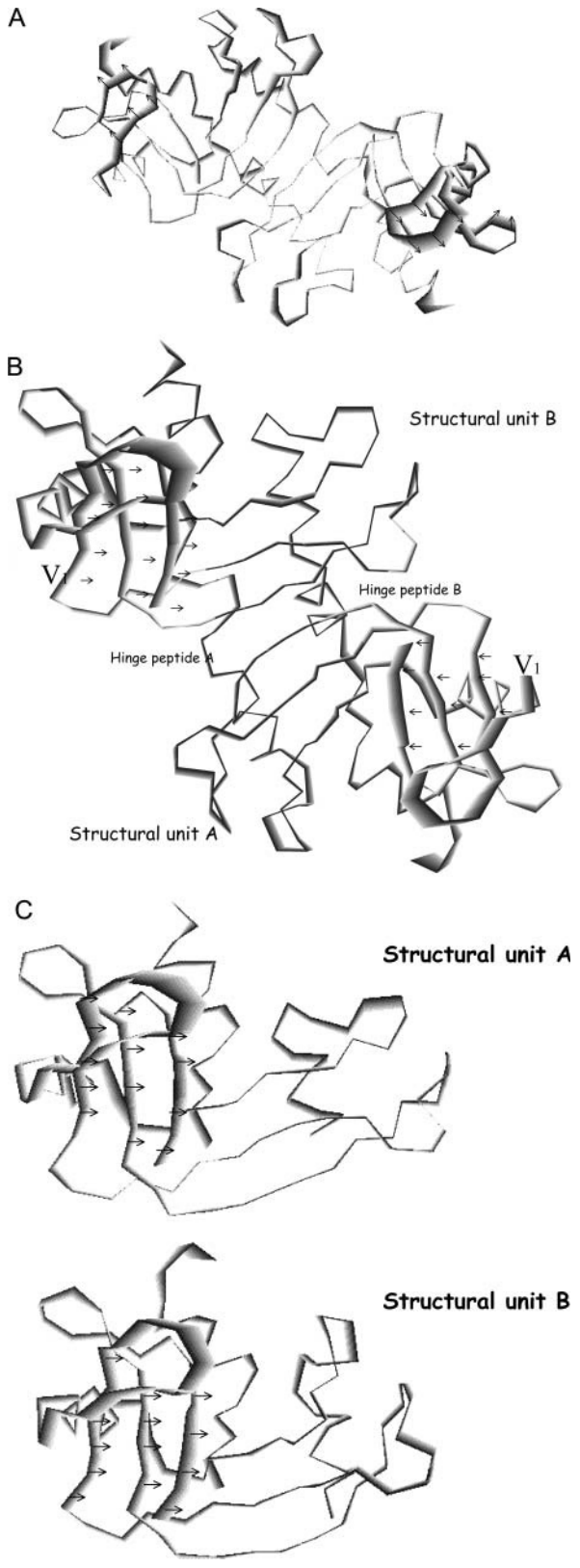

FIGURE 6 Protein motions along the first two eigenvectors. A grayscale is used to represent motion in a film-like fashion. Arrows are used as qualitative indicators of the motion direction. C^{α} trace of N-Dimer as it moves along the first (*A*) and the second (*B*) eigenvector. In *C*, the same representation is reported as for *B*, with the two N-Dimer structural units (see Fig. 1 for the definitions) oriented in the same way.

The two structural units of N-Dimer show the functional breathing motion observed in RNase A

The collective motions of MD-NDimer were analyzed by essential dynamics (Amadei et al., 1993). The essential dynamics analyses of most atomic displacements were contained in the first few eigenvectors. The essential subspace spanned by the first 10 eigenvectors covers ~80% of the fluctuations of the dimer. To visualize the regions involved in the motions described by the first two eigenvectors of MD-NDimer, the conformational evolution of the protein along these principal components was plotted in a film-like fashion (Fig. 6, *A* and *B*). The atomic displacements observed for the first eigenvector are mainly concentrated in the regions of the loops 65–72 and 91–95 (Fig. 6 *A*) and of the N-terminal portion of each subunit. The analysis of the fluctuations corresponding to the second eigenvector (Fig. 6 *B*) shows that the V_1 arms of the β-sheets of both subunits display high mobility. This finding indicates that the β-sheet displays a breathing motion in both subunits. The comparison of the motions between the two N-Dimer structural units carried out by using the root mean-square inner product (Amadei et al., 1999; Ceruso et al., 1999b, 2003; Merlino et al., 2003) (RMSIP, see Methods for details) between the first 10 eigenvectors derived from the diagonalization of the covariance matrices (Table 1), reveals that they exhibit the same dynamic behavior. The high value of RMSIP indicates that the essential subspace spanned by the first 10 eigenvectors of the two N-Dimer structural units is largely overlapped (Table 1). A similar analysis (Table 1) also shows that the breathing motion of these regions closely resembles that observed in RNase A (Berisio et al., 2002; Vitagliano et al., 2002; Merlino et al., 2002, 2003). Interestingly, the V_1 arms of the two subunits move coherently in relation to the core formed by the two V_2 arms. Therefore, the two N-Dimer structural units open and close in phase (Fig. 6 *C*). This finding is supported by the inspection of the correlation matrices that show that motions of the V_1 arms are coupled in the two subunits (data not shown). Thus, the six-stranded β-sheet interface, being the most rigid part of the N-Dimer (Fig. 6, *A* and *B*), may play an important role in transmitting the motion from one structural unit to the other, although other regions of the enzymes, such as the hinge peptides, may also be involved in this process.

Dynamic coupling between the breathing motions of the two structural units: implication for modulated activity of N-Dimer

Since in ribonucleases the breathing motion has been linked to the protein function (Vitagliano et al., 2002), the cor-

relation between the breathing motion of the two structural units of the N-Dimer could be functionally relevant.

Under conditions in which the native monomeric enzyme shows Michaelis-Menten kinetics, RNase A dimers exhibit nonhyperbolic saturation curves for the substrate (Piccoli and D'Alessio, 1984). This finding indicates the possibility of modulation of the function. Although these results may be influenced by the fact that these experiments were carried out on an unresolved mixture of the two RNase A dimers, these findings are in line with the results of the present MD analysis. In this context, a mechanism can be proposed which explains the negative cooperativity of N-Dimer. Experimental (Vitagliano et al., 2002) and theoretical (Nadig and Vishveshwara, 1997) results on RNase A suggest that the binding of the substrate at the first catalytic site of N-Dimer produces a small closure-like motion of the structural unit in which the catalytic site is located. Here we show that β-sheet motions of the two subunits of the N-Dimer are coupled in such a way that the closure of one structural unit favors the closure of second one. Therefore, the binding of the substrate to one subunit may limit the accessibility and hence the binding of the substrate to the second active site. Finally, the present MD/ED analysis provides an example of a mechanism, which may give rise to cooperativity in dimeric systems.

CONCLUSIONS

In the present article the results of 3-ns molecular dynamics simulation of RNase A N-terminal swapped dimer in solution have been reported. This is the first MD/ED study of a three-dimensional domain-swapped protein. When compared to the x-ray structure, the average conformation derived from the MD simulation presents a slightly more compact structure, characterized by a larger number of hydrogen bonds at the intersubunit interface. The local and global dynamic behavior of the two structural unit of N-Dimer closely resembles that of RNase A. Each structural unit exhibits hinge-bending motion of its β-sheet structure as previously revealed by crystallographic (Vitagliano et al., 2002) and computational studies performed on the monomeric form (Merlino et al., 2002, 2003). This finding demonstrates that in the N-Dimer the three-dimensional domain swapping does not perturb the dynamic properties of each structural unit compared to RNase A. These data show that the dynamic properties of each subunit are regulated by their β-sheet topology rather than by the hinge peptide regions. This result is in line with previous investigations carried out on topologically similar proteins (Ceruso et al., 1999b; Keskin et al., 2000; Merlino et al., 2003).

Interestingly, the breathing motion of the two structural units of N-Dimer is dynamically coupled, as they open and close in phase. This coupling is favored by the rigid structure of the six-stranded β-sheet interface and highlights a putative mechanism for the communication between the two active sites of the dimer. It appears that, at least in this case, domain swapping is not directly involved in the motion transmission through the different units of the dimer, but instead constitutes a structural constraint that favors the formation of a rather rigid intersubunit assembly.

In conclusion, these analyses suggest a speculative mechanism for negative cooperativity of this dimeric system (Piccoli and D'Alessio, 1984) and provide an example of how three-dimensional domain swapping can develop modulation of the functional properties in oligomeric enzymes by coupling the functional movements of a monomeric protein.

Finally, the present MD/ED analysis holds intriguing implications on a general belief, which assumes that allosteric concerted models preserving structural symmetry during the allosteric transitions cannot explain negative cooperativity. This paradigm relies on the assumption that, in a system preserving the overall structural symmetry during the allosteric transition, the binding of the substrate to the first active site shifts the equilibrium toward states with higher substrate affinity. The data here presented opens a new possibility. Indeed, the coupling of motion between the two subunits in N-Dimer preserves the overall structural symmetry of the system. Nevertheless, as described in the previous section, the binding of the substrate to the first subunit may hamper its binding to the other active site. In other words, although the preservation of the overall symmetry during the structural transition guarantees that the local structure of each active site is optimal for the substrate binding, it cannot be excluded that the structural transition might limit the accessibility of the substrate to the unbound active site.

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