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Correlative motions and memory effects in molecular dynamics simulations of molecules: principal components and rescaled range analysis suggest that the motions of native BPTI are more correlated than those of its mutants

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Abstract

In this work MD simulations of the native bovine pancreatic trypsin inhibitor (BPTI) and 16 mutants were done in vacuum in order to study memory effects in the mutants using principal component analysis (PCA) and the rescaled range analysis (Hurst exponents). Both PCA and the rescaled range analysis support our previous proposition, based on PCA of lysozyme, that the motions of a native protein are more correlated than those of mutants. The methods are compared, the nature and applications of the rule and the role of the long-range correlations in MD time series (i.e. memory) are discussed in the context of collective motions. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Proteins are often thought as dynamical constructs having internal motions important for their biological function [1]. They are known to possess collective low-frequency motions, like hinge bending [2] or domain rearrangements [3–6]. More recently, correlated motions have been suggested to be important for enzymes' catalytic activity [7–9] and even binding of ligands to protein receptors. For example, substrate specificity of α-lytic protease may be, at least partially, under dynamic control: the change in specificity upon mutation appears to be driven not only by the change in specific interactions, but also by the change in the vibrations of the active-site residues [10]. Unfortunately the experimental analysis and characterization of the motions is very tedious. However, theoretical methods together with progress in computer technology and algorithms have enabled simulations of the dynamics of biomole-

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cules with reasonable efforts and accuracy. The huge data produced in such calculations can be analyzed and compressed efficiently by such methods like principal components analysis (PCA) and, thus, the combination of MD and the different statistical methods forms an efficient computational tool for analysis of intramolecular motions.

Principal components analysis (PCA), often under different names such as essential dynamics or quasi-harmonic analysis, has been used to divide the conformational space of a protein into two major subspaces: (i) the essential subspace including only a few degrees of motional freedom but explaining most of the atomic fluctuations; and (ii) the subspace in which the fluctuations have a narrow Gaussian distribution with nearly random characteristics. Consequently, PCA has become a standard tool for investigating important motions of biomolecules [11].

Besides the collective motions in the essential subspace, there are considerable memory effects present in the MD data of biomolecules. For example, the question of how long a particular subunit of a DNA oligomer keeps the memory of its conformation or location has been explored by using a time-dependent canonical correlation analysis [12]. The hidden peculiarities in the potential-energy time series of peptides have been highlighted by employing a recurrence plot analysis [13,14]. Interestingly, it has been shown via a Fourier analysis that the potential energy fluctuations during an MD simulation of a protein plastocyanin is colored (flickering) $1/f$ noise, i.e. the power spectrum depends on the frequency [15–17]. The presence of $1/f$ noise in the potential energy time series of a protein is a hallmark of self-organization and indicates that the fluctuations actually reduce to a few collective degrees of freedom, as suggested by the essential dynamics of the co-ordinate space. Hence, it seems reasonable to suggest that both the collective motions and memory effects may be closely related to the biological function of a protein. It is also reasonable to suggest that the two effects may be related.

In addition to PCA, two other methods have been used to examine the collective motions of proteins. One is the frequency filtering method, in which the molecular dynamics spectrum is filtered to determine the collective motions of different frequencies [18]. The other method is the normal mode analysis, in which the conformational energy surface of a protein is assumed to obey the harmonic approximation and is represented by the normal modes of an energy minimum structure of a protein [19]. However, from a number of studies, both experimental and computational, it is known that proteins explore a very anharmonic energy surface with multiple minima. In such a case, or in the presence of water, PCA is a powerful method enabling one to determine collective variables from a protein's molecular dynamics trajectory [20].

Recently, we have presented a hypothesis, based on the PCA of the MD data of lysozyme that the internal motions of a native enzyme are more organized than those of mutants [21]. Essential dynamics studies on the effect of mutations on protein motions have been otherwise rare [22]. In this work we explore the topics further by studying the native bovine pancreatic trypsin inhibitor (BPTI), some selected in-house mutants and mutants whose X-ray structures are available in the Protein Data Bank [23].

BPTI is one of the best-characterized proteins. It has served as an important model system for the examination of almost all aspects of protein structure and dynamics and its structural and physicochemical properties and mechanism of inhibition have been extensively documented [24–26]. However, its function is completely different to lysozyme for which our hypothesis was tested for the first time. From that point of view, for BPTI the hypothesis might even be expected to fail. On the hand, success of the rule would mean that the motions have a role in molecular recognition or that the high correlation of motions is just a general intrinsic property of proteins. In this work, we also wanted to explore the possible computational methods and strategies for a system which is computationally more feasible than lysozyme.

2. Materials and methods

2.1. MD simulations

The modeling of the proteins was done using the Quanta version 4.1 (Molecular Simulation Inc.,

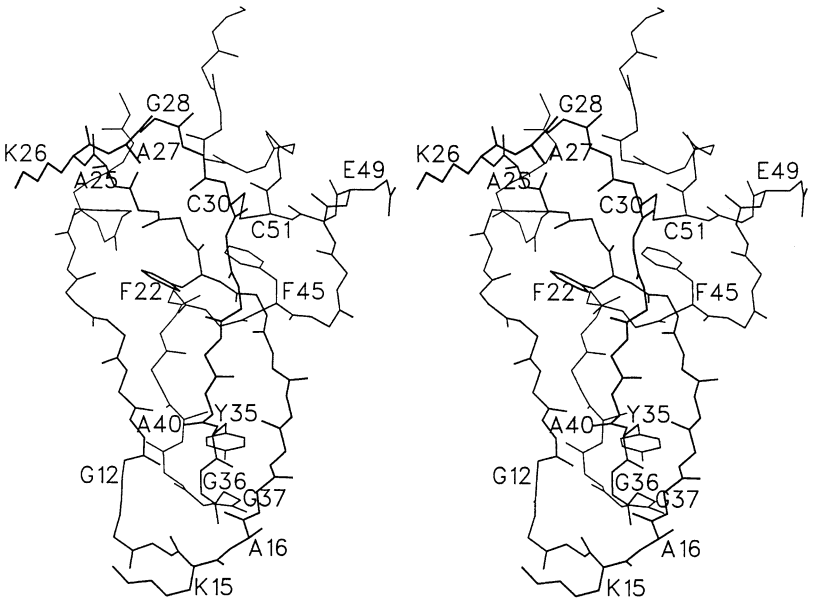

Fig. 1. A stereoview of BPTI showing positions of the mutated amino acid residues studied by PCA and *R*/*S* analyses. This figure was prepared with SETOR [42].

San Diego, CA). The MD simulations in vacuum were carried out on Cray C94 with the CHARMM version 23 [27] and the water box simulations on Cray T3E with the CHARMM version 25b2 [28] running parallel on 64 processors. The starting co-ordinates for simulations of the native BPTI (entry 1BPI) and the X-ray mutants of BPTI (entries 1AAL, 1BTI, 1FAN, 7PTI, and 8PTI) were obtained from the Protein Data Bank [23]. The crystallographic waters of the X-ray structures were removed for vacuum simulations but included in the water box simulations. A set of selected Ala→Ser, Glu→Ala, Gly→Ala, and Lys→Leu mutants were created starting from the co-ordinates of the native BPTI (Fig. 1). Mutations were done with the Protein Design module of the Quanta program.

For the vacuum simulations the protein co-ordinates were first minimized for 500 steps with the adopted-basis Newton Raphson (ABNR) method. Then the protein was heated from 0 to 300 K in 20 ps. After that the protein was equilibrated for 40 ps in 300 K. The production MD simulations of 1 ns were done in 300 K using the Berendsen temperature-coupling algorithm [28] and a coupling constant of 0.4 ps. On the basis of some test calculations on native BPTI, the values of the Hurst exponents calculated from the total energy depend on the coupling method and parameters. However, the result that the values calculated from potential energy using the couplings times of 0.1, 0.4 and 5.0 ps were practically identical (0.72, 0.73 and 0.73), indicates that our calculations measure real memory effects. The values computed from the total and kinetic energies were 0.79, 0.92, 1.0 and 0.54, 0.57, 0.62, respectively. The cut-off distance was set to 13 Å for the non-bonded interactions, and all bond lengths were constrained with the SHAKE algorithm [29]. Co-ordinates of C_α atoms were saved for analysis every 50 step.

The periodic boundary water box simulations were done for the native BPTI and its Gly-36→Ala and Gly-12→Ala mutants. The simulation box had dimensions of 59.0×43.5×49.7 Å. The effect of ions on water box dynamics was tested by running MD simulations for the native BPTI with and without ions. Water box simulations for Gly-36→Ala and Gly-12→Ala mutant were done with ions. BPTI was neutralized by adding six

chloride ions close to charged arginine residues on the protein's surface. In the simulation of the native BPTI there were 3885 water molecules (including 164 crystallographic water molecules). The MD simulations in the water box were initialized by minimizing the water molecules for 500 steps while keeping the protein fixed. Then the water molecules were heated in 5 ps from 0 to 300 K and equilibrated for 10 ps at 300 K. A time step of 2 fs and the Verlet MD integration algorithm [30] was used. The Berendsen temperature coupling algorithm and the temperature coupling constant of 0.4 ps were used to keep the temperature at 300 K. After that the protein atoms were released and the whole system (protein, ions, if present, and water molecules) was minimized for 1000 steps. Then the system was heated from 0 to 300 K in 10 ps and equilibrated for 40 ps at 300 K. The actual production simulations of 1 ns were started after the equilibration. We used a cut-off distance of 12.5 Å for the non-bonded interactions and the van der Waals switching algorithm. The SHAKE option was set to 0.0004 in the MD runs. Co-ordinates were saved every 50 steps for analysis.

In order to ensure that all the systems reached thermal equilibrium, we monitored the kinetic and potential energy, and the r.m.s. deviation of the C_α atoms (data not shown). It appeared that it took 700 ps for some of the systems to reach equilibrium and, therefore, data from the last 300 ps of the 1-ns simulations were subjected to PCA and other analyses.

2.2. Principal components analysis

If P is a co-ordinate matrix (P_{ij} is the deviation of the jth co-ordinate from its average at the time i) obtained by MD calculation, the diagonalization of the correlation matrix C $[C_{ij}=D_{ij}/(D_{ii}D_{jj})^{1/2}$, where D_{ij} is an element of the matrix $P^{\mathrm{T}}P]$ gives principal components (PCs). They define an orthogonal set of dynamic states, so that any MD data point can be expressed as their linear combination. The eigenvalue of a state gives the variance explained by the state. The PC can be understood as a motion: the eigenvalue represents the amplitude of the motion and a large value of a loading means a large contribution of the co-ordinate to the corresponding motion. If only a few PCs are needed to explain a large part of the total variance, the motions are highly correlated, i.e. collective. Instead, if many PCs are needed, the motions are more random. PCAs were performed by using the MATLAB program package (The MathWorks, Natick, MA 01760-1500, USA).

2.3. Rescaled range analysis (*Hurst exponents*)

Many dynamic processes in nature, including MD simulations, are not independent random processes, but show significant long-term correlations in time (memory). Rescaled range analysis, R/S analysis, initiated by Hurst [31] already in the 1950s, is a means of characterizing a time series that provides a sensitive method for revealing long-term correlations in even seemingly random processes. The basics of the R/S analysis are briefly given here. More details are found for example in Feder and co-workers [32,33].

In the R/S analysis, a time series ξ_t is divided into the periods of length τ and the averages $\langle\xi\rangle_\tau$ are computed for each period. After that the cumulative sums (1),

$$X(t,\tau)=\sum_{i=1}^{t}\left(\xi_i-\langle\xi\rangle_\tau\right) \tag{1}$$

and standard deviations (2),

$$S(\tau)=\left[\frac{1}{\tau}\sum_{t=1}^{\tau}\left(\xi_t-\langle\xi\rangle_\tau\right)^2\right]^{1/2} \tag{2}$$

and ranges (3)

$$R(\tau)=\left[X(t,\tau)\right]_{\max}-\left[X(t,\tau)\right]_{\min}(1\le t\le\tau) \tag{3}$$

are used for the computation of $R(\tau)/S(\tau)$. The average over the τ-periods gives an estimate for a rescaled range for that τ-value. The process is then repeated for a new, larger τ-value. If a time series exhibits considerable memory effects, the $R(\tau)/S(\tau)$ is asymptotically given by a power law, i.e. $R/S=\tau^H$. The corresponding exponent H is called the Hurst exponent. Persistent behavior is characterized by a Hurst exponent $0.5<H<1$ and antipersistent by a H value $0<H<0.5$, respectively. For a random process, H is equal to 0.5. In general,

R/S analysis has quite poor convergence properties, requiring approximately 200 data points for 10% accuracy and 2000 for 5% accuracy [33], but for a sufficiently long MD simulation this requirement is easily fulfilled. The MATLAB script required to perform the rescaled range analysis was written by the authors.

3. Results

Most of the calculations of this study were carried out in vacuum. Although the solvent plays an important role in the motions and structure of the protein, the main features of the protein dynamics and the differences between the proteins studied are believed to be reproduced for the purposes of this study. It has also been previously shown that the contributions of the principal components to the fluctuations of BPTI are similar in water and vacuum simulations [20]. Importantly, the omission of the solvent enhances the correlations and allows fast MD simulations. These both points are important for converged statistics of PCA and have been discussed more in our previous publication [21].

The largest PC eigenvalue (EV1, Table 1) shows that the motions of the native BPTI are generally more organized than most of the mutants agreeing with the corresponding results for lysozyme [22]. There are only three mutants, A25S, A27S, and the double mutant C30A, C51A, out of 16 studied whose EV1 values of the vacuum simulations are of the same magnitude as those of the native BPTI. The double mutant, whose X-ray structure is also available, has a clearly larger EV1 (50.0) than the native BPTI (44.0). It seems that the removal of a disulfide bridge has changed the dynamics of BPTI drastically and resulted in a double mutant protein with large structural fluctuations. It is likely that the remaining two disulfide bridges keep the mutant protein compact and at the same time allow large-scale motions. The mutations A25S and A27S are located in the same loop (Fig. 1). Also the third mutant of this loop, K26L, has a higher than average EV1 (32.4). This surface loop is loosely packed with the rest of the protein and this probably explains why the three mutations have only a small effect on the correlated motions of the protein. Furthermore, the contributions of these amino acids (residues 25–27) to the first principal component of the native BPTI simulation are small (data not shown). This suggests that if these amino acid residues were mutated, the effects on the dynamics of the protein would be small, as observed in the mutant simulations. In general, the results indicate that even small changes in the amino acid sequence may alter significantly the eigenvalues of the MD data matrix. Hence, if the internal motions are important for the biological activity of the molecule, it can be changed considerably.

The eigenvalues of the water simulations are considerably larger than those of the vacuum simulations and show much smaller differences between the native and mutant structures. That

Table 1
Descriptors in co-ordinate and energy spaces

Mutant	Co-ordinates			Energies (Hurst exponents)		
	EV1	r.m.s.d.	Hurst_{ave}	E_{tot}	E_{kin}	E_{pot}
Gas phase, in-house						
Native	43.98	0.3534	0.79	0.95	0.59	0.73
G12A	19.71	0.2555	0.62	0.87	0.50	0.57
K15L	22.61	0.2784	0.61	0.79	0.46	0.56
A16S	26.48	0.2900	0.56	0.85	0.44	0.60
A25S	44.65	0.3817	0.75	0.88	0.49	0.72
K26L	32.40	0.3011	0.68	0.93	0.48	0.72
A27S	40.64	0.3796	0.73	0.85	0.52	0.71
G28A	22.50	0.2662	0.66	0.81	0.56	0.67
G36A	20.26	0.2886	0.54	0.82	0.52	0.67
G37A	22.15	0.2645	0.59	0.82	0.48	0.64
A40S	21.90	0.2667	0.61	0.89	0.39	0.59
E49A	25.09	0.2888	0.66	0.86	0.54	0.65
Gas phase, X-ray[a]						
C30V (1aal)	18.19	0.2728	0.63	0.95	0.52	0.67
F22A (1bpti)	26.84	0.2920	0.73	0.95	0.49	0.78
F45A (1fan)	18.64	0.2874	0.74	0.94	0.48	0.75
C30A, C51A (7bti)	50.02	0.4108	0.79	0.88	0.39	0.69
Y35G (8pti)	15.53	0.2663	0.67	0.84	0.48	0.62
Water, in-house						
Native	90.84	1.8796	1.02	0.82	0.49	0.82
Native[b]	91.25	2.1170	1.03	0.80	0.47	0.79
G12A[b]	80.17	2.4752	1.03	0.85	0.54	0.86
G36A[b]	80.03	2.0775	1.03	0.82	0.47	0.82

[a] Protein databank code in parentheses.
[b] Simulations were done with counterions.

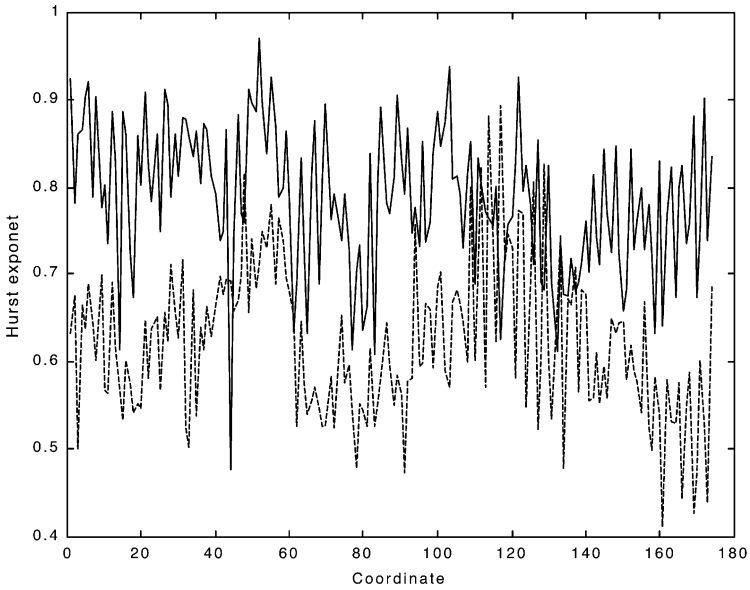

Fig. 2. Hurst exponents as a function of C_α co-ordinates for the native BPTI (solid line) and mutant G12A (dashed line).

G12A and G36A have smaller EV1s than the native BPTI and that the eigenvalues of the two mutants are similar are in line with the results of the vacuum simulations.

The Hurst exponents of the total energy time series of BPTIs lie in the range of 0.79–0.95 indicating that the fluctuations present time correlated processes. This means that the sequences have an intrinsic memory and the subsequent values are determined by the preceding ones. To a lesser extent, the same is true for the potential energy time series (0.59–0.73). In contrast, the kinetic energy exhibits no memory effects and the corresponding Hurst exponents were near 0.5 for every BPTI mutant (mean = 0.49). Interestingly, the native BPTI has the largest Hurst exponent. This type of persistent behavior is probably characteristic for biomolecules, in contrast to random thermal fluctuations that are present in all molecular matter. Furthermore, the Hurst exponents of the individual Cartesian co-ordinates vary along the polypeptide chain considerably (Fig. 2), depending on the site of the mutation. In the future, it might be possible to link this variation to the biological activity of a protein by employing for example PLS (partial least squares) algorithm [34], which is applicable also to the underdetermined equation systems, i.e. number of columns can be much larger than that of rows.

4. Discussion

In this work, a main question was on how PCA and time series analysis can be applied to analysis of correlated motions. The above results show that PCA and Hurst time series analyses give here parallel results in characterizing the mutational effects on molecular dynamics trajectory. The advantage of the Hurst analysis is that it can be used to follow time-dependent processes where vibration-like motions are to be separated from large motions like protein folding. A disadvantage is that the latter method seems to be unable to make a difference between the mutants for MD calculations in solution. It is also interesting that the largest EVs and the average standard deviations are closely related in the vacuum simulations (Fig. 3); this is discussed below.

There are several ways by which the motions may affect the protein function. First, large-scale domain movements and arrangements are involved in the function of many proteins [4,5,35] and such

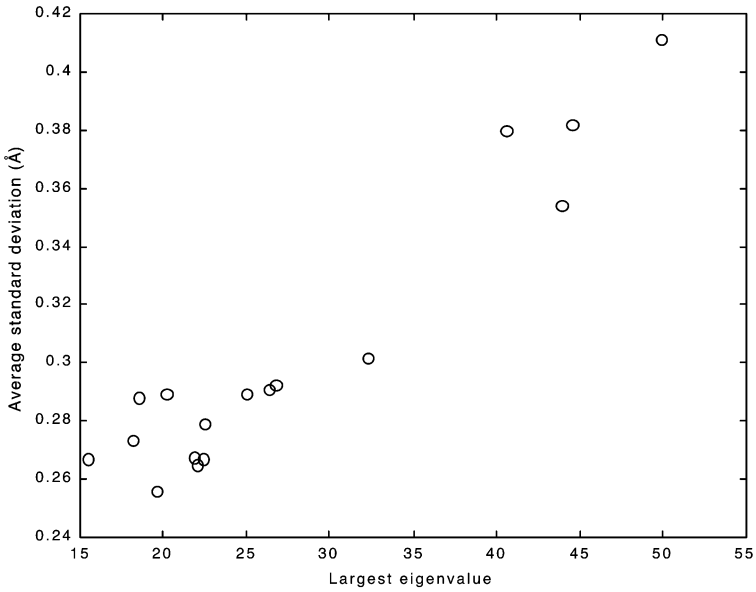

Fig. 3. Correlation between the largest EVs and the average standard deviations of C_α atoms of the gas-phase MD simulations of BPTI mutants. The correlation coefficient (r) is 0.95.

movements are needed, for example, in ligand-binding process or interaction between a protein and other macromolecule. These kinds of processes are well known, such as induced fit and allosteric movements. Second, in addition to these relatively straightforward examples of correlated motions of protein, the protein dynamics can be directly involved in the activity of many proteins and be partly responsible for the rate enhancement and specificity of some enzymes [9,36–39]. The observations can be related to the reaction co-ordinate and the kinetic energies connected to them: energetic and strongly coherent motion in the direction of reaction co-ordinate obviously reduces the reaction activation free energy, improving also the enzymatic specificity. It is notable that the PCA and Hurst methods measure also fast and vibration-like energy rich motions. Third, the motions may have an important role in protein–protein and protein–ligand interactions: matching vibrational modes with high correlations of the motions obviously improve the binding affinity. On the other hand, the binding of a ligand may also either increase or decrease motions in the direction of the reaction co-ordinate. In the latter case the ligand is an inhibitor of the protein function and the effectivity of the inhibition can be expected to be better the more organized are the motions of the ligand. In the particular case of BPTI, the motions may explain the 1–2 orders of magnitude of inhibition not explained by the static energetics model [26]. The proof of this would demand calculations that are beyond the scope of this work.

As to the applicability of the above tools, even at the present technology the above described motion analyses of a medium size protein can be done within reasonable efforts. For example, one 1-ns MD run for a 194-residue protein (xylanase) in vacuum, suitable for PCA, demands approximately 20-h cpu time on normal PC with a 1.4-GHz processor. The cpu time needed for the PCA analysis is negligible. Recently the use of MD calculation has been described for assess ‘foldability of mutants’ [40]; this is a potential application of the present tools. If dynamics are important for enzyme function, and if the protein regions most responsible for the motion can be identified, they present a potential target for small-molecule drug binding and inhibition [10] and, thus, MD together with the tools described here can be used in protein

engineering and in drug design, and in general, to cast some light upon questions of how protein and ligand dynamics are actually coupled.

Our results also bring out light on the nature of the correlations and the inherent nature of the protein structure that leads to correlated motions and memory effects. It seems obvious that a compact tightly packed protein with extensive van der Waals and hydrogen bond interactions have correlated dynamics. If such a structure is disturbed by a mutation, correlated motions are probably affected. There is a good correlation between the C_α r.m.s. value and efficient packing of native proteins [41]. Because the largest eigenvalues correlate with the C_α r.m.s. values (Fig. 3), PCs reflect also good packing of native proteins. The connection between high correlations and the packing effects is supported also by comparison of the gas and solvent phase calculations: the rule performs better in the former case (where the structures are more tightly packed). One could now question whether the good correlations only reflect good packing or, vice versa, whether the good packing is necessary for good correlations. An interesting question is also how the C_α r.m.s. value is connected to these measures; do they all measure just the same thing? Although this may be much the case, the observations that also the Ala→Ser and Ser→Ala mutations, affecting the motions mainly via Coulombic interactions, have clear effects on the correlations of the motions [21], indicating that the question is not solely about spatial packing effects.

5. Conclusions

In general, we believe that the correlation analysis brings some unique semiquantitative information about the actual relationships between the memory effects and biological activity and even about the atomic level mechanism of how amino acid mutations affect these processes. The present results also support our hypothesis that, in general, the motions of native proteins are more organized than those of the mutants [21]. The result that the rule holds for the two rather different proteins, proposes that the rule is independent of the function of the protein. Because there are mutants for which motions are even more correlated than for the native protein, one could reformulate the rule as follows: high correlations of the intramolecular motions are an intrinsic property of native proteins. One may also conclude that the property can be measured by using a simple MD calculation in vacuum. The generalization of the rule to other proteins of different function and catalytic mechanism is of theoretical interest and would have considerable consequences and applications in protein structural chemistry. Of course, the generalization and full understanding of the rule and its applications demands more work on different types of proteins.

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