Received 6 November; accepted 5 January 1979.

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Protein structural fluctuations during a period of 100 ps

A RECENT 9-ps molecular dynamics simulation[1] of the bovine pancreatic trypsin inhibitor (PTI) at 295 K revealed a rich variety of motional phenomena at the atomic level on a picosecond time scale. To obtain information about longer time processes, and to characterise more accurately the short time results, a 96-ps dynamical simulation of PTI at an average temperature of 306 K has been completed with the techniques used previously[1]; an extended equilibration period of 72 ps before simulation served to eliminate internal stresses. Analysis of the present simulation has confirmed most of the conclusions of the earlier study but has shown in addition that there are significant features of the dynamics that can be observed only over a longer period, ~100 ps.

An overall measure of the correspondence between the time-average dynamical and X-ray structures[2] is given by the radius of gyration. It equals 10.22 Å (with fluctuations of ±0.1 Å) for the former and 10.96 Å for the latter. The primary source of difference between the two are the positions of the charged external side chains, which may in fact have different average positions in solution and in the crystal[3,4]. The dynamics simulation also shows a strong coupling between the amino and carboxyl termini, in agreement with NMR solution results[5] but different from the crystal structure (see Fig. 1 of ref. 1). The core of the protein has a heavy-atom number density close to the X-ray value; within a radius of 8 Å from the protein centre, the calculated number density is 0.063 $Å^{-3}$, compared with 0.060 $Å^{-3}$ for the X-ray structure.

Analysis of individual atom position fluctuations shows that, as a result of the more careful equilibration, they are slightly smaller than in the previous simulation. The average r.m.s. fluctuation for the α carbons is 0.60 Å (previously 0.74 Å) and that for all atoms 0.75 Å (previously 0.90 Å); the corresponding X-ray results from isotropic temperature factors are 0.68 Å and 0.74 Å, respectively (J. Deisenhofer, personal communication). The calculated mean-square positional fluctuations are found to be highly anisotropic for most of the atoms. For the α carbons, the overall averages are $\langle z^2 \rangle = 0.252$ $Å^2$, $\langle x^2 \rangle = 0.051$ $Å^2$ and $\langle y^2 \rangle = 0.099$ $Å^2$, where a local principal axis system is used for each atom; in some cases, the largest component is more than 10 times the smallest. The differences in the orientations of the local systems for atoms in the same region, relative to a space-fixed axis system, make clear that the anisotropy is reflecting structural constraints rather than overall protein motion. Evaluation of the positional distribution functions shows that many of them differ significantly from the standard gaussian form, a consequence of the fact that the potential of mean force experienced by the atoms is not harmonic.

Because of their well-defined nature and possibility for direct experimental investigation, the aromatic side chain motions in PTI were studied in detail[6]. Tyrosine ring orientation fluctuations of ±30° from the average (r.m.s. value of 12°) were found for residues in the interior of the protein; this corresponds to a torsional potential of mean force considerably softer than that of the rigid protein. The time-dependence of the torsional motion approximates that expected from a Langevin equation for an angular oscillator with near critical damping; the calculated relaxation times for this motion are of the order of tenths of ps; for example, for Tyr 21 it is 0.2 ps. Comparison between the tyrosine side chain in a dipeptide and in the protein clearly demonstrates the importance of matrix damping effects due to the surrounding atoms in the latter.

An important result of the new simulation is the presence of long-lived fluctuations in the properties of PTI. Making use of 1-ps dynamic averages ('coarse-graining') to damp out rapid oscillations, it was found that fluctuations of 5–15 kcal mol^{-1} with a duration of 2–15-ps occur in the potential energy associated with the bond-stretching and bond-angle-bending coordinates, as well as with the non-bonded interactions. It seems that these energy fluctuations represent transitions of the protein from one potential minimum to another in the neighbourhood of the time-averaged structure. Direct evidence for this is given by the correlation of the potential energy fluctuations with transitions between minima of side chain dihedral angles and other internal coordinates.

A consequence of the infrequent structural transitions is that long periods of averaging are required to establish the fluctuations in global properties. Using the entire 96-ps period, we find the r.m.s. fluctuation in the kinetic energy to be 11.51 ± 0.1 kcal mol^{-1}, which yields a classical heat capacity of 3.14 ± 0.1k per protein atom (k is the Boltzmann constant). The previous simulation resulted in a smaller value for the r.m.s. kinetic energy fluctuation (9.3 kcal mol^{-1}) and a correspondingly smaller heat capacity (2.3 k per atom); test calculations show that such low values are due to the short time interval used for averaging. Apparently the heat capacity contains a contribution from equilibration among the different available states and so cannot be related as simply to models for protein dynamics as assumed in ref. 1. Long-lived fluctuations also occur in the atom positions, so that values obtained by averaging over less than 10 ps tend to be too low; for example, for the α carbon atoms, the r.m.s. fluctuation from 2-ps intervals are ~0.40 Å as compared with the value of 0.60 Å for the complete simulation.

The present calculation supports the earlier conclusion that a dynamic simulation can provide realistic and highly informative descriptions of the detailed atomic motions in the interior of a protein. The existence of large fluctuations in the structure and energy components indicates that the properties of proteins result from the occupation of a variety of thermally accessible states, even for times as short as 10–100 ps. It will be of interest to assess the role of these states in the biological activity of proteins and to determine the importance of alterations in the available states and transitions among them due to the solvent environment and ligand or substrate binding.

A full account of the extended simulation will be presented elsewhere. We thank John Ramsdell, Barry Olafson, and Thomas Jordan for their assistance and the NIH for support. J. A. McC. thanks the NIH for a postdoctoral fellowship.

Note added in proof: Frauenfelder, Petsko, and Tsernoglou (personal communication) have recently determined isotropic temperature factors for myoglobin at a series of temperatures and have used them to obtain atomic positional fluctuations; a dynamical simulation to compare with their results is in progress.

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Received 5 July; accepted 1 December 1978.

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0028–0836/79/0277—0578$01.00