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Molecular dynamics of an α-helical polypeptide: Temperature dependence and deviation from harmonic behavior

(protein dynamics/temperature factor/quantum corrections/fluctuations/normal modes)

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ABSTRACT **The mean square amplitudes of atomic fluctuations for a polypeptide (decaglycine) α-helix evaluated from molecular dynamics simulations at seven temperatures between 5 and 300 K are compared with analytic harmonic results and with experimental values. Above 100 K the harmonic approximation significantly underestimates the amplitudes of the displacements. Analysis of the time dependence of the fluctuations shows that low-frequency modes (<75 cm^{-1}) dominate the atomic fluctuations and that there is a contribution with a very long relaxation time (>10 ps). Quantum corrections to the amplitude of the fluctuations are found to be small above 50 K. The mean square amplitudes obtained from the molecular dynamics simulations are compared with the values derived from x-ray temperature (Debye–Waller) factors for metmyoglobin (80, 250, and 300 K) and ferrocytochrome *c* (300 K).**

It is now recognized that significant internal mobility exists in proteins at ambient temperature and that some of the motions that occur play a role in the biological activity (1–3). Theoretical studies (1, 4, 5) have shown that room temperature fluctuations on the picosecond time scale lead to root mean square (rms) atom displacements on the order of 0.5 Å (0.2–1.5 Å), a magnitude in overall agreement with estimates from x-ray diffraction and Mössbauer studies of protein crystals (6–8). Consideration of the closely packed nature of the protein interior requires that the motions have a strongly cooperative character; i.e., only by correlated fluctuations can the observed magnitudes be realized. Two extreme models for such condensed-phase dynamic behavior can be formulated (9). In the first, analogous to a liquid, the atomic fluctuations can be described as localized diffusion in which the collisions between atoms play the essential role; in the second, analogous to a solid, the atomic fluctuations involve the coupling of a large number of normal modes. It is likely that the interiors of proteins contain both fluid-like and solid-like regions (6, 9, 10); e.g., it has been suggested that hydrophobic clusters are more fluid-like, whereas parts with considerable secondary structure (α-helices, β-sheets) are more solid-like (9).

In this paper we describe a study of the full molecular dynamics of an isolated α-helix as a function of temperature (5–300 K) and compare the results obtained with those for the same system in the harmonic approximation (11–14). The analysis is of interest for itself, in that it determines quantitatively the anharmonic contribution to the internal motions of a macromolecule. Further, it provides a basis for interpreting the dynamics of this important structural element as part of a globular or fibrous protein. Results are given for both the magnitude and the frequency dependence of the fluctuations as a function of temperature, and the importance of quantum corrections is determined. The values obtained for the isolated helix are compared with experimental and calculated results for α-helices in proteins.

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METHOD

The system considered is α-helical decaglycine with the amino and carboxyl ends blocked by CH_3CO and $NHCH_3$ groups, respectively; the decaglycine helix contains eight hydrogen bonds. All atoms and degrees of freedom of the molecule are treated explicitly except that the CH_2 and CH_3 groups are represented as extended atoms. The details of the model, including the empirical potential energy function, have been presented (11). A series of temperatures between 5 and 300 K defined by the mean kinetic energy of the system was studied. For each temperature, 20 trajectories, each 1 ps in length, were calculated by solving simultaneously the classical equations of motions for the atoms of which the helix is composed. Up to and including 100 K (5, 50, and 100 K), a kinetic energy twice the final desired temperature was imparted to the system in a single step; in all cases the temperature dropped to its equilibrium value within 50 to 100 steps (step size = 0.5×10^{-3} ps). At temperatures above 100 K, the system was heated by 25–50 K every 200 steps until the desired temperature was reached. The total kinetic energy imparted to the system for the 20 trajectories with a given temperature was the same; the values of the average temperature of individual trajectories varied by up to 20%. In all cases, the initial coordinates of the system corresponded to the minimal energy geometry of the decaglycine helix and the individual trajectories of a set differed only in the initial velocity components assigned to each atom; that is, the initial velocity components were chosen by using a set of random numbers corresponding to the appropriate Maxwellian distribution. Once the final temperature was reached in each case, the analysis of the trajectory was begun.

Twenty short trajectories with different initial conditions were used in an attempt to cover a larger portion of phase space than would a single extended trajectory in this relatively harmonic system. A set of 20 trajectories was chosen after a series of test calculations in which it was found that this number gave consistent results when repeated with a different distribution of initial velocities; sets of 5 or 10 trajectories showed deviation between different samples that were too large. For comparison, a single longer trajectory was calculated at 300 K; it was equilibrated for 9 ps and the analysis period lasted 10 ps.

The mean square amplitude of the fluctuations of a given atom, $\langle \Delta R^2 \rangle$, is given by

$$\langle \Delta R^2 \rangle = \frac{1}{n} \sum_I \langle |\vec{r}_I - \langle \vec{r} \rangle|^2 \rangle, \qquad [1]$$

in which $\vec{r}_I$ is the instantaneous coordinate of the atom in the

*I*th trajectory and n is the number of trajectories in the set; the brackets represent time averages and $\langle \vec{r} \rangle = n^{-1} \Sigma_I \langle \vec{r}_I \rangle$ is the mean value of the coordinate for the 20 trajectories. The harmonic approximation to the amplitudes of atomic vibrations are obtained from the eigenvectors and eigenvalues of the decaglycine α-helix normal modes (11); that is,

$$\langle \Delta R^2 \rangle_{\text{har}} = k_B T \sum_{j=1}^{3N-6} \frac{|\vec{\alpha}_j|^2}{\omega_j^2}, \quad [2]$$

in which k_B is the Boltzmann constant, T is the absolute temperature, N is the number of atoms, ω_j is the frequency of the jth normal mode, and $\vec{\alpha}_j$ is the vector of the projections of the jth normal mode onto the Cartesian components of the vector $\vec{r}$ for the atom of interest. To obtain the normal modes, the energy of the α-helix was minimized by using the full potential, and the second-derivative matrix of the energy with respect to the Cartesian coordinate displacements relative to the minimal-energy structure was determined. Details concerning the harmonic dynamics method as applied to the α-helix have been reported (11).

Because quantum effects are expected to be important at low temperatures, the harmonic approximation was used to estimate the quantum correction to the rms fluctuations as a function of temperature for the α-helix. Instead of Eq. 2 we have (15)

$$\langle \Delta R^2 \rangle_{\text{qm}} = \sum_{j=1}^{3N-6} |\vec{\alpha}_j|^2 \left[\frac{\hbar}{2\omega_j} \coth\left(\frac{\hbar \omega_j}{2k_B T} \right) \right]. \quad [3]$$

RESULTS

To determine the difference between the molecular dynamics calculation and the harmonic approximation, we compare the mean square displacements obtained for α-helical decaglycine as a function of temperature between 5 and 300 K. The classical formulation is used for both calculations to permit an unequivocal comparison; quantum corrections in the harmonic approximation are described below. Table 1 lists the averages for the helix (excluding the terminal residues 1 and 10) of the mean square fluctuations obtained for all atoms and for the C^α atoms alone from the dynamics runs and from the harmonic approximation to it. All-atom values for the interior residues (2–9) and for only the central residues (5 and 6) are given. From the table, the all-atom and C^α values are very similar (molecular dynamics) or essentially identical (harmonic dynamics). Comparing the α-carbon molecular dynamics and harmonic results, we see that the former values increase from 0.0025 Å^2 at 5 K to 0.372 Å^2 at 300 K, whereas the latter increase from 0.0025 Å^2 to only 0.152 Å^2. Up to 50 K, the harmonic approximation is very close to the exact results, but the difference between the two methods is already significant at 100 K (ratio of 1.74), and it increases to a ratio of 2.45 at 300 K.

The mean-square all-atom displacements are plotted as a function of temperature in Fig. 1, where we give separately the results for the central residue averages (residues 5 and 6) and the other interior residue averages (residues 2–4 and 7–9). In the harmonic approximation, the mean square displacements increase linearly with temperature as expected for motion about a single minimum (Eq. 2) and the difference between the central and other interior residues is rather small. The complete dynamics results are considerably different. The displacements are more sensitive to the position in the helix; e.g., at 250 K, the ratio of the central residue fluctuations to those of the other residues is 0.43 in the molecular dynamics and 0.75 in the harmonic approximation. As to the temperature dependence of the dynamics results, it is clearly nonlinear for both the central and the other interior residues. Further, the slope is generally greater than it is for the harmonic calculation; the results between 250 and 300 K are indicative of structural changes in the helix. If an effective spherically symmetric, *temperature-independent* potential of the form r^μ is assumed for the positional fluctuations (6), the temperature dependence between 50 and 250 K of the all-atom averages in Table 1 yields $\mu \approx 1.4$ instead of the value $\mu = 2$ that corresponds to the harmonic potential. Thus a relatively small deviation from harmonic behavior in this model leads to an important change in the value of the mean square fluctuations. An alternative interpretation would introduce a *temperature-dependent* effective force constant and assume that the potential is harmonic at each temperature (quasi-harmonic approximation) (16). The calculated temperature dependence for the central residues (5 and 6) in the full dynamics would require that the force constant decreases from 5.97 kcal/mol·Å^2 at 100 K to 4.65 kcal/mol·Å^2 at 250 K (1 kcal = 4.18 kJ).

To analyze the origin of the anharmonic behavior, we write $\langle \Delta R^2 \rangle$ obtained from the full dynamics as a sum of two terms ($\langle \Delta R^2 \rangle = \langle \Delta R^2 \rangle_1 + \langle \Delta R^2 \rangle_2$), in which $\langle \Delta R^2 \rangle_1$ is the result obtained by calculating the mean square fluctuations for each 1-ps trajectory with respect to its mean and then averaging over the 20 trajectories, and $\langle \Delta R^2 \rangle_2$ is the mean square fluctuation of the mean over the 20 trajectories (i.e., $\langle \Delta R^2 \rangle_2 = n^{-1} \Sigma_I |\langle \vec{r}_I \rangle - \langle \vec{r} \rangle|^2$). Table 2 gives the results obtained for the C^α atoms. Comparing $\langle \Delta R^2 \rangle_1$ with the harmonic results, we find that the two are very similar even at 300 K. This makes clear that the local high-frequency (<1 ps) fluctuations around a given mean structure obtained in a dynamics trajectory are close to those obtained from a harmonic model. However, the range of mean structures, corresponding to $\langle \Delta R^2 \rangle_2$, that are populated by the 1-ps trajectories with different initial velocity distributions is such that important deviations from harmonic behavior are introduced. This is confirmed by the 10-ps 300 K trajectory, which yields results very close to those obtained from Eq. 1 with the 20 1-ps trajectories; e.g., the 10-ps run has a value of 0.54 Å for

Table 1. Temperature dependence of mean square displacements: Molecular dynamics and harmonic approximation*

Temperature, K	Molecular dynamics (Eq. 1)			Harmonic approximation (Eq. 2)		
	All atoms (residues 2–9)	All atoms (residues 5 and 6)	α-Carbons (residues 2–9)	All atoms (residues 2–9)	All atoms (residues 5 and 6)	α-Carbons (residues 2–9)
5	0.0025	0.0025	0.0025	0.0025	0.0020	0.0025
50	0.032	0.023	0.032	0.026	0.020	0.026
100	0.078	0.053	0.084	0.048	0.040	0.048
150	0.152	0.096	0.176	0.073	0.058	0.073
200	0.230	0.130	0.260	0.102	0.078	0.102
250	0.303	0.160	0.348	0.130	0.102	0.130
300	0.336	0.240	0.372	0.152	0.123	0.152

* All values in Å^2.

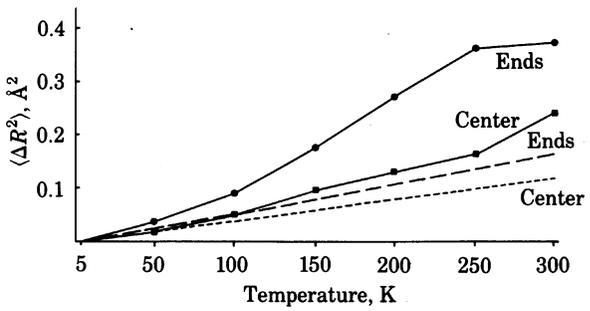

FIG. 1. Temperature dependence of mean square atomic displacements obtained from molecular dynamics and the harmonic approximation. ■——■, Molecular dynamics (atoms in residues 5 and 6); ●——●, molecular dynamics (atoms in residues 2–4 and 7–9); -----, harmonic approximation (atoms in residues 5 and 6); and ———, harmonic approximation (atoms in residues 2–4 and 7–9).

the rms fluctuation of C^α of residue 6, whereas the 20 1-ps trajectories give 0.52 Å at 300 K.

Some details concerning the magnitudes of the fluctuations of specific atoms of the helix are contained in Fig. 2. It shows the rms values obtained from the molecular dynamics and the harmonic approximation for the individual C^α, O, and N atoms at 5, 100, and 300 K; included in the figure are the fluctuations of the amino-terminal and carboxyl-terminal blocking groups. For 5 K the two sets of results are identical. At 100 K the fluctuations of the central atoms of the helix are close to the harmonic results, whereas at 300 K all the atoms have substantially larger fluctuations. As expected, the fluctuations increase from the center of the helix towards the ends in the complete dynamics, particularly at 300 K. There is a much smaller effect of position in the harmonic approximation. Further, the different atoms generally show similar behavior. The carboxyl-terminal carbonyl oxygens, which are not hydrogen bonded (residues 8, 9, and 10), have larger fluctuations than the oxygens in the other residues.

It is of interest to compare the isolated helix rms values with those for helices in proteins. Table 3 lists the calculated α-carbon rms fluctuations for the decaglycine helix and for the helices in ferrocytochrome *c* and experimental estimates from x-ray temperature factors for the helices in metmyoglobin and ferrocytochrome *c*. For metmyoglobin (6), values for 250 and 300 K are given with an average disorder correction based on Mössbauer data for the heme iron; for ferrocytochrome *c* (8), the results are for a single temperature (300 K) and the disorder correction is made by a comparison with the average results for the protein interior from a molecular dynamics simulation (8). The metmyoglobin values are close to those calculated for the central residues of decaglycine by the full molecular dynamics; i.e., 0.41 Å and 0.48 Å at 250 and 300 K for metmyoglobin, as compared with 0.42 Å and 0.52 Å from the isolated helix simulation. A recent x-ray analysis (17) of metmyoglobin at 80 K yields (with the Mössbauer disorder correction) (18) a value of 0.28 Å for the average C^α rms displacement in the helices as compared with the calculated value of 0.22 Å for the central helix residues at 80 K.

Table 2. Decomposition of dynamic results for C^α atoms*

Temperature, K	Residues 2–9			Residues 5 and 6		
	Harmonic	$\langle \Delta R^2 \rangle_1$	$\langle \Delta R^2 \rangle_2$	Harmonic	$\langle \Delta R^2 \rangle_1$	$\langle \Delta R^2 \rangle_2$
100	0.048	0.053	0.031	0.040	0.040	0.013
300	0.152	0.168	0.204	0.123	0.144	0.136

* All values in Å^2.

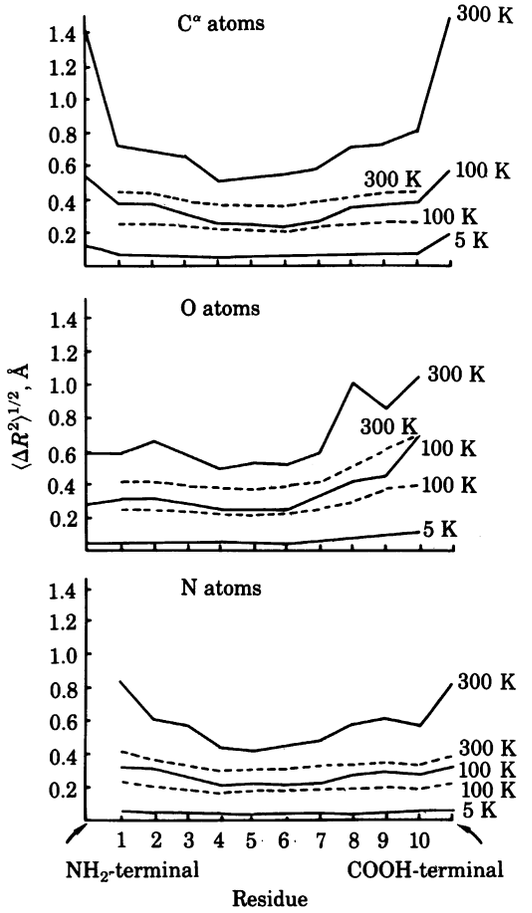

FIG. 2. rms atomic fluctuations by residue for the C^α, O, and N atoms; the amino- and carboxyl-terminal blocking groups are included. ——, Molecular dynamics; ----, harmonic approximation.

In comparing with the x-ray results, only the central helix residues results were used. When residues closer to the ends of the model α-helix are included in the averaging, the calculated displacements are larger than the metmyoglobin helix values (except at 80 K). This suggests that the protein environment (including the fact that helices in proteins are generally bonded at both ends to other parts of the polypeptide chain) can play a role in reducing the helix fluctuations. Moreover, a decaglycine helix might be expected to have somewhat larger fluctuations than helices composed of amino acids with side chains. It should be noted, however, that the experimental estimates of helix atom fluctuations in metmyoglobin and ferrocytochrome *c* are significantly different. As to the rms values calculated from the harmonic dynamics, they are clearly too small.

Mössbauer spectroscopy (18, 19) measurements of the ^{57}Fe temperature (Debye–Waller) factor for ^{57}Fe in myoglobin as a function of temperature suggest that at approximately 200 K there is a transition leading to increased motion in the interior. From the present study of the temperature dependence of the atomic fluctuations in the isolated helix, it appears that part of the observed increase may be due to the greater importance of anharmonic contributions to the motion at higher temperatures.

To determine the time dependence of the positional fluctuations, the normalized autocorrelation function, $C_{\Delta R(t)} = \langle \Delta R(o) \Delta R(t) \rangle / \langle |\Delta R(o)|^2 \rangle$, was evaluated. The results for the C^α atom of a central residue (6) are shown in Fig. 3 *Upper*; the curve was obtained from the full dynamics 10-ps trajectory at 300 K. It can be seen that the correlation function is dominated by an

Table 3. rms fluctuations for C^{α} atoms in α-helices*

Temperature, K	System	Estimated from *B* factors	System	Molecular dynamics	Harmonic dynamics
250	Myoglobin	0.41†	Decaglycine	0.42–0.59‡	0.32–0.36‡
300	Myoglobin	0.48†	Decaglycine	0.52–0.61‡	0.35–0.39‡
300	Cytochrome *c*	0.57§	Cytochrome *c*	0.54§	

* All values in Å.
† From ref. 6.
‡ The smaller value is for the central residues (5 and 6) and the larger value is for all the interior residues (2–9).
§ From ref. 8.

oscillation with a frequency of about 30 cm^{-1} ($\tau \approx 1$ ps). Superposed on this oscillatory function is a much slower decay with an apparent relaxation time on the order of ≈18 ps, though a longer simulation would be required to obtain an accurate value. These results for the isolated α-helix contrast somewhat with those obtained for the positional fluctuations in proteins. For pancreatic trypsin inhibitor, the main-chain atom correlation functions generally show a rapid decay with a relaxation time between 0.5 and 5 ps and the oscillations in the correlation function of most, but not all, atoms are weak (20); the correlation functions of the atoms in the terminal helix of the pancreatic trypsin inhibitor have the normal rapid decay with some oscillations (unpublished). Thus, although the amplitudes of the positional fluctuations in the middle of the isolated α-helix and the protein interior are very similar, their time dependence appears to be different. This is likely to be due to the fact that, in the protein interior, high-frequency collisions occur between the atom under examination and the surrounding protein atoms; an illustration of such interactions is given in figures 2 and 3 of ref. 21 for the torques due to collisions involving a tyrosine ring in bovine pancreatic trypsin inhibitor.

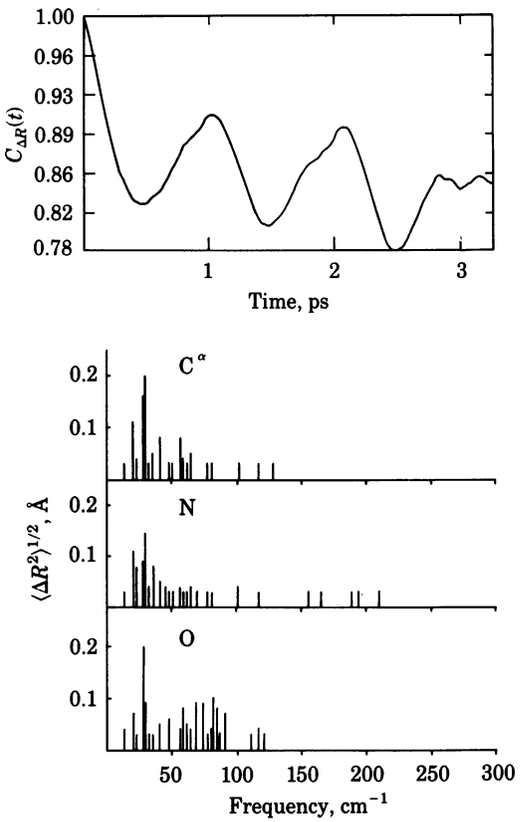

FIG. 3. Time dependence of the fluctuations of a C^{α} atom (residue 6) at 300 K. (*Upper*) Positional correlation function $\langle \Delta R(o) \Delta R(t) \rangle / \langle |\Delta R(o)|^2 \rangle$ as a function of time in full dynamics. (*Lower*) Spectral density in terms of normal modes obtained from harmonic dynamics at 300 K.

To examine the α-helix motions in more detail, we have used the harmonic model and determined the spectral densities corresponding to the positional fluctuations. The results for C^{α} of residue 6 are shown in Fig. 3 *Lower*. It is clear that all of the contributions come from frequencies below 150 cm^{-1} and that the most important terms are below 75 cm^{-1}. In fact, the largest contributions are from vibrations with frequencies of 28 and 30 cm^{-1}, in approximate agreement with the dominant oscillation in the correlation function for the full dynamics. These low-frequency modes have large contributions from the backbone dihedral angles.

The results of the spectral analysis make it possible to determine the quantum corrections to the mean square position fluctuation in the harmonic approximation. Using Eq. 3 and summing over the modes that make a nonnegligible contribution to the displacements, we find that for the C^{α} atom of residue 6, the rms displacement is 0.143 Å at 50 K and 0.3303 Å at 300 K; the corresponding classical values are 0.135 Å at 50 K and 0.3295 Å at 300 K. Thus, it is clear that although the quantum values are always larger, as expected, the magnitude of the corrections to the rms fluctuations is small at 50 K and negligible at 300 K in the harmonic approximation. In the full dynamics, the quantum corrections should be even less important. These conclusions are in accord with qualitative arguments given previously (1, 22).

Although quantum effects are insignificant for the evaluation of positional fluctuations at room temperature, other properties such as the heat capacity and absolute entropy (16, 23) have more important quantum corrections. For the α-helix, the energy as a function of temperature from the complete molecular dynamics calculation yield a classical heat capacity, C_V, very close to the harmonic value of $3k_B$ per atom. Quantum corrections are expected to reduce this value by a significant amount (1).

CONCLUSIONS

This study provides a quantitative theoretical analysis of the accuracy of harmonic models for polypeptide dynamics. By a comparison of the mean square amplitudes of the positional fluctuations obtained as a function of temperature between 5 and 300 K for a model decaglycine α-helix by a full molecular dynamics simulation and by the harmonic approximation to the dynamics, we have shown that there are significant anharmonic effects above 100 K; the harmonic results are found to underestimate the amplitudes of the mean square atomic fluctuations by more than a factor of 2 at 300 K. Comparison with x-ray temperature factor results for helices in protein confirms the importance of the anharmonic contribution. A more detailed anal-

ysis of the equilibrium and time-dependent properties of α-helices obtained in the full dynamics will be given in a future publication.

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1. Karplus, M. & McCammon, J. A. (1981) *CRC Crit. Rev. Biochem.* **9,** 293–349.
2. Gurd, F. R. N. & Rothgeb, T. M. (1979) *Adv. Protein Chem.* **33,** 73–165.
3. Williams, R. J. P. (1979) *Biol. Rev.* **54,** 389–437.
4. McCammon, J. A., Gelin, B. R. & Karplus, M. (1977) *Nature (London)* **267,** 585–590.
5. McCammon, J. A. & Karplus, M. (1980) *Annu. Rev. Phys. Chem.* **31,** 29–45.
6. Frauenfelder, H., Petsko, G. A. & Tsernoglou, D. (1979) *Nature (London)* **280,** 558–563.
7. Artymiuk, P. J., Blake, C. C. F., Grace, D. E. P., Oatley, S. J., Phillips, D. C. & Sternberg, M. J. E. (1979) *Nature (London)* **280,** 563–568.
8. Northrup, S. H., Pear, M. R., McCammon, J. A., Karplus, M. & Takano, T. (1980) *Nature (London)* **286,** 304–305.
9. McCammon, J. A., Wolynes, P. G. & Karplus, M. (1979) *Biochemistry* **18,** 927–942.
10. Lifshitz, I. M. (1969) *Soviet Phys. JEPT (Engl. Transl.)* **28,** 1280–1286.
11. Levy, R. M. & Karplus, M. (1979) *Biopolymers* **18,** 2465–2495.
12. Peticolas, W. L. (1979) *Biopolymers* **18,** 747–755.
13. Gō, M. & Gō, N. (1976) *Biopolymers* **15,** 1119–1127.
14. Gō, N. (1978) *Biopolymers* **17,** 1373–1379.
15. Cohen-Tannoudji, C., Diu, B. & Laloë, F. (1973) *Mecanique Quantique* (Hermann, Paris), Vol. 1, p. 634.
16. Karplus, M. & Kushick, J. (1981) *Macromolecules* **14,** 325–332.
17. Hartmann, H., Parak, F., Steigemann, W., Petsko, G. A., Ringe Ponzi, D. & Frauenfelder, H. (1982) *Proc. Natl. Acad. Sci. USA* **79,** in press.
18. Keller, H. & Debrunner, P. G. (1980) *Phys. Rev. Lett.* **45,** 68–71.
19. Parak, F., Frolov, E. N., Mössbauer, R. L. & Goldanskii, V. I. (1981) *J. Mol. Biol.* **145,** 825–833.
20. van Gunsteren, W. F. & Karplus, M. (1981) *Biochemistry,* in press.
21. McCammon, J. A. & Karplus, M. (1979) *Proc. Natl. Acad. Sci.* **76,** 3585–3589.
22. Landau, L. D. & Lifshitz, E. M. (1969) *Statistical Physics* (Permagon, New York), 2nd Ed.
23. Gō, N. & Scheraga, H. A. (1969) *J. Chem. Phys.* **51,** 4751–4767.