Using Macromolecular Dynamics Simulations to Interpret Experiments

RONALD M. LEVY
Department of Chemistry, Rutgers University, New Brunswick, NJ 08903, USA

(*Received 2 May 1986*)

Abstract. The use of molecular dynamics simulations to study the internal motions and structural flexibility of proteins and nucleic acids has received a great deal of attention in recent years. The attraction of the method is, in part, its potential for studying detailed properties of biological macromolecules that are only indirectly accessible to experiment. Equally important is the use of the method to provide a more fundamental understanding of the molecular information contained in various kinds of experiments on these complex systems. In this paper we review recent work in our laboratory concerned with the use of computer simulations for the interpretation of experimental probes of molecular structure and dynamics of proteins and nucleic acids. The interplay between computer simulations and three experimental techniques are emphasized: (1) nuclear magnetic resonance relaxation spectroscopy; (2) refinement of macromolecular X-ray structures; and (3) vibrational spectroscopy.

INTRODUCTION

Molecular dynamics simulations provide the most powerful theoretical method for studying the atomic mobility of proteins and nucleic acids at the atomic level (see references 1–4 for recent reviews). The molecular dynamics methodology as implemented for these macromolecules was originally developed to study structural and dynamical aspects of liquids and the two disciplines — macromolecular and liquid state simulations-still maintain a very close relationship. Packing considerations play a major role in determining the molecular properties of both liquids and biological macromolecules; because packing can be described by simple potential functions of the kind used in liquid state and macromolecular simulations there is a good foundation for a computer simulation based approach to the study of macromolecular structure and dynamics. For both simple and complex liquids, molecular dynamics simulations have been demonstrated to provide accurate descriptions of structural and dynamical properties.[5] Several factors, however, distinguish molecular dynamics simulations of macromolecules from liquid state simulations so that it is difficult to use experience gained from molecular dynamics simulations of liquids to estimate the precision inherent in the protein simulations. For liquid simulations the basic system contains at least 100 identical molecules so that it is possible to take advantage of considerable statistical averaging in the calculation of quantities for comparison with experiment. For protein and nucleic acid molecular dynamics simulations in contrast, the computational effort required to evaluate the large number of interatomic interactions within a single molecule limits the simulated system to one or at most a very small number of macromolecules. The highly anisotropic nature of the protein or nucleic acid interior and intrinsic interest in extracting site specific information further complicates the computational problem.

Additional features of macromolecular simulations which make them different from and more difficult than simulations of liquids and solids include the difficulty in obtaining exact results for comparison with trajectory averages, the slow convergence of the macromolecular simulations and the problem of treating quantum effects for large systems which lack a high degree of symmetry. Despite these difficulties, computer simulations of biological macromolecules are playing an increasingly important role in biophysical chemistry. One important use involves the modeling of biochemical processes via computer simulation.[6–8] Another major area of research concerns the relationship between computer simulations and experiment.[4] There are four aspects of this area of research which have been stressed: (1) the use of experimental results to refine empirical potentials; (2) the use of simulations as a testing ground for models used to interpret experiment; (3) the use of simulations to better understand the molecular information contained in experiment; and (4) the use of simulations to suggest new experiments. In this paper recent work in my laboratory concerned with the use of computer simulations to provide a theoretical foundation for the interpretation of experimental probes of macromolecular dynamics is reviewed. The interplay between computer simulations and three experimental techniques are emphasized: (1) nuclear magnetic resonance relaxation spectroscopy, (2) X-ray refinement of macromolecular structures and (3) vibrational spectroscopy.

NUCLEAR MAGNETIC RESONANCE RELAXATION SPECTROSCOPY

High resolution NMR spectroscopy and X-ray diffraction provide the primary experimental probes of protein and nucleic acid dynamics at the atomic level. The interpretation of these experiments however is difficult because these macromolecules have many coupled internal degrees of freedom, and because the structural fluctuations occur over a wide range of frequencies. NMR relaxation parameters for proteins in solution are determined by dynamics on the picosecond-nanosecond time scale so that comparisons of simulations lasting 100 ps with NMR experiments are more straightforward than with experiments involving much longer time scales.

The NMR relaxation parameters probe angular time

correlation functions (the spherical harmonics) of the relaxing nucleus. These correlation functions and their Fourier transforms, the spectral densities, can be obtained directly from the macromolecular trajectories. For the C^{13} nucleus, the dynamical quantities of interest are the spherical polar coordinates (θ, ϕ) with respect to the laboratory frame of the C–H internucleus vectors. Because of the highly restricted nature of the motion in the protein interior, the NMR correlation functions do not decay to zero on a picosecond time scale. We have observed from an analysis of protein trajectories that for many carbons the NMR correlation functions decay to a plateau value within a time period τ_p which is short compared with the total length of the trajectory (see Fig. 1). The plateau value is an important quantity because it can be related directly to experimental relaxation times and because it provides a measure of the motional freedom (order parameter) of the NMR probe. We have shown how to calculate the order parameter for a dipolar NMR probe from conventional molecular dynamics simulations.[10,11] The results of detailed studies of order parameters and comparisons with experiment have been reported for dipolar C^{13} NMR relaxation for protonated[11] and non-protonated[10] carbons and for dipolar cross relaxation (transient Overhauser effect).[12]

Since the initial decay of the NMR correlation functions occur in a very short time, the spectral density at the Larmor frequency is reduced uniformly. If the relaxation is fast enough ($\tau_p < 10$ ps) the following simple formula for the measured spin lattice relaxation is valid:[9-11]

$$\frac{1}{T_1} = \mathcal{S}^2 \left(\frac{1}{T_1}\right)^{\text{Rigid}} \qquad (1)$$

where T_1 is the spin lattice relaxation rate for a rigidly tumbling protein and $\mathcal{S}^2$ is the order parameter for the probe. Lipari and Szabo have demonstrated that for internal motions that relax on a time scale less than 300 ps, the measured relaxation rates can be fit more generally to a two parameter equation involving $\mathcal{S}^2$ and a parameter τ_p which is proportional to the area under the correlation function for internal motion.[13]

The experimental [14] order parameters for PTI have been compared with those calculated from a 96 ps molecular dynamics simulation.[15] The relative flexibility of the residues in the simulation is in good agreement with the experimental results.[11] Of potential biological importance is the observation that the region of the inhibitor which appears most flexible by NMR (smallest order parameters) is the region which is in contact with trypsin in the complex. In a recent molecular dynamics simulation of another protease inhibitor (turkey ovomucoid inhibitor), it was also observed that the enzyme binding site was the most flexible portion of the free inhibitor.[16]

Fluorescence depolarization can also be used to probe reorientational dynamics of residues in proteins and nucleic acids on a time scale accessible to molecular dynamics simulations. The time correlation functions probed by fluorescence experiments have a form similar to that for NMR relaxation. For reviews of the analysis of the effects of fast motions on fluorescence depolarization using macromolecular simulations see references 17 and 18.

X-RAY REFINEMENT SIMULATIONS

It has become clear during the last five years that there is much valuable information concerning the spatial distribution of atomic fluctuations contained in high resolution X-ray diffraction data on proteins and nucleic acids.[19] Crystallographic refinement is a procedure which iteratively improves the agreement between structure factors derived from X-ray intensities and those calculated from a model structure. The refinement models usually include four parameters per atom, the three positional coordinates and one temperature factor. Information concerning the spatial distribution of atomic fluctuations is contained in the temperature factor. For isotropic, harmonic motion of atoms, the relationship between the mean square displacement and temperature factor B is:

$$B_j = \frac{8\pi^2}{3} \langle (\Delta r_j)^2 \rangle. \qquad (2)$$

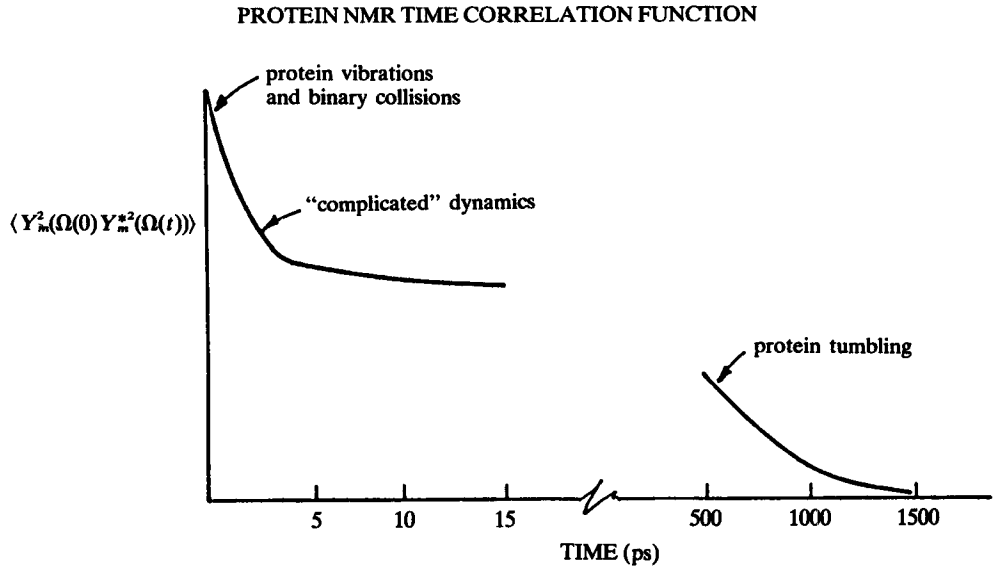

Fig. 1. Schematic diagram of the general behavior of the decay of NMR correlation functions in proteins. The physical origins for the decay resulting from the different kinds of protein motions are indicated in the figure (Levy *et al.*, Ref. 9).

The results of several protein molecular dynamics simulations have been compared with experimental temperature factors.[1-3,20-22] While there is qualitative agreement between the displacements calculated from the simulations and experimental values extracted from temperature factors, atoms with the largest displacements appear to exhibit much larger fluctuations in the simulations than in the X-ray experimental results. As an example, comparisons between a recent 300 ps simulation of myoglobin and experimental values are presented in Table I.[22] When the macromolecular simulation results do not agree with experiment, it is always assumed the discrepancies are due to approximations or errors in the simulations. However, in order to resolve quantitative discrepancies between the simulations and experimental results, it is necessary to evaluate possible sources of error in both. With regard to experiment, the quantitative analysis of atomic displacements using temperature factors for proteins is not straightforward. A number of assumptions concerning protein motions are incorporated into the crystallographic refinement programs which affect the subsequent refinement and the temperature factors. Furthermore, in light of the recent attempts to correlate protein antigenicity with mobility as reflected in temperature factors[23,24] it is particularly important to establish the extent to which temperature factors for macromolecules reflect the underlying molecular flexibility. Molecular dynamics simulations can be used to evaluate the effects of approximations contained in the refinement on the final structure and temperature factors. Two such studies have recently been carried out: one for a protein at a single temperature,[25] and another for a nucleic acid oligamer at several temperatures.[26] We briefly describe here the basic idea employed.

The structure factor $F(Q)$ in X-ray crystallography is the fourier transform of the electron density for the molecule:

$$F(\boldsymbol{Q}) = \int d\boldsymbol{r}\rho(\boldsymbol{r})\exp(i\boldsymbol{Q}\cdot\boldsymbol{r}) \qquad (3)$$

where $\rho(\boldsymbol{r})$ is the electron density at $\boldsymbol{r}$. In a crystallography experiment the electron density varies with time due to thermal motion and the observed structure factor amplitude is the time average of Eq. (3):

$$F_0(\boldsymbol{Q}) = \langle F(\boldsymbol{Q})\rangle = \int d\boldsymbol{r}\langle\rho(\boldsymbol{r})\rangle\exp(i\boldsymbol{Q}\cdot\boldsymbol{r}). \qquad (4)$$

In order to generate a set of calculated structure factors $F_0(Q)$ from a set of coordinates, it is necessary to introduce a model for the time variation of the electron density. The usual assumptions in macromolecular crystallography include harmonic isotropic motion of the atoms and, in addition, the molecular scattering factor is expressed as a superposition of atomic scattering factors. With these assumptions the calculated structure factor is given by:

$$F_c(\boldsymbol{Q}) = \sum_{j=1}^{N}\exp(i\boldsymbol{Q}_j\cdot\boldsymbol{r}_j)\exp W_j(\boldsymbol{Q})F_j(\boldsymbol{Q}) \qquad (5)$$

where $F_j(\boldsymbol{Q})$ is the atomic scattering factor for atom j and r_j is the position of atom j in the model structure. The thermal averaging of atomic motion is contained in the atomic Debye–Waller factor $\exp(W_j(Q))$. W_j is given by:

$$W_j(\boldsymbol{Q}) = -B_j|\boldsymbol{Q}|^2 \qquad (6)$$

where B_j is the atomic temperature factor. The mean square atomic fluctuation for atom j is obtained from the refined temperature factors through Eq.1. The thermal averaging implicit in Eq. 4 is accomplished by averaging the atomic structure factors obtained from coordinate sets sampled along the molecular dynamics trajectories at each temperature:

$$F_0(\boldsymbol{Q}) = \langle F(Q)\rangle = \frac{1}{M}\sum_{k=1}^{M}\sum_{j=1}^{N}F_j(\boldsymbol{Q})\exp(-i\boldsymbol{Q}\cdot\boldsymbol{r}_j^k) \qquad (7)$$

where r_j^k is the position of the jth atom in the kth coordinate set along the trajectory and M is the number of coordinate sets sampled.

Table I. Root Mean Square Atomic Fluctuations* Averaged Over Different Portions of the 300 ps Myoglobin Simulation

Time interval, ps	$(\Delta R)^{2+}$
0–100	0.829
100–200	0.829
200–300	0.707
0–100	0.829
0–200	0.960
0–300	1.071
Experiment	0.650

* Average of rms fluctuations backbone N, C^{α}, C' atoms, Å.
+ $\langle(\Delta R)^2\rangle^{1/2} = \{(\Delta x)^2 + (\Delta y)^2 + (\Delta z)^2\}^{1/2}$.
Levy *et al.*, Ref. 22.

X-RAY REFINEMENT SIMULATION OF MYOGLOBIN

A 300 ps molecular dynamics simulation of myoglobin[22] was used to test the X-ray refinement procedure as outlined above.[25] The simulated X-ray intensities at the Bragg reciprocal lattice points were used as input to the restrained least squares refinement program PROLSQ of Hendrickson and Konnert.[27] Several refinements were carried out using varying stereochemical and temperature factor restraints and including alternate conformations of selected side chains. The final R factors, coordinates and temperature factors were analyzed and compared with the "exact" trajectory reults. The final R factors varied between 16%–19%. These high R-factors reflect the neglect of anisotropy and especially anharmonicity in the atomic fluctuations. The overall rms errors in atomic positions ranged from 0.24 Å to 0.29 Å in the different refined structures. For the backbone atoms the largest errors exceeded 0.5 Å while for the side chains errors as large as 1.0 Å were observed. One of the most significant results was that the refinement with tight stereochemical restraints produced the lowest rms error in backbone positions (0.10 Å) of all the refinements.

The refined mean square fluctuations were found to be systematically smaller than the fluctuations calculated directly from the simulation. The average temperature factors calculated from the myoglobin molecular dynamics trajectory are compared with the temperature factors obtained from the refinement of the simulated intensities and with B factors for two experimental crystal structures in Table II. For the side chain atoms which have the largest rms displacement ($\langle(\Delta r)^2\rangle \geq 1$ Å^2) the refined temperature factors are 50% smaller than the exact values. It is of interest to note that when the side chain atomic fluctuations are calculated directly from the molecular dynamics simulation they appear much too large compared with values estimated from experimental

Table II.* B-Factors for Various Crystal Structures of Myoglobin Compared with Molecular Dynamics Values

Source	Backbone Average Temperature Factor	Sidechain Average Temperature Factor
1. Met-myoglobin, (Ref. 28)	11.80	13.1
2. Oxy-myoglobin, (Ref. 38)	11.50	21.12
3. 25 ps molecular dynamics	12.33	26.80
4. 25 ps molecular dynamics restrained refinement	11.35	16.51
5. 25 ps molecular dynamics refinement with tight restraints	12.49	14.48
6. 25 ps molecular dynamics unrestrained refinement	11.74	17.61
7. 300 ps molecular dynamics	25.64	48.63
8. 300 ps molecular dynamics restrained refinement	16.81	21.08
9. 300 ps molecular dynamics restrained refinement with higher initial B	19.20	23.46

* Kuriyan *et al.*, Ref. 25.

refinements. However, when the trajectory data is "refined" in the same way as experimental intensities the theoretical and experimental temperature factors are in close accord. This provides an important example of the difficulty encountered in facile attempts to compare the results of molecular dynamics simulations with experimental quantities and of the need to analyze errors that may be introduced by the experimental analysis itself.

TEMPERATURE DEPENDENT X-RAY REFINEMENT SIMULATIONS OF A Z-DNA HEXAMER

Analysis of the temperature dependence of Debye–Waller factors can provide information concerning the potential within which the atoms move.[19,28-30] We have used the X-ray refinement simulation technique to explore the extent to which the data from X-ray refinements provides an accurate description of the temperature dependence of the underlying atomic mobility. Molecular dynamics simulations of a *Z*-DNA hexamer were performed at several temperatures between 100 K and 300 K. At each temperature the trajectories were used to construct a set of X-ray diffraction intensities which were then refined. At the highest temperature and for the most mobile atoms, the effect of the refinement is to significantly reduce the mean square atomic fluctuations as described above for myoglobin. This is most evident for the phosphate atoms of the *Z*-DNA backbone (see Fig. 2). Although the errors introduced by the approximations inherent in the refinement of the nucleotide are consistent with the results obtained from the myoglobin study at a single temperature, we found that the temperature dependence of the atomic mobilities as estimated from temperature factors provides a reasonably accurate description of the true temperature dependence of the system. In Fig. 2b and 2c the mean square atomic fluctuations extracted from the refinement simulations at each temperature and averaged by group are plotted as a function of temperature for comparison with the exact results shown in Fig. 2a. As to the refinement without temperature factor restraints (Fig. 2b) except for the phosphates at the highest temperature, the extent of anharmonicity (curvature) is in good agreement with the exact result despite the fact the refinement model assumes isotropic, harmonic motion. It is clear from Fig. 2c that when strong temperature factor restraints are introduced in the refinement, differences in the temperature dependence of the atomic fluctuations among the groups are suppressed.

One useful approach to an analysis of the anharmonicity implicit in the results shown in Fig. 2 is to model the atomic potential function by an effective spherically symmetric, temperature independent potential of the form $V(r) = Cr^{\mu}$. The probability of a displacement by r from equilibrium is according to the Boltzmann distribution $\rho(r) \sim \exp(-cr^{\mu}/k_B T)$ and the average mean square displacement is $\langle(\Delta r)^2\rangle \sim T^{2/\mu}$. The value of μ is obtained from a $\log r$ versus $\log T$ plot of the results shown in Fig. 2. Experimental temperature factors for proteins have been analyzed in this way[18,28-30] as have the results of temperature dependent molecular dynamics simulations of a polypeptide α-helix.[29] The temperature dependence of the *Z*-DNA atomic fluctuations between 113 and 300 K yields values of $\mu < 1$ with the largest anharmonicity (smallest value of μ) calculated for the GpC phosphates for which $\mu = 0.6$. It is of interest to note that the atoms with the largest atomic fluctuations (GpC phosphates) exhibit the greatest temperature dependence.

The present results provide a theoretical foundation for the use of Debye–Waller factors obtained from refinements at several temperatures to extract information concerning the anharmonicity of the atomic displacements and underlying potential surface.

REFINEMENT OF MD AVERAGE STRUCTURES AGAINST EXPERIMENTAL X-RAY INTENSITIES

The refinement of macromolecular structures in X-ray crystallography involves the minimization of a function of many variables. During the refinement it is possible to get trapped in a local minimum of this function. Molecular dynamics trajectories provide one possible approach to escaping from a local minimum in the iterative refinement of an X-ray structure. We have refined the average structure obtained from the molecular dynamics simulations of the *Z*-DNA hexamer against experimental structure factors. At low temperature, the effect of the restrained refinement of the MD average structure against the experimental structure factors is to move the MD average coordinates closer to the crystal structure (the average rms displacement between the refined MD coordinates and crystal is 0.08 Å as compared with 0.14 Å rms between the MD coordinates and the crystal). At the same time, however, the number of bad distances has decreased from 92 with an *R* factor of 14% for the converged experimental refinement starting from an ideal model *Z*-DNA structure to 37 with an *R* factor of 13.7% for the refinement against molecular dynamics

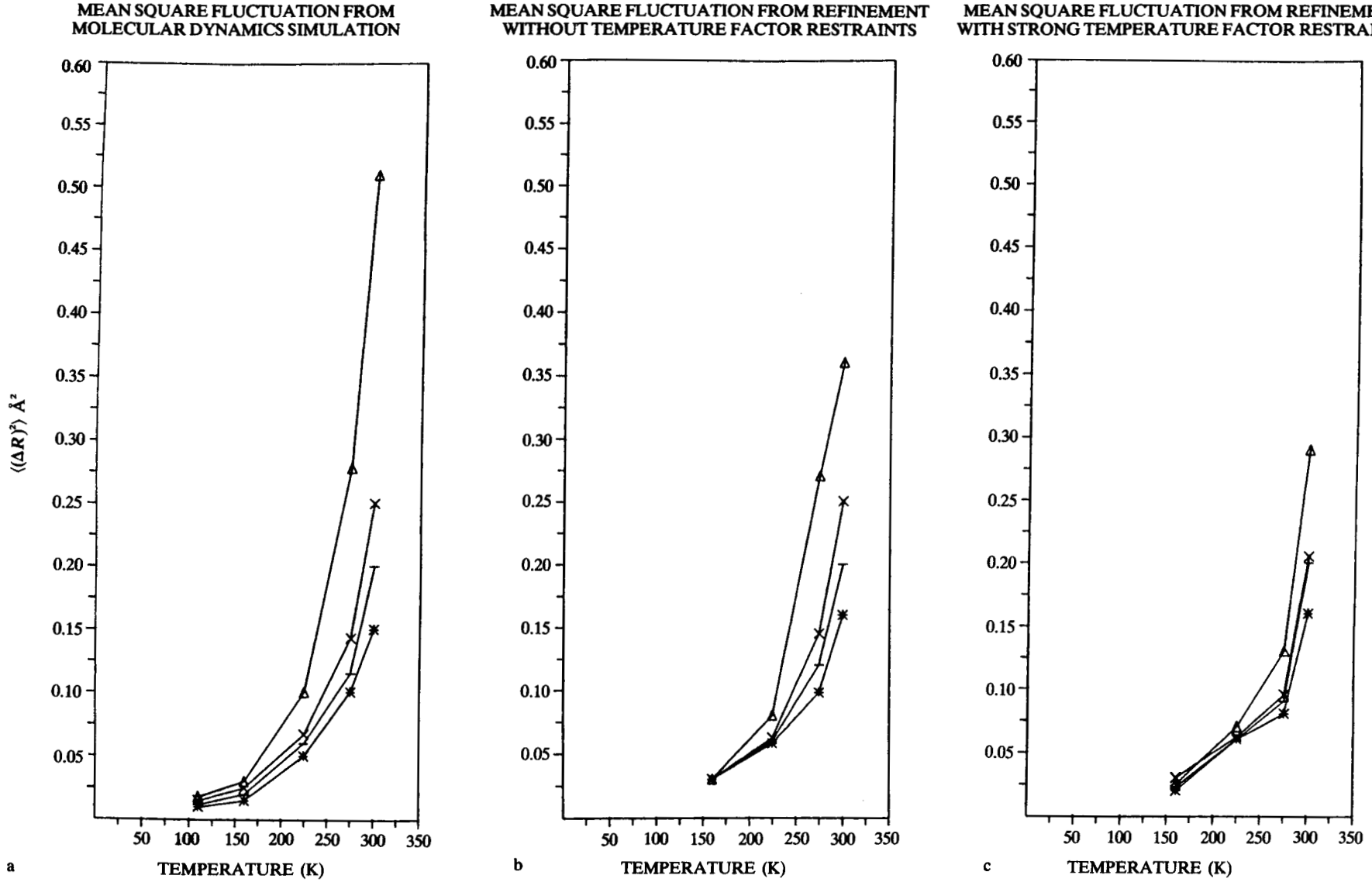

Fig. 2. Temperature dependence of mean square atomic fluctuations of the Z-DNA hexamer. Fig. 2a, mean square atomic fluctuations calculated directly from the molecular dynamics trajectories. Fig. 2b and Fig. 2c mean square atomic fluctuations calculated using $(\Delta r)^2 = (3/8\pi^2)B$ with the Debye–Waller temperature factors obtained from the X-ray refinement of the molecular dynamics trajectories with no restraints (Fig. 2b) and with tight restraints (Fig. 2c).
Symbols:* bases, – sugars, × CpG phosphates, Δ GpC phosphates. (Westhof *et al.*, Ref. 26).

average coordinates. By the R-factor and stereochemistry criteria, the refined low temperature MD coordinates represent a better structure than the initial crystal structures. Molecular dynamics simulations at low temperatures may thus provide a useful tool for optimizing refined structures.

VIBRATIONAL SPECTROSCOPY

As a final example of the fruitful interaction which can exist between simulations and experiment I review some recent work in my laboratory concerned with the use of simulations to provide a clearer structural interpretation of environmental effects on vibrational spectra. One approach to the interpretation of vibrational spectra for biopolymers has been a harmonic analysis whereby spectra are fit by force constant changes. There are a number of reasons for developing other approaches. The consistent force field (CFF) potentials developed by Lifson and co-workers,[31] which are used in macromolecular simulations, are meant to model the motions of the atoms over a range of conformations without reparameterization. It is desirable to develop a formalism for interpreting vibrational spectra which takes into account the variation in the conformations of the chromophore and surroundings which occur due to thermal motions. Furthermore, much of the interesting structural information that can be extracted from experiments involves understanding the origin of spectral shifts as a function of some experimentally adjustable parameter, often the temperature. If computer simulations on realistic potential surfaces are to be useful for interpreting these experiments, methods must be developed which incorporate these anharmonic effects on the spectra. Semi-classical trajectories provide one possible approach to this problem. Here we present an alternative approach.

THE QUASIHARMONIC MODEL FOR VIBRATIONAL SPECTRA

We have introduced a new method for calculating vibrational spectra from classical molecular dynamics or Monte-Carlo simulations.[32,33] The method involves a quasiharmonic oscillator approximation in which a temperature dependent quadratic Hamiltonian is parameterized from the results of a simulation on the complete (anharmonic) potential. The parameterization is accomplished by fitting the first and second moments of the coordinate and momentum distributions obtained from a simulation on the exact surface to a harmonic model.

As a first illustration of the method, we recently reported the results of a vibrational analysis of a small molecule (butane) with six internal degrees of freedom using the quasiharmonic oscillator model.[32] The empirical potential contained all the terms present in the potential for macromolecules, viz bond stretching, bending, and torsional terms as well as non-bonded interactions. A novel aspect of the simulation procedure was the use of normal-mode eigenvectors as the independent coordinates for Monte-Carlo sampling which was demonstrated to substantially increase the convergence rate of the simulation. From a conventional normal mode analysis we extracted the frequencies of the model which ranged from 119 cm^{-1} for a pure torsional vibration to 1044 cm^{-1} for a mixed bond stretch-angle bend vibration. Classical simulations were performed on the complete surface at a series of temperatures between 5 K and 300 K. We demonstrated how anharmonic effects at higher temperatures can rotate the normal coordinates and shift the frequencies with respect to the harmonic values. The quasiharmonic frequencies calculated from Monte-Carlo trajectories on the anharmonic potential surface for trans and gauche butane are listed in Table III. The effective frequency of the torsional mode is lowered by 25 cm^{-1} to 91 cm^{-1} at 300 K. The anharmonicity of the exact potential results in the decreasing curvature of the quasiharmonic potential and the lowering of the effective torsional frequency with temperature.

We have initiated a study of intramolecular vibrations in liquid water using the quasiharmonic analysis method.[33,34] The important experimental observation is that on going from the gas phase to the liquid the OH stretching vibration is red shifted by 300 cm^{-1} while the HOH bending vibration is blue shifted by 100 cm^{-1}.[35] In the simplest form of quasiharmonic analysis the effective frequencies are constructed from the probability distributions for the vibrational displacements of OH bond and HOH bond angle in the gas and liquid phases respectively. In the gas phase these probability distributions were calculated by direct numerical integration of the partition function while for the liquid, the distributions were constructed from a 5 ps simulation of 216 water molecules with periodic boundary conditions in the TVN ensemble. For the OH stretching vibration, the gas phase quasiharmonic frequency is 3,970 cm^{-1}, the liquid phase vibrational frequency is calculated to be 3,792 cm^{-1}, resulting in a red shift of 200 cm^{-1}. In contrast, the HOH bending quasiharmonic frequency is blue shifted by 75 cm^{-1} in solution compared to the gas phase calculation ($\omega_{eff} = 1{,}274$ cm^{-1} gas phase, $\omega_{eff} = 1{,}348$ cm^{-1} liquid). It is very encouraging that the simplest quasiharmonic approximation qualitatively reproduces the directions of the water vibrational frequency shifts on going from the gas to the liquid state. In should also be possible to use this simple approximation to estimate the inhomogeneous contribution to the vibrational linewidths by constructing a distribution of quasiharmonic frequencies from a quasiharmonic analysis of individual water molecules.

The approach to the evaluation of vibrational spectra described above is based on classical simulations for which quantum corrections are possible. The incorporation of quantum effects directly in simulations of large molecular systems is one of the most challenging re-

Table III.* Quasi-Harmonic Frequencies[a] Calculated from Monte Carlo trajectories[b] on the Exact Potential Surface for Trans and Gauche Butane

trans			gauche
100 K	200 K	300 K	300 K
113 (5)[c]	102 (17)[c]	91 (25)[c]	99 (33)[c]
407	406	407	419
435	437	436	602
899	911	892	857
1008	1014	1002	966
1045	1055	1043	1034

a. Frequency in cm^{-1}.
b. Monte Carlo trajectories constructed with Q_k (mass-weighted Cartesian) as independent coordinates.
c. Numbers in parentheses indicated percent deviation from harmonic normal-mode eigenvalues; only deviations greater than 1% indicated.
* Levy *et al.*, Ref. 32.

search areas in theoretical chemistry today. The direct evaluation of quantum time correlation functions for anharmonic systems is extremely difficult. We are pursuing an approach to the incorporation of quantum effects on vibrational lineshapes based upon the fact that the moments of the vibrational spectrum can be expressed as functions of equilibrium averages of positional operators. These expectation values can in turn be evaluated using new and efficient techniques for evaluating discretized path integrals.[36,37] The results obtained for small model systems are encouraging[33] and extensions to macromolecular simulations are underway.

CONCLUSIONS

New methods have been developed for comparing the results of macromolecular dynamics simulations with a variety of experiments and for testing the approximations inherent in models used to extract molecular information from experimental results. A number of comparisons between computer simulations and experimental results have been made. The connections between different approaches to the study of macromolecular dynamics continue to be strengthened. It is clear that the relative mobility of the atoms within proteins and nucleic acids is accurately reflected in the molecular dynamics simulations. For proteins, a qualitative picture is emerging from both theoretical and experimental studies of the vibrations of atoms about distinct time averaged conformations. The presence of many local potential minima in the vicinity of the X-ray structure severely complicates the problem of achieving fully converged simulations. The availability of supercomputing facilities is requied for systematic studies of possible solutions to the convergence problem in macromolecular simulations.

Acknowledgements. I am grateful to my scientific colleagues who have collaborated with me on several of the papers listed in the references for the discussions and collaborations concerning the relationship between computer simulations and experimental probes of macromolecular dynamics. This work has been supported in part by a National Institutes of Health Grant GM-30580 and by the award of an Alfred P. Sloan Foundation Fellowship and NIH Research Career Development Award. I thank the members of the Laboratoire du Cristallographie Biologique, I.B.M.C., C.N.R.S, Strasbourg, France for their hospitality during the writing of this manuscript.

REFERENCES

1. M. Karplus and J. A. McCammon, *Ann. Rev. Biochem.*, **52**, 263 (1983).
2. J. A. McCammon, *Rep. Prog. Phys.*, **47**, 1 (1984).
3. M. Levitt, *J. Mol. Biol.*, **186**, 595 (1983).
4. R. M. Levy and J. Keepers, *Comments on Molecular and Cellular Biophys.*, in press (1986).
5. J. P. Hansen and I. R. MacDonald, *Theory of Simple Liquids*, Academic Press, New York, 1976.
6. T. P. Lybrand, J. A. McCammon and G. Wipff, *Proc. Natl. Acad. Sci USA*, **83**, 833 (1986).
7. A. Bruenger, C. L. Brooks and M. Karplus, *Proc. Natl. Acad. Sci. USA*, **82**, 8458 (1985).
8. O. Rojas, R. M. Levy and A. Szabo, *J. Chem. Phys.*, in press.
9. R. M. Levy, M. Karplus and J. A. McCammon, *J. Am. Chem. Soc.*, **103**, 994 (1981).
10. R. M. Levy, C. M. Dobson and M. Karplus, *Biophys J.*, **39**, 107 (1982).
11. G. Lipari, A. Szabo and R. M. Levy, *Nature*, **300**, 197 (1982).
12. E. T. Olejniczak, C. M. Dobson, M. Karplus and R. M. Levy, *J. Am. Chem. Soc.*, **106**, 1923 (1984).
13. G. Lipari and A. Szabo, *J. Am. Chem. Soc.*, **104**, 4546 (1982).
14. R. Richarz, K. Nagayama and K. Withrich, *Biochemistry*, **19**, 5189 (1980).
15. M. Karplus and J. A. McCammon, *Nature*, **267**, 585 (1977).
16. J. Keepers and R. M. Levy, to be published.
17. R. M. Levy and A. Szabo, *J. Am. Chem. Soc.*, **104**, 2073 (1982).
18. T. Ichiye and M. Karplus, *Biochemistry*, **22**, 2884 (1983).
19. G. Petsko and D. Ringe, *Ann. Rev. Biophys.*, **13**, 331 (1984).
20. S. Northrup, M. R. Pear, J. D. Morgan, J. A. McCammon and M. Karplus, *J. Mol. Biol.*, **153**, 1087 (1981).
21. W. F. van Gunsteren, H. J. C. Berendsen, J. Hermans, W. G. J. Hol and J. P. M. Postma, *Proc. Natl. Acad. Sci. USA*, **80**, 4315 (1983).
22. R. M. Levy, R. P. Sheridan, J. W. Keepers, G. S. Dubey, S. Swaminathan and M. Karplus, *Biophys J.*, **48**, 509 (1985).
23. E. Westhof, Altschuh, D. Moras, A. C. Bloomer, A. Mondragon, A. Klug and M. H. V. Van Regenmortel, *Nature*, **311**, 123 (1984).
24. J. Tainer, E. D. Getzoff, H. Alexander, R. A. Houghten, A. J. Olson A. J. Lerner and W. A. Hendrickson *Nature*, **312**, 127 (1984).
25. J. Kuriyan, G. A. Petsko, R. M. Levy and M. Karplus, *J. Mol. Biol.* (1986) in press.
26. E. Westhof, B. Chevrier, S. L. Gallion, P. K. Weiner and R. M. Levy, *J. Mol. Biol.* (1986) in press.
27. W. A. Hendrickson and J. Konnert in R. Diamon, S. Ramasheshan and K. Venkatesan, eds., *Computing in Crystallography*, Indian Institute of Science, Bangalore, 1981, pp. 1301–1323.
28. H. Frauenfelder, G. Petsko and D. Tsernoglu, *Nature*, **280**, 558 (1979).
29. R. M. Levy, D. Perahia and M. Karplus, *Proc. Natl. Acad. Sci. USA*, **79**, 1346 (1982).
30. J. Hartmann, F. Parak, W. Steigman, G. A. Petsko, Ponzi D. Ringe and H. Frauenfelder, *Proc. Natl. Acad. Sci. USA*, **79**, 4967 (1982).
31. A. Warshel, M. Levitt and S. Lifson, *J. Mol. Spect.*, **33**, 84 (1970).
32. R. M. Levy, O. Rojas and R. A. Friesner, *J. Phys. Chem.*, **88**, 4233 (1984).
33. R. M. Levy, *Ann. N. Y. Acad. Sci.* (1986), **282**, 24 (1986).
34. S. Nakai, F. Hirata and R. M. Levy, to be published.
35. D. Eisenberg and Kauzmann, *The Structure and Properties of Water*, Oxford University Press, Oxford, 1969.
36. R. A. Friesner and R. M. Levy, *J. Chem. Phys.*, **80**, 4488 (1984).
37. R. M. Levy, P. Zhang and R. A. Friesner, to be published.
38. S. E. V. Phillips, *J. Mol. Biol.*, **142**, 531 (1980).