

A SIMULATED COOLING PROCESS FOR PROTEINS

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Received 8 January 1990; in final form 14 January 1990

We have developed a molecular dynamics simulation method to cool proteins in. The method uses a stochastic boundary layer which surrounds the protein and simulates a contact with a coolant. The atoms in this layer are subject to fluctuating and dissipative forces and follow a prescribed temperature schedule. In this arrangement the protein is cooled from the boundary into the interior. The temperature change can be described by the heat conduction equation assuming a thermal diffusivity $D=7\times 10^{-4}$ cm² s⁻¹. We have applied the cooling method suggested to a segment of the photosynthetic reaction center of *Rhodospseudomonas viridis*. Cooling leads to a contraction of this structure, evidenced by a decrease in the radius of gyration.

1. Introduction

Biological macromolecular systems are intended to function optimally in a very narrow range of physiological temperatures. To elucidate the mechanisms underlying their function, one often applies perturbations and monitors the resulting macromolecular properties. One such perturbation can be a lowering of temperature. In fact, many low temperature experiments have been made on proteins and interesting results were obtained. For example, Frauenfelder et al. investigated the thermal expansion of myoglobin by X-ray analysis [1]. For the same protein they also systematically investigated the dynamics of ligand binding in the temperature range between 40 and 300 K [2] and Doster et al. [3] carried out inelastic neutron scattering between 160 and 280 K. The kinetics of nitric oxide recombination to myoglobin and cytochrome c oxidase were investigated by means of EPR techniques by LoBrutto et al. [4]. In ref. [5] Privalov describes temperature effects on protein stability and hydrophobic interactions. Various experiments related to the thermal behavior of proteins are discussed in ref. [6].

A protein complex which exhibits interesting temperature effects is the photosynthetic reaction center of *Rhodospseudomonas viridis* (*Rps. viridis*) [7-9].

For this system, Fleming et al. [10] and Kirmaier and Holtz [11] measured the temperature dependence of the primary and secondary electron transfer reactions and observed a highly non-classical, i.e. non-Arrhenius, behavior. A detailed review of the reactions in the photosynthetic reaction center can be found in ref. [12].

Molecular dynamics (MD) simulations are considered today as an important information source of protein function (for reviews see refs. [13,14]). In this respect, it appears desirable to complement laboratory investigations of the low temperature behavior of proteins by MD simulations. An example of such an investigation considering a glass transition in a protein-water solution was carried out by Wong et al. [15]. Naturally, one questions how far such simulations correctly reproduce the equilibrium structure of proteins at low temperatures. Problems may arise in this respect due to the short time scale, a few hundred picoseconds at best, which MD simulations can cover. In this paper, we will address the problem stated and suggest both a method to properly cool proteins as well as to monitor in how far proteins equilibrate during a cooling schedule.

The key idea of our method of cooling proteins is to reproduce faithfully the cooling processes as they occur under laboratory conditions. In the laboratory, the system to be studied is brought into contact with a coolant, and a temperature decrease develops from

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the surface inwards. We model the laboratory method of cooling on a microscopic volume of a single protein: the protein to be cooled is surrounded by a layer of atoms, which can be part of the protein or part of a water-membrane environment. The atoms in this layer constitute the coolant. For this purpose, the ordinary Newtonian equations of motion for the coolant atoms are replaced by Langevin equations which contain dissipative and fluctuating forces. The relationship between dissipative and fluctuating forces, as dictated by the fluctuation-dissipation theorem, contains a temperature factor and can be used to clamp the temperature of the coolant to a desired value. When the temperature of the boundary is initially below that of the protein, the mechanical coupling between atoms in the boundary layer and atoms in the interior region should lead to a conduction of heat from the protein interior towards the boundary. This cooling method does not involve any artificial dynamics for the atoms in the protein interior and, thus this should avoid trapping of artificial structures. On a mesoscopic scale, i.e. on a scale which averages over, let say, a hundred atoms, the spatial and temporal distribution of temperature in the simulated protein should follow closely the phenomenological behavior described by the heat conduction equation. By comparing MD results with the predictions of this equation we can ascertain in how far thermal equilibration is taking place during the simulation. As a by-product of such comparison, one can determine the effective thermal conductivity K of the simulated protein.

We applied our method of simulated cooling to the photosynthetic reaction center of *Rhodospseudomonas viridis* (*Rps. viridis*), a large protein complex. In section 2, we will describe the arrangement of the reaction center in our simulations. In section 3, we will demonstrate that the cooling method can actually be applied successfully. An important structural response of the reaction center to the cooling process will be analysed by monitoring the radius of gyration of the simulated protein segment as a function of temperature.

2. Cooling in a molecular dynamics simulation

2.1. Our set-up of the reaction center

The method of cooling by MD outlined in section 1 is based on treating atoms in a layer surrounding a protein or a protein segment by fixed temperature Langevin dynamics. This treatment corresponds to the stochastic boundary method [16,17] as implemented in XPLOR [18], the program we adopted for our studies. The calculations were carried out on a segment of the photosynthetic reaction center of *Rps. viridis* [7-9] #1. The stochastic boundary was chosen as a spherical shell with inner radius 26.5 Å and outer radius 29 Å around the center-of-mass of the special pair and the bacteriopheophytins, the latter being prominent prosthetic groups in the photosynthetic reaction center. By choosing this arrangement about 5800 of the approximately 13000 reaction center atoms were simulated, 1190 of those were located in the boundary region.

2.2. Cooling through stochastic boundary

The cooling method as briefly outlined in section 1 is based on temperature-dependent forces in the boundary region. In this region, the motion of atom i is governed by the Langevin equation

$$m_i \frac{d^2}{dt^2} \mathbf{r}_i = \mathbf{F}_i^{\text{nat}} + \mathbf{F}_i^{\text{hrm}} + \mathbf{F}_i^{\text{frict}} + \mathbf{F}_i^{\text{fluc}}. \quad (1)$$

Atom i is subject to the native forces $\mathbf{F}_i^{\text{nat}}$, i.e. the forces which represent chemical bonds, Coulomb and van der Waals forces etc., as well as subject to three temperature-dependent forces: an harmonic force which confines each atom to its position in the X-ray structure,

$$\mathbf{F}_i^{\text{hrm}} = -m_i \Omega_i^2 (\mathbf{r}_i - \mathbf{r}_i^{\text{ref}}),$$

$$\Omega_i^2 = \frac{3k_B T_{\text{bath}}}{m_i \langle \Delta r_i^2 \rangle}, \quad (2)$$

a frictional force

#1 The X-ray structure used in our calculations has a 2.3 Å resolution and contains 201 watermolecules.

$$F_i^{\text{frict}} = -m_i \beta_i \frac{d}{dt} r_i, \\ \beta_i = \frac{3k_B T_{\text{bath}}}{m_i} \left(\int_0^\infty \langle \dot{r}_i(0) \cdot \dot{r}_i(t) \rangle dt \right)^{-1}, \quad (3)$$

and a fluctuating force

$$F_i^{\text{fluc}}(t) = \sqrt{\frac{2m_i \beta_i k_B T_{\text{bath}}}{\Delta t}} f_i(t). \quad (4)$$

The vector $f_i(t)$ contains Gaussian-distributed ($(1/\sqrt{2\pi}) \exp(-r^2)$) random numbers r in each component, and Δt represents the integration time step. In the above expressions, T_{bath} is the temperature of the coolant. The harmonic force, as given by eq. (2), is chosen such that the resulting mean quadratic deviation of each atom $\langle \Delta r_i^2 \rangle = \langle |r_i(t) - \langle r_i(t) \rangle|^2 \rangle$ agrees with that implied by the B factors of the X-ray structure: $\langle \Delta r_i^2 \rangle = B_i/8\pi^2$. We assumed the same $\langle \Delta r_i^2 \rangle$ values for all temperatures. The atoms not in the boundary region are subject only to the native forces F_i^{nat} .

When the temperature of the coolant is lower than the temperature of the protein interior, a heat conduction process towards the boundary is initiated. The thermal equilibration of the protein interior during the simulation can be monitored by means of an averaged temperature defined by

$$\langle T_{\text{sim}} \rangle = \frac{1}{N_{\text{deg}} k_B} \sum_{i=1}^N m_i v_i^2, \quad (5)$$

where N_{deg} is the numbers of degrees of freedom for N atoms. By summing over either a few or all the atoms in the system one can keep track of the local temperature or of the mean temperature, respectively.

2.3. Cooling through velocity scaling

An alternative method which simulates very rapid cooling involves a scaling of atomic velocities everywhere in a protein. All velocities may be multiplied by a factor which enforces a mean kinetic energy with a prescribed temperature. After rescaling the kinetic energy degrees of freedom are at the desired temperature, however, not necessarily the potential energy degrees of freedom. In fact, during the dynamics following the velocity rescaling energy flows from the potential to the kinetic energy degrees of free-

dom and the rescaling needs to be applied repeatedly. One can scale velocities after each integration step adopting a schedule of temperatures which gradually cool the system from an initial temperature to a target final temperature. Our choice of the scaling factor s_i for a specific atom i , which relates kinetic energies E_i^{old} and E_i^{new} before and after the scaling, implies that atoms with higher kinetic energy are losing more energy than those with lower kinetic energy

$$s_i = \frac{3 k_B T_{\text{new}}}{2 \langle E_i \rangle}, \\ E_i^{\text{new}} = s_i E_i^{\text{old}}. \quad (6)$$

Here T_{new} is the new temperature of the system, and $\langle E_i \rangle$ is the average kinetic energy of this atom.

Simulated cooling by velocity rescaling has the disadvantage that it implies artificial dynamics since the cooling method interferes with the integration steps for the Newtonian equation of motion. As a result, the velocity correlation acquires faulty features. The errors are particularly strong when the bath temperature deviates significantly from the temperature of a system simulated. For this reason we apply velocity rescaling only in the asymptotic regime of the simulated cooling process, i.e. at a time when the initial rapid phase of cooling from outside to inside is completed and the average temperature of the protein interior has reached values close to the bath temperature. Since the asymptotic relaxation time $\tau_\infty = R^2/\pi^2 D$ for the temperature derived below is rather long, e.g. about 10 ps for the segment simulated, it is attractive to accelerate the asymptotic cooling phase when rather small changes in mean temperature result for each picosecond simulated.

A further alternative to simulate cooling of macromolecules involves a random assignment of new velocities to all atoms according to a Maxwell distribution at the desired temperature. This method ignores any velocity autocorrelation. The ensuing artificial dynamics result in large deviations from initial equilibrium structures, deviations which do not relax upon further cooling of the system. We have not adopted this method.

3. Simulations and results

In this section we present the results obtained for simulated cooling by the stochastic boundary method. In the example presented the simulation started from a reaction center structure, which had been first energy-minimized and then equilibrated over 40 ps at 300 K. The temperature of the coolant was fixed at 200 K. During the simulation we determined the mean temperature $\langle T_{\text{sim}} \rangle$ of the protein. This temperature is presented as a function of time in fig. 1. Fig. 1 shows that the temperature $\langle T_{\text{sim}} \rangle$ initially drops within 1 ps from 300 to 280 K. It takes another 3 ps for the temperature to drop to 260 K. A further drop by 25 K would have required another 10 ps of simulated time. To accelerate the cooling process after 8 ps, we employed "velocity scaling". The temperature schedule chosen involved a reduction by 5 K every half picosecond, starting from the value of $\langle T_{\text{sim}} \rangle$ at 8 ps and halting when the temperature of 200 K was reached. At the end of the cooling process described, we obtained a conformation with Maxwell-distributed velocities. In fig. 2, the kinetic energy distribution resulting from the simulation is compared with the Maxwell distribution

$$p(E_{\text{kin}}) \propto (k_{\text{B}}T)^{-3/2} \sqrt{E_{\text{kin}}} \exp\left(-\frac{E_{\text{kin}}}{k_{\text{B}}T}\right). \quad (7)$$

A MD simulation of 40 ps appended to the cooling trajectory of the protein revealed that the mean ener-

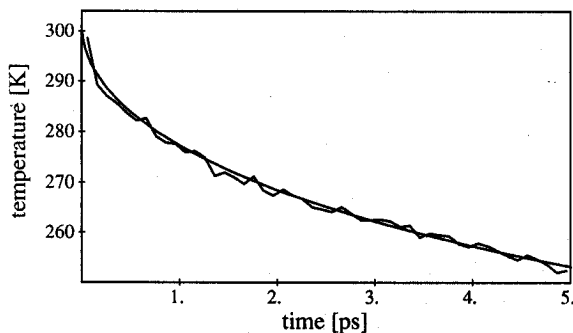


Fig. 1. Comparison of $\langle T_{\text{sim}} \rangle$ and $\langle T \rangle$: to determine $\langle T \rangle$ from the solution of the heat conduction equation, we used one hundred terms in the expansion (8) and chose $D=7 \times 10^{-4} \text{ cm}^2 \text{ s}^{-1}$; $\langle T_{\text{sim}} \rangle$ was averaged over all atoms and over 0.1 ps time intervals.

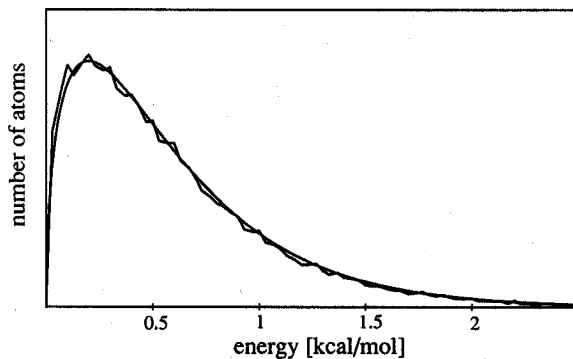


Fig. 2. Kinetic energy distribution at 200 K: compared are a distribution resulting from the simulation to the Maxwell distribution.

gies remained stable and yielded no major deviations from the starting structure.

We have applied the technique described to generate equilibrium structures of the photosynthetic reaction center at six temperatures down to 10 K [19].

3.1. Comparison based on the heat conduction equation

In this section we demonstrate that the temperature decay presented in fig. 1 follows a time dependence which is in agreement with predictions by the heat conduction equation

$$\frac{\partial T(r, t)}{\partial t} = D \nabla^2 T(r, t),$$

$$D = K/\rho c, \quad (8)$$

where $T(r, t)$ is the local temperature. D is the thermal diffusivity, K the thermal conductivity, ρ the density, and c the specific heat. This equation was solved previously for the geometry and boundary conditions corresponding to those of our simulation [20]:

$$T(r, t) = T_{\text{bath}} + \sum_{n=1}^{\infty} \alpha_n \exp\left[-\left(\frac{n\pi}{R}\right)^2 Dt\right] \times \frac{\sin(n\pi r/R)}{r}, \quad (9)$$

$$\alpha_n = 2\Delta T \frac{R}{n\pi} (-1)^{n+1}. \quad (9 \text{ cont'd})$$

Here R is the radius of the stochastic boundary and ΔT the difference between the initial temperature $\langle T_{\text{sim}} \rangle = 300$ K and $T_{\text{bath}} = 200$ K. The temperature $\langle T \rangle$, averaged over the interior region, is

$$\langle T \rangle = T_{\text{bath}} + 6 \frac{\Delta T}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp \left[- \left(\frac{n\pi}{R} \right)^2 Dt \right]. \quad (10)$$

In fig. 1, $\langle T_{\text{sim}} \rangle$ is compared to $\langle T \rangle$. The best fit is obtained with $D = 0.7 \times 10^{-3} \text{ cm}^2 \text{ s}^{-1}$. This value, which lies in the range of the corresponding value [21] for brain tissue ($D = (0.5\text{--}1.3) \times 10^{-3} \text{ cm}^2 \text{ s}^{-1}$), apple sauce #2 ($D = 1.1 \times 10^{-3} \text{ cm}^2 \text{ s}^{-1}$) and water ($D = 1.4 \times 10^{-3} \text{ cm}^2 \text{ s}^{-1}$), appears of be of the magnitude expected for proteins.

3.2. Radius of gyration

A typical structural response to cooling is volume decrease. This response can be monitored in a MD simulation by means of the radius of gyration defined by

$$R_{\text{gyr}} = \sqrt{\langle |r_i - \langle r_i \rangle|^2 \rangle}. \quad (11)$$

In this expression, the brackets denote averaging over a set of atoms. In this set all atoms in the X-ray structure were included that had a distance from the center of the protein segment simulated of less than 16.6 Å. For simulations at three temperatures, which were carried out by repeatedly applying the cooling method described above, the radius of gyration is shown in fig. 3. The results show that the radius of gyration contracts with decreasing temperature, i.e. the protein segment decreases upon cooling. For each temperature the radius of gyration is stable in time, i.e. fluctuates around a mean value. From 300 to 80 K the radius of gyration decreases by 1%. This value is in agreement with experimental observations for myoglobin [1].

To ascertain that the contraction is not an artifact produced by the stochastic boundary, we performed a second 5 ps simulation at 300 K, but with harmonic constraints corresponding to a temperature

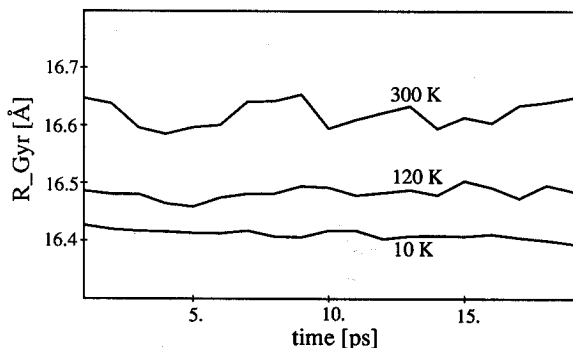


Fig. 3. The fluctuations of the radius of gyration at 300, 120, and 10 K over a 20 ps time interval.

factor $T_{\text{bath}} = 10$ K (see eq. (2)). The resulting radius of gyration was identical to that obtained for the 300 K simulation discussed above.

The contraction can be explained as follows: the reaction center exhibits local neutrality of charge resulting from a charge distribution which surrounds a charged atom by charges of opposite sign [22]. This local ordering has a typical length of 5–6 Å and actually exhibits several shells of charge distributions with alternating sign around a charged atom. This charge distribution implies that it is energetically favourable for the reaction center to contract during a cooling process. This is underlined by the observation that the contraction is accompanied by a decrease of the total electrostatic energy of the system. Due to the decreasing amplitudes of the atomic displacements upon cooling – equivalent to smaller energy gradients – the non-Coulombic forces are decreasing. As a result, the electrostatic forces are gradually dominating the non-Coulombic forces upon cooling which leads to a contraction of the system.

Acknowledgement

We like to thank J. Deisenhofer for kindly providing us with the X-ray structure of the reaction center of *Rps. viridis*, H. Treutlein for a equilibrated 300 K conformation for the reaction center, A. Brünger for making upgraded versions of the program XPLOR available to us, J. Berendzen and A. Shinzaki for helping us preparing this paper. Computation time was granted to us by the National Cen-

#2 Moisture content 37.3%.

ter of Supercomputer Applications supported by the National Science Foundation.

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