COMMENTS

Distribution of Temperature in Globular Molecules, Cells, or Droplets in Temperature-Jump, Sound Velocity, and Pulsed LASER Experiments

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Introduction

A wide range of practically important heat diffusion phenomena may approximately be modeled by the scenario of the heat exchange of homogeneous, spherical bodies that are heated in a constant energy flow and are in heat exchange with a large reservoir of solvent: This situation may occur in LASER spectroscopic experiments[1−3] (Figure 1) where the knowledge about local heating effects is important and in rapid kinetic measurements using temperature-jumping (T-jumping)[4−6] where it is important to know how fast the molecules reach the desired temperature.

In particular, there is a strong current interest in the extension of the time scale of protein folding studies using T-jumping.[7−11] The high structural and kinetic resolution of the early, fast folding events is expected to contribute significantly to the unraveling of the folding paradox,[12−14] i.e., the question how in the course of the folding reaction the gigantic number of unfolded conformations can so rapidly be funneled into the unique native conformation. The maximum time resolution of the new method involving T-jumping of protein, starting from the cold-denatured state,[7−11] is essentially given by the speed of temperature equilibration in the sample.

An analytical solution of this heat diffusion problem has been given and applied on sound velocity measurements.[15] Since the sound wave represents a pressure wave, a small temperature change of the protein solution will be involved.[15,16] The theory predicts that under the usual experimental conditions there is a significant heat exchange between the interior of the molecule, its solvent shell, and the bulk solvent.[15] Qualitatively, this has been pointed out earlier by Kharakoz and Sarvazyan, who called the estimated compressibility of the interior of the protein molecule pseudoadiabatic.[16] Since usually there is only a very small difference between adiabatic and isothermal compressibility of proteins,[16] this prediction could not be confirmed experimentally yet.

Here it is shown how the same theory can be applied on a wide range of important experiments, for example, in which intense LASER pulses or rapid electrical discharges are used. The limits given by the analytical solution can now be compared with direct experimental evidence,[7,8,17] showing a good agreement. It is concluded that kinetic folding studies which use T-jumping may safely be extended to the time scale below 100 ps.

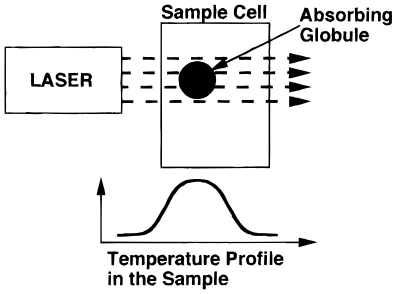

Figure 1. Pulsed LASER experiments on spherical bodies, e.g., molecules, cell organelles, cells, micelles, droplets, etc. The sample is excited by an intense LASER beam. Chemical or physical changes may be monitored with a second beam (not shown). Question: what is the temperature change in the sample?

Temperature Distribution in the Sample

The temperature change relative to the bulk solvent, ΔT, as function of time, t, and radius, r, of an approximately homogeneous, spherical body that is heated in a constant energy flow is given by eq 1 (see eq 18 in ref 15):

$$\Delta T(r<r_0,t) = \theta(I_+ + I_- - 1 + J_+ - J_-) \quad (1a)$$

$$\Delta T(r>r_0,t) = \theta(I_+ - I_- + J_+ - J_-) \quad (1b)$$

$$I_\pm = (1 + w_\pm^2)\Phi(w_\pm) + (2\pi)^{-0.5} w_\pm \exp(-0.5 w_\pm^2) - w_\pm^2 \quad (1c)$$

$$J_\pm = 2\xi\,(-w_\pm^2\Phi(w_\pm) + (1 - w_\pm^2)(2\pi)^{-0.5} w_\pm^{-1} \exp(-0.5 w_\pm^2) + w_\pm^2) w_\pm r_0/(3r) \quad (1d)$$

$$w_\pm = |1 \pm r/r_0|/\xi \quad (1e)$$

$$\xi = (2\Lambda t/\rho C r_0^2)^{1/2} \quad (1f)$$

where Λ, ρ, C, and r_0 are the heat conductivity, density, specific heat capacity, and radius of the body, respectively, and Φ is the Gauss function. θ is the increase in temperature that would occur in the absence of thermal diffusion. Equation 1 is valid for an approximately constant product $\Lambda\rho^{-1}C^{-1}$ within the sample and no significant heat radiation, heat convection, or shock waves which may occur for very high ΔT. It may be applied on a variety of objects and sources of heating, such as pressure-induced heating, but also heating caused by light absorption or an electrical discharge. Temperature profiles according to eq 1 for different values of the parameter ξ are shown in Figure 2. As one can see, for $\xi \ll 1$ there is little heat exchange with the bulk solvent, and for $\xi \gg 1$ there is significant exchange with the solvent.

Results

Sound Velocity and Compressibility Measurements. Sound velocity measurements are an important tool for the determination of compressibility,[18−21] solvation,[16,22−24] and conformational relaxation[25−30] of protein molecules. Adiabatic com-

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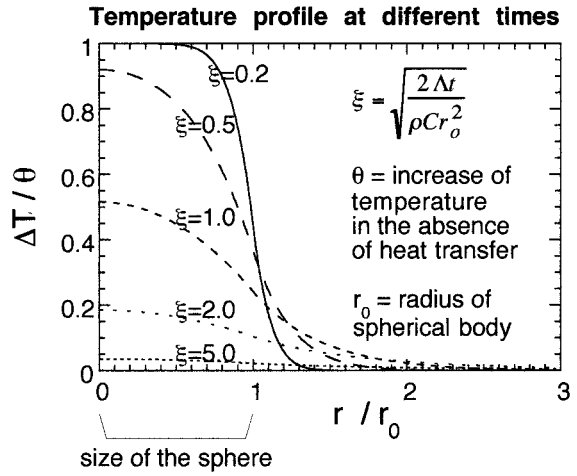

Figure 2. Temperature profiles according to eq 1 for different values of the parameter $\xi = (2\Lambda t/(\rho C r_0^2))^{0.5}$, where Λ, ρ, and C are the heat conductivity, density, and specific heat capacity of the molecule, respectively, and t is the time, are shown. The temperature difference relative to the bulk solvent is ΔT; θ is the increase in temperature that would occur in the absence of thermal diffusion; r and r_0 are the radius under consideration and the radius of the spherical body, respectively.

pressibility, β, density, ρ, and sound velocity, u, of a solution are related to each other by the *Laplace* equation:[31]

$$\beta^{-1} = \rho u^2 \tag{2}$$

The picture of the physical properties of the protein molecule that emerges from recent progress is as follows:[16]

1. The interior of protein molecules has an average compressibility of about $(14 \pm 3) \times 10^{-11}$ Pa^{-1}, which is 3 times less than that of water and comparable to that of soft organic solids.

2. The molecule is surrounded by a hard solvent shell which comprises several hundred water molecules that are significantly changed in their physical and chemical properties, in particular hydrogen bonding and structure, relative to bulk water. The average compressibility of the solvent shell is 35×10^{-11} Pa^{-1}, 20% less than that of bulk water.

The parameter ξ in eq 1 increases with time and decreases with the size of the molecule. At the beginning of the compression or expansion half-cycle, corresponding to a small ξ, the pressure-induced temperature change of the protein molecule is proportional to the time and as large as in the absence of thermal diffusion, i.e., $\Delta T = \theta$ (Figure 2). Since the thermodynamic parameters of protein differ from that of water, a temperature difference between the interior of the protein molecule and the water will build up. For large ξ, corresponding to a small radius of the molecule and a large time, the further growth of the temperature difference, ΔT, of the interior of the protein molecule relative to the solvent is significantly slowed by heat diffusion, i.e., $\Delta T \ll \theta$. For the usual conditions of sound velocity measurement of proteins, $r_0 < 10$ nm, $C \approx 1300$ J kg^{-1} K^{-1}, $\rho \approx 1400$ kg m^{-3}, $\Lambda \approx 0.17$ J m^{-1} s^{-1} K^{-1}, and sound frequencies of less than 100 MHz, one finds $\xi \gg 1$ and thus significant heat exchange of the molecule with bulk solvent.

Because a system is adiabatic when no heat exchange occurs between the system and its surrounding,[32] usually the protein molecule is not under adiabatic conditions,[1] even though the whole protein solution is still under adiabatic conditions, unless there is significant heat exchange of the solution with the walls of the cell. Kharakoz and Sarvazyan pointed out that their

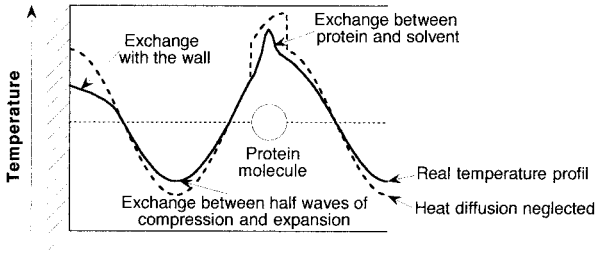

Figure 3. Snapshot of a sound wave passing through a sample cell that contains a protein molecule. The temperature profile of the sample is shown with a solid line. The dashed line represents the profile that would be obtained if heat transfer is neglected. Three types of heat exchange that may cause a deviation from adiabatic conditions of the interior of the protein molecule are illustrated: (1) At the usually applied low and medium frequencies significant heat transfer occurs between the interior of the protein molecule, its solvent shell, and the solvent. (2) At unusually low frequencies there may be a significant heat transfer between protein solution and the walls of the sample cell. (3) Significant heat transfer between the half-wave of compression and the half-wave of expansion is only possible at exceedingly high frequencies.

estimate of the intrinsic compressibility is a pseudoadiabatic value.[16] For a two-component system of solvent and solute, the effect of thermal diffusion on the compressibility may be accounted for in a thermal scattering theory which has been validated for bodies as small as 130 nm.[33] For small proteins it may be necessary to consider as a third component the solvent shell which has a relatively larger contribution to the overall sound velocity and compressibility than typically for much larger bodies.

Pinfield and Povey mentioned a further possible source for a deviation from adiabatic conditions: At high sound frequencies there may be a heat transfer from the half-cycle of expansion to the half-cycle of compression of the sound wave.[33,34] This is because at very short sound wavelengths, heat diffusion can efficiently cross the small distance between half-wave of expansion and half-wave of compression. This effect occurs because the wavelength of sound changes linearly with the reciprocal of frequency, $1/f$, but the thermal decay length, l, changes with the square root of $1/f$:[33]

$$l = (\Lambda/(\pi\rho C f))^{0.5} \tag{3}$$

where Λ, ρ, and C are the heat conductivity, density, and specific heat capacity, respectively. However, under the usual conditions of compressibility measurement this mechanism does not apply.[33] In water at 20 °C this mechanism would cause a deviation from adiabatic conditions at a frequency of above roughly 10^{13} Hz.[33]

The different causes for a departure from adiabatic conditions in compressibility measurements via sound velocity are illustrated in Figure 3 which represents a snapshot of a cross section through the sample cell. Only the heat exchange between protein and solvent appears to be significant under the usual conditions of measurement. It should be pointed out that for most proteins isothermal and adiabatic compressibility differ only slightly, and there appears to be no significant effect on former conclusions. Estimates of the compressibility of the interior of the protein molecule from sound velocity data[16] are in good agreement with those from mechanic measurements of protein crystals[35] or from the packing density in crystals.[36]

LASER Spectroscopic Experiments. Equation 1 applies to experiments in which an intense LASER beam is used to provide an optical trigger of chemical or physical changes[3,37−42] in a suspension of approximately homogeneous, spherical bodies that contain a light-absorbing dye in case of no strong extinction

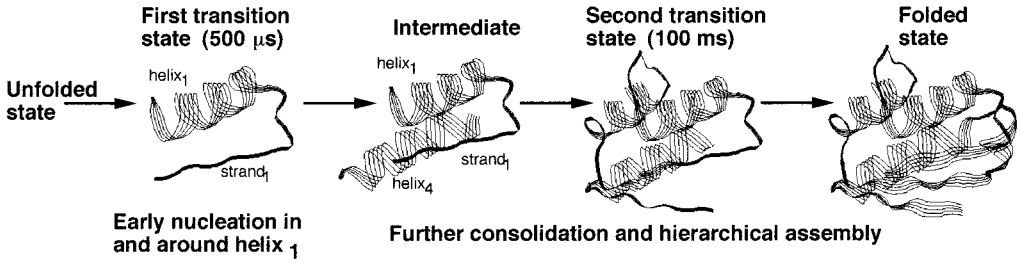

Figure 4. In temperature-jump experiments, rapid protein folding under essentially isothermal conditions may be studied in the time scale from about 20 ps to seconds. The folding pathway of barstar, the inhibitor of the ribonuclease barnase, is shown. The ribbon represents the part of the structure that is significantly consolidated. The early events of folding of barstar are characterized by a nucleation in and around helix$_1$ in about 500 μs. Then further structure grows step by step around the nucleus.

over the length of the single absorbing bodies. In each case one needs to calculate the parameter θ, i.e., the temperature which would occur in the absence of any heat diffusion, now given by eq 4:

$$\theta = 2.3 I_o \epsilon c t/(\rho C) \tag{4}$$

where I_o, ϵ, and c are the light intensity (in W m^{-2}), decimal molar extinction coefficient of the dye, and concentration of the dye, respectively. For short light pulses, i.e., $\xi \ll 1$, there is no significant heat exchange with the environment and $\Delta T(r<r_0,t) = \theta$. The pulse length, t, above which heat exchange starts to become important is approximately given by eq 5:

$$t \approx 0.5 r_0^{\,2} \rho C \Lambda^{-1} \tag{5}$$

For example, for a suspension of cells with $r_0 = 1$ μm and using the values for water, $\rho = 1000$ kg m^{-3}, $C = 4200$ J kg^{-1} K^{-1}, and $\Lambda = 0.6$ J m^{-1}, $t = 3$ μs. Thus, for light pulses of about 3 μs and longer, the temperature increase of the cells is significantly lowered by heat diffusion. On the other hand, when using nanosecond or picosecond pulses on suspensions of chloroplasts or components of the photosynthesis apparatus,[2] there might be local heating effects because of a low degree of temperature equilibration in the sample. Since a superposition law applies to eq 1, the distribution of temperature may easily be calculated for any arrangement of spherical bodies of different sizes and properties.[15]

Temperature-Jumping. Electrical discharge[4−6,9−11] and LASER-induced[7,8,17,43−45] T-jumping are two of the fastest known methods for the induction of fast protein folding reactions.[46−49] In these experiments it is important that the folding reaction proceeds under isothermal conditions. In T-jumping one usually encounters the inverse problem to that described in the previous paragraphs; i.e., the solvent is heated first, and the temperature of the protein lags behind. Equation 1 may analogously be applied for an approximately constant and homogeneous heating with ΔT still being the temperature difference of the protein molecule relative to bulk solvent, only θ now has a negative sign. For diluted solutions where the solvent is heated, θ is simply given by the change of the temperature of the bulk solvent. In case that only the solute is heated by radiation, θ is given by eq 4.

The rapid equilibration of temperature after a T-jump has enabled the development of a new method to solve transient protein structures with only microsecond lifetimes at the resolution of individual residues.[9,10] Application of this new method has led to a better understanding of the early events of protein folding (Figure 4). The high speed of the folding reaction may be explained by the highly anisotropic folding behavior, described in the nucleation−condensation model of protein folding.[50−53] Like in the growth of a crystal, a part of the molecule, the so-called nucleation site, forms very early. In the course of the folding reaction, the folding nucleus becomes increasingly stabilized as further structure condenses around it[9,10] (Figure 4).

A requisite for the precise measurement of the temperature-dependent kinetic rate constants is a good temperature equilibration. There is a strong interest in the rapid events of protein folding and in the extension of the time scale in which folding can be initiated. According to eq 5, the critical pulse duration for sufficient temperature equilibration is estimated to be about 10−20 ps for a protein molecule of the size of barstar.

Comparison of Theory and Experiment. Now the theory can be compared with the experiment: When rapidly heating an aqueous solution of a small protein molecule, myoglobin, with an infrared LASER beam at 1.54 μm which excites the water, it is found that the temperature of the protein molecule equilibrates with the buffer in less than 20 ns,[7,8] as judged by the change of the fluorescence signal of the protein.

An even lower limit for the minimum speed of thermal equilibration has been given by picosecond T-jumps via exciting a solution of crystal violet, used to study the ultrafast unfolding of ribonuclease A.[17] The "instant" change of the amide I region of the protein occurs in faster than 300 ps (from Figure 3 in ref 17). The equilibration of the temperature of the buffer after applying a LASER pulse of roughly 35 ps duration at a concentration of 1.5 mM of the dye takes less than 45 ps, as measured by absorption at 2270 cm^{-1}.[17] A lower limit of the temperature equilibration in this experiment may be calculated using eq 1: When approximating the solution as a cube of water with 10 nm length of the edges and a dye molecule located on each of its eight corners and using the thermodynamic parameters of water for the solution, $C = 4000$ J kg^{-1} K^{-1}, $\rho = 1000$ kg m^{-3}, and $\Lambda = 0.6$ J m^{-1} s^{-1} K^{-1}, one finds for $t = 45$ ps that the temperature increase in the coldest point, the center of the cube, is greater than 60% of the average temperature increase in the cube. This shows a good agreement between experiment and theory.

Conclusions

The analytical solution for the heat exchange of a spherical molecule with solvent (ref 15 and eq 1) is applicable on a wide range of problems involving heating of approximately spherical bodies that are in heat exchange with solvent: (1) determining the heat exchange of the interior of the protein molecule with its solvent shell and the bulk solvent in sound velocity and compressibility measurements to address the question whether the conditions of measurement are adiabatic; (2) calculation of heating effects in globular macromolecules, cell organelles, cells, micelles, droplets, etc., in spectroscopic experiments with high

light intensities; (3) estimation of the speed of temperature equilibration between protein and buffer in electrical discharge or LASER induced T-jumping.

In contrast to simple solutions for point sources, eq 1 enables to obtain a profile of temperature in and around suspensions of spherical bodies of different sizes and thermodynamic properties and is applicable on experiments where the size of the bodies cannot be neglected. The limits for the temperature equilibration observed in T-jump experiments[8,17] match well with the prediction from the theory. Further extension of the study of rapid reaction kinetics of small macromolecules to below 100 ps is possible when using T-jumping.

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