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Multiple time scales for dispersive kinetics in early events of peptide folding

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Abstract

Early events involving local collapse and helix formation manifested by the disulphide recombination of a de-novo peptide, over the time scale from 1 ps to 10 μs, are shown to fit equally well to a stretched exponential ($\alpha = 0.086 \pm 0.003$) and by an asymptotic power-law decay ($\beta = 0.331 \pm 0.004$). For inhomogeneous recombination kinetics each decay pattern leads to a different distribution of relaxation times.

1. Introduction

The current conceptual basis for protein folding [1,2] focuses on the description of its energy landscapes, roughness and existence of funnels [1–5]. Some central issues in the structure-dynamics-function relation of folding pertain to: (1) protein sequence dependence of folding efficiency, (2) time scales and hierarchy for various processes and (3) static or/and dynamic spread of the rates for specific folding processes. Inhomogeneous kinetics, originating from static disorder, were documented for other processes in globular proteins, i.e. low-temperature O_2 or CO recombination to myoglobin and hemoglobin [6] and in membrane proteins, i.e. the primary charge separation in photosynthesis [7]. There is a quantitative difference between inhomogeneous kinetics in globular and membrane proteins manifested in the spread of the kinetic parameters (e.g. activation energies and/or energy gaps), which is considerably larger in the former case. The sequence of events during the early steps of the folding of globular proteins involves [2,8,9]

$$\rightarrow \text{Helix formation} \rightarrow \text{Tertiary collapse} \rightarrow \text{Molten globule} \rightarrow \text{Native State}.$$

The individual steps are characterized by a separation of time scales, with the native state being formed on the time scale of seconds to minutes, the collective tertiary collapse and the molten globule formation occurring on

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the time scale of a few microseconds to milliseconds, while the local collapse involving non-native hydrophobic contacts and helix formation constitutes the fastest process [2,9]. Obligatory steps in the local collapse of proteins, i.e. side chain packing and helix formation, where most of the real organization prevails, are fast on the time scale of the overall process [8,9]. It has recently been demonstrated that the overall process of folding and unfolding of the secondary structural elements of proteins can occur on the time scale of nanoseconds [9–12]. Experimental studies of Volk et al. [13] explored the early folding process involving local collapse and helix formation of de-novo peptides with a disulphide bond between two modified tyrosines (Y′) linking the ends of a 17-amino-acid polypeptide chain $Y'(AAAAK)_3Y'$ (with A ≡ alanine and K ≡ lysine), constraining it to a non-native, more randomly coiled conformation. The ultrafast (sub-picosecond) process triggering the folding constitutes the photodissociation of the disulphide S–S bond. These experiments provide information on the kinetics of α-helix formation, which is interrogated by the recombination dynamics of the thiyl radical pair at the time scale from 1 ps to 10 μs. A significant novel result emerging from the studies of Volk et al. [13] is the failure of conventional kinetic schemes for the description of this process. Over a time range of 7 orders of magnitude the radical concentration exhibits an extremely non-exponential time dependence, which will be discussed in this Letter. Volk et al. [13] analyzed their experimental observation of the time-dependent radical concentration by a stretched exponential (Fig. 1a)

$$C(t) = C(0)\exp\left(-\frac{t}{\tau_0}\right)^{\alpha} \tag{1}$$

with the parameters $\tau_0 = 1.5 \pm 1.2$ fs and $\alpha = 0.086 \pm 0.003$ and the average decay time $\langle\tau\rangle = 326$ ns, where

$$\langle\tau\rangle = \int_0^\infty \mathrm{d}t C(t)/C(0)\,.$$

This phenomenological analysis implies that the recombination kinetics is characterized by a low value $\alpha = 0.086$ of the power of the stretched exponential. In spite of the ubiquity of kinetic processes amenable to description in terms of a stretched exponential [14], such a low α value is rare. This value of α is close to the low value $\alpha = 0.1$ obtained from the analysis of the kinetics of low-temperature CO–myoglobin recombination [6] in terms of a stretched exponential. This representation [15–18] of the experimental data in terms of Eq. (1)

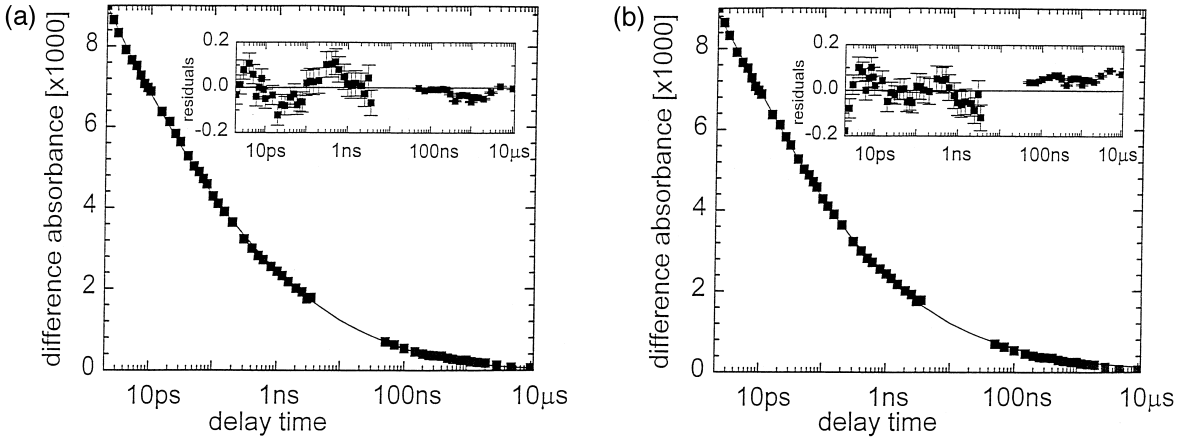

Fig. 1. Analysis of the experimental results of Volk et al. for the early events of local collapse and folding in the de-novo peptides studied by the time dependence of the absorption due to the transient absorption of the thiyl radicals [13]. The kinetic data are analysed by: (a) fitting by a stretched exponential (Eq. (1)); (b) fitting by an asymptotic power law (Eq. (3)). The fitting parameters are given in the text.

can be expressed by a time-dependent recombination rate coefficient $k(t)$, which is defined by assuming the first-order reaction equation

$$\frac{\mathrm{d}C(t)}{\mathrm{d}t} = -k(t)C(t)\,, \tag{2a}$$

with

$$k(t) = \frac{\alpha\tau_0^{-\alpha}}{t^{1-\alpha}}\,. \tag{2b}$$

Rewriting the stretched exponential in terms of Eqs. (2a) and (2b) does not imply, of course, any specific recombination mechanism. Klafter and Shlesinger [14] have demonstrated that the stretched exponential can be obtained by several models which correspond to different physical realizations and mechanisms. The unifying feature of the models is the generation of a scale invariant distribution of relaxation times.

An alternative description of the kinetics of the experimental results for the disulphide recombination in the de-novo polypeptide can be given in the functional form of an asymptotic power law

$$\frac{C(t)}{C(0)} = \left[1 + \left(\frac{t}{\tau_0'}\right)^{\beta}\right]^{-1}\,. \tag{3}$$

This power-law Eq. (3) does not necessarily imply second-order kinetics for equal concentrations, which was previously analyzed [16–19]. The functional form in Eq. (3) is also referred to as an asymptotic fractal [20]. A related fitting function (with $\beta \geqslant 1$) was already proposed in 1910 for the case of O_2 dissociation from hemoglobin [21]. Here again, a time-dependent recombination rate coefficient can be defined as

$$k(t) = \frac{(\beta/\tau_0')(t/\tau_0')^{\beta-1}}{\left[1 + (t/\tau_0')^{\beta}\right]}\,. \tag{4}$$

Over a broad time domain the kinetic data can be well fitted by Eq. (3) (Fig. 1b) with the parameters $\tau_0' = 7 \pm 1$ ps and $\beta = 0.331 \pm 0.004$. τ_0' is about three orders of magnitude larger than τ_0. Note also that τ_0 for the stretched exponential is not within the data set, but far off towards the origin. The value of the power β for the fit, according to Eq. (3), is considerably larger than the value of α for the stretched exponential case (Eq. (1)) indicating different distribution functions of relaxation times. The phenomenological description of the kinetics, which is characterized either by Eq. (1) or Eq. (3), is not sufficient to determine a particular mechanism for the folding reaction. In general, two classes of mechanisms, i.e. inhomogeneous or homogeneous kinetics, can lead to such non-exponential long-tailed time dependence. Here we focus mainly on the inhomogeneous kinetics.

2. Inhomogeneous mechanism

Each peptide molecule recombines exponentially in time, with a characteristic time-independent rate (a Debye process), but each molecule is characterized by a different rate. Such a distribution of rates may originate from an inhomogeneous distribution of initial structures [22]. The total time evolution in the inhomogeneous system is then given as a superposition of the simple individual decays with a distribution function of the lifetimes $f_j(\tau)$, with $j = 1$ and $j = 2$ for the stretched exponential and the power law, respectively. The stretched exponential description (Eq. (1)) is then given in terms of the Laplace transform

$$\exp\left[-\left(\frac{t}{\tau_0}\right)^{\alpha}\right] = \int_0^{\infty} \mathrm{d}\tau\, f_1(\tau)\, \mathrm{e}^{-t/\tau}\,, \tag{5}$$

and in a similar way

$$\left[1+\left(\frac{t}{\tau_0'}\right)^{\beta}\right]^{-1} = \int_0^\infty \mathrm{d}\tau f_2(\tau)\mathrm{e}^{-t/\tau}. \tag{6}$$

Let $g(t/\tau_0) = C(t)/C(0)$ be the relaxation function. Its decomposition into single Debye processes of rates τ is given as follows:

$$g\left(\frac{t}{\tau_0}\right) = \int_0^\infty \mathrm{d}\tau f(\tau)\mathrm{e}^{-t/\tau}. \tag{7}$$

Introducing the substitution $p = 1/\tau$, i.e. $\mathrm{d}\tau = -p^{-2}\mathrm{d}p$, one finds:

$$g\left(\frac{t}{\tau_0}\right) = \int_0^\infty \mathrm{d}p \frac{f(1/p)}{p^2}\mathrm{e}^{-pt} \equiv L\left\{\frac{f(1/p)}{p^2}; t\right\}, \tag{8}$$

i.e. $g(t/\tau_0)$ is the Laplace transform of $p^{-2}f(1/p)$. Thus we obtain $f(\tau)$ via:

$$f(1/p) = p^2 L^{-1}\left\{g\left(\frac{t}{\tau_0}\right); p\right\} \tag{9}$$

and

$$f(\tau) = f(1/p \to \tau). \tag{10}$$

Introducing the Fox function representations (see Appendix A) [23–25], the inverse transform can be calculated as a closed form solution. The Fox functions are

$$g_1(t) = \mathrm{e}^{-(t/\tau_0)^\alpha} = \frac{1}{\alpha} H_{0,1}^{1,0}\left[\frac{t}{\tau_0}\middle|\left(0,\frac{1}{\alpha}\right)\right] \tag{11}$$

and

$$g_2(t) = \frac{1}{1+(t/\tau_0')^\beta} = \frac{1}{\beta} H_{1,1}^{1,1}\left[\frac{t}{\tau_0'}\middle|\begin{matrix}(0,1/\beta)\\(0,1/\beta)\end{matrix}\right] \tag{12}$$

for our two relaxation functions, respectively. Applying the inverse Laplace transform, we obtain, after some steps, the closed form solution [23–25]

$$f_1\left(\frac{\tau}{\tau_0}\right) = \frac{1}{\alpha\tau} H_{1,1}^{1,0}\left[\frac{\tau}{\tau_0}\middle|\begin{matrix}(0,1)\\(0,1/\alpha)\end{matrix}\right] \tag{13}$$

and

$$f_2\left(\frac{\tau}{\tau_0}\right) = \frac{1}{\beta\tau} H_{1,2}^{1,1}\left[\frac{\tau_0'}{\tau}\middle|\begin{matrix}(1,1/\beta)\\(1,1/\beta),(1,1)\end{matrix}\right]. \tag{14}$$

Asymptotically one finds for $\tau \gg \tau_0$ [25]

$$f_1\left(\frac{\tau}{\tau_0}\right) \sim \frac{1}{\alpha\tau_0(\tau/\tau_0)} \frac{\alpha^{\frac{2\alpha-1}{2-2\alpha}}}{\sqrt{2\pi}\sqrt{1-\alpha}} \left(\frac{\tau}{\tau_0}\right)^{\frac{\alpha}{2-2\alpha}} \exp\left\{-(1-\alpha)\alpha^{\frac{\alpha}{1-\alpha}}\left(\frac{\tau}{\tau_0}\right)^{\frac{\alpha}{1-\alpha}}\right\}, \tag{15}$$

which differs from the expression in Refs. [16,17] by the prefector, and

$$f_2\left(\frac{\tau}{\tau_0}\right) \sim \frac{1}{\tau_0'\Gamma(\beta)}\left(\frac{\tau}{\tau_0'}\right)^{-1-\beta}. \tag{16}$$

Eq. (16) displays a power-law distribution of relaxation times which is often referred to as a temporal fractal, or Lévy distribution [26–30]. From the numerical data for $f_1(\tau)$ and $f_2(\tau)$ (Fig. 2), calculated for the α and β values from the fit of the kinetic data, it is apparent that the distributions of the rates for both $f_1(\tau)$ and $f_2(\tau)$ are broad.

A model can be constructed, although somewhat oversimplified for the complex protein dynamics, which leads to a stretched exponential or to a power-law recombination depending on the competition between energetic and entropic trends. The model is based on recent calculations of first passage times in the presence of an energy funnel, which directs the recombination reaction and competes with a random walk process which tends to explore the configuration space [4,5,31]. Assume that upon cleavage of the S–S bond each peptide starts recombining from a 'distance' L of an unrecombined configuration. L is defined in some space of possible configurations. The typical recombination time starting from L is $\tau(L)$ which depends on the nature of the search for the recombined state

$$\frac{C(t)}{C(0)} = \int_0^\infty \mathrm{d}L f(L) \mathrm{e}^{-t/\tau(L)}, \tag{17}$$

where $f(L)$ is the distribution of distances. Following Palmer et al. [32] we assume

$$f(L) = f_0 \lambda^{-L} = f_0 \exp(-L \ln \lambda). \tag{18}$$

If the first passage time for recombination behaves as

$$\tau(L) = \tau_0 \exp(aL), \tag{19}$$

which results from an exponential increase in the number of accessible configurations [4,5,30], or more generally from biasing the random walk away from the recombined configuration, then

$$C(t) \sim t^{-\ln\lambda/a}. \tag{20}$$

The constants λ and a are independent of L and t.

On the other hand, if $\tau(L)$ scales with L, as we expect, for instance in the simple diffusive case, or when an energy funnel directs the motion towards recombination,

$$\tau(L) = \tau_0 L^\eta \tag{21}$$

Eqs. (17), (18) and (21) result in

$$C(t) \sim \exp\left[-(Kt)^{1/1+\eta}\right] \tag{22}$$

where K is independent of λ. Note that in the diffusive case $\eta = 2$. From this analysis we infer that the exponential increase of $\tau(L)$ according to Eq. (19) results in the asymptotic power law, while the scaled case Eq. (21) results in the stretched exponential behavior.

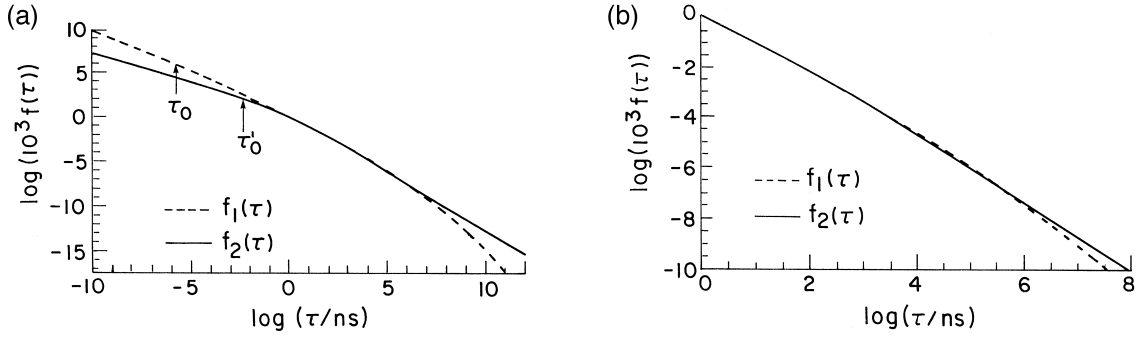

Fig. 2. The lifetimes distribution functions $f_1(\tau)$ with $\alpha = 0.086$, $\tau_0 = (1.5 \pm 1.2)$ fs (dashed curve) and $f_2(\tau)$ (solid curve) with $\beta = 0.331$, $\tau_0' = (7 \pm 1)$ ps for the description of the inhomogeneous kinetics. (a) The distribution functions over the broad range $\tau/\mathrm{ns} = 10^{-10} - 10^{10}$ with the values of τ_0 and τ_0' being marked. Note the different asymptotic behavior. (b) The distribution functions over the range $\tau/\mathrm{ns} = 1 - 10^8$, where they coincide except for their asymptotic behavior.

3. Homogeneous mechanism

The experimental result cannot rule out, however, homogeneous relaxation kinetics. Namely, here all proteins are dynamically identical, with the time evolution within each protein being non-exponential. An example of a homogeneous, sequential, mechanism characterized by a non-exponential decay, is the hierarchically constrained dynamics proposed by Palmer et al. [32]. This sequential mechanism can lead to either a stretched exponential or to a power-law behavior, depending on the scaling properties of the internal relaxation times [14,32].

The available experimental data do not allow one to distinguish between the inhomogeneous and the homogeneous mechanisms, which give rise to multiple time scales. The distinction between the inhomogeneous and homogeneous mechanisms can and should be obtained by kinetic hole burning, i.e. the interrogation of the disulphide recombination kinetics under repeated illumination. This technique was applied to CO–myoglobin recombination [33], establishing that for this process the kinetics is inhomogeneous, originating from static disorder.

We have shown that the relaxation time distributions can be expressed in closed forms via Fox functions. For the asymptotic power-law fit this distribution has a power-law tail and thus follows Lévy statistics [29,30,34]. To speak of Lévy flights in the process of protein local collapse and helix formation is still of a speculative nature. The observed dynamics is surely influenced by both, the folding dynamics and the motion of the chain ends (sulphur contacts) which has a random feature, but is not totally independent of the intra-chain dynamics.

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Appendix A

Fox functions are defined via a generalized Mellin–Barnes integral [22,25]

$$H_{p,q}^{m,n}\left[x\left|\begin{matrix}(a_1,A_1),(a_2,A_2,\ldots,(a_p,A_p)\\(b_1,B_1),(b_2,B_2,\ldots,(b_q,B_q)\end{matrix}\right.\right]=\frac{1}{2\pi i}\int_L \mathrm{d}s\,\chi(s)\,x^s \tag{A1}$$

with

$$\chi(s)=\frac{\prod_1^m \Gamma(b_j-B_j s)\prod_1^n \Gamma(1-a_j-A_j s)}{\prod_{m+1}^q \Gamma(1-b_j+B_j s)\prod_{n+1}^p \Gamma(a_j-A_j s)}\,. \tag{A2}$$

They comprise a rich class of functions, Maitland's generalised hypergeometric or Bessel functions, (confluent) hypergeometric functions, or Lommel functions amongst them. If a Fox function $H_{p,q}^{m,n}$ has $n=0$, it will decay exponentially for large arguments. On the other hand, if $n>0$, the decrease will be a power law. Fox functions

were introduced into physics by Schneider [35] as exact representations of Lévy stable distributions [34]. They occur often as solutions of fractional differential equations. The asymptotic expansions are as follows:

$$f_1\left(\frac{\tau}{\tau_0}\right) \sim \frac{1}{\alpha\tau}\begin{cases} \displaystyle\sum_{\nu=1}^{\infty} \frac{(-1)^{\nu}}{\nu!\Gamma(-\alpha\nu)}\left(\frac{\tau}{\tau_0}\right)^{\alpha\nu} & \tau \ll \tau_0 \\ \displaystyle\frac{\alpha^{\frac{2\alpha-1}{2-2\alpha}}}{\sqrt{2\pi}\sqrt{1-\alpha}}\left(\frac{\tau}{\tau_0}\right)^{\frac{\alpha}{2-2\alpha}} \exp\left\{-(1-\alpha)\alpha^{\frac{\alpha}{1-\alpha}}\left(\frac{\tau}{\tau_0}\right)^{\frac{\alpha}{1-\alpha}}\right\} & \tau \gg \tau_0 \end{cases} \tag{A3}$$

and

$$f_2\left(\frac{\tau}{\tau_0}\right) \sim \frac{1}{\beta\tau}\begin{cases} \displaystyle\sum_{\upsilon=1}^{\infty} \operatorname{res}\left(\chi(s)\left[\frac{\tau_0'}{\tau}\right]^{s}\right)_{|s=-\beta\nu} \sim \left(\frac{\tau}{\tau_0'}\right)^{\beta} & \tau \ll \tau_0' \\ \displaystyle\frac{\beta}{\Gamma(\beta)}\left(\frac{\tau_0'}{\tau}\right)^{\beta} & \tau \gg \tau_0' \end{cases} \tag{A4}$$

This means for the stretched exponential, the distribution function is also of stretched exponential shape. For as small a stretched exponential parameter as $\alpha \approx 0.09$, the parameter $\alpha/(1-\alpha)$ of the distribution function (A3) becomes 0.1, which is still small. On the other hand, the long tail of the distribution function f_2 scales as a power of $-(1+\beta) \approx -1.33$.

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