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ORIGINAL PAPER

Temperature and solvent dependence of the dynamical landscape of tau protein conformations

Antonio Bianconi · Gabriele Ciasca · Alexander Tenenbaum · Anna Battisti · Gaetano Campi

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Abstract We report the variation with temperature of the ensemble distribution of conformations spanned by the tau protein in its dynamical states measured by small-angle X-ray scattering (SAXS) using synchrotron radiation. The SAXS data show a clear temperature variation of the distribution of occupied protein conformations from 293 to 318 K. More conformations with a smaller radius of gyration are occupied at higher temperature. The protein–solvent interactions are shown by computer simulation to be essential for controlling the dynamics of protein conformations, providing evidence for the key role of water solvent in the protein dynamics, as proposed by Giorgio Careri.

Keywords Protein conformations · Intrinsic disordered proteins · Water effect in conformational landscape · Temperature effects in protein fluctuations

1 Introduction

1.1 From the "lock-and- key" model to the dynamics-function relation

The dogma that the function of a protein depends critically on its fixed three-dimensional structure was rapidly accepted after the discovery of the DNA and protein structures by

A. Bianconi (✉) · G. Ciasca · A. Tenenbaum
Physics Department, Sapienza University of Rome, Piazzale A. Moro 2, 00185 Roma, Italy
e-mail: antonio.bianconi@roma1.infn.it

A. Battisti
Physics Department, University of Trento, Via Sommarive 14, 38123 Povo (Trento), Italy

A. Battisti
LISC, FBK-CMM and University of Trento, Via Sommarive 18, 38123 Povo (Trento), Italy

G. Campi
CNR, Institute of Crystallography, Via Salaria Km 29.300, 00016 Monterotondo (Roma), Italy

X-ray diffraction in the 1950s. Enzymes have been described as being able to bind to specific substrates because their shapes match perfectly, with the *'lock-and-key'* mechanical model first proposed by chemist Emil Fischer in 1894. The genome encodes a molecular primary structure that, once expressed, folds in a secondary and tertiary structure. The expressed structure, following the *lock-and-key* paradigm, allows protein–protein or protein–ligand recognition, and therefore the protein function in the living cell. In the 1970s, the general validity of the *'lock-and-key'* paradigm was questioned by Giorgio Careri with Paolo Fasella and Emilio Gratton [1, 2], who proposed a key role for fluctuations of protein conformations in biochemical reactions, described as statistical events. The main point was the scenario of proteins as macromolecules fluctuating between quasi-stationary states [3], their dynamics being a key feature for understanding living matter. As a matter of fact, it was observed that in some proteins the binding site was not uniquely well defined. Paolo Fasella, a close collaborator of Giorgio Careri and inspired by Giorgio's discussions, focused on the brain protein calmodulin (CaM: CALcium MODULated proteIN). Calmodulin is expressed in all eukaryotic cells that bind to and regulate a number of different protein targets, thereby affecting many different cellular functions such as short-term and long-term memory, nerve growth and the immune response, inflammation, metabolism, apoptosis, smooth muscle contraction, and intracellular movement. The instantaneous structure of the conformations of the active site of calmodulin and other Ca-binding proteins in solution [4, 5] was measured by a new fast structural method "X-ray absorption near-edge structure" (XANES), probing the higher-order correlation functions of the local structure with a time scale of 10^{-13} s [6, 7]. The instantaneous structure of these proteins in solution was found to deviate from the averaged structure of the crystallized protein detected by X-ray diffraction [8], showing the existence of fast protein fluctuations between different conformations. Calmodulin and parvalbumin [9–11] were later identified as flexible biological macromolecules that show an intrinsic dynamical state spanning a wide set of protein conformations determined by NMR [12]. The set of time-resolved protein conformations and the percent of occupation time of the multiple protein conformational states has been confirmed by other physical methods probing the protein dynamics such as inelastic neutron scattering and dielectric methods [13], a method that was used by G. Careri to investigate the dynamics of proteins controlled by the solvent [14].

In the first 10 years of this century, the key role of protein dynamics and flexibility has attracted a wide scientific audience due to the identification of a large number of proteins called intrinsic disordered proteins (IDPs) [15, 16]. The number IDPs that perform their biological function having no fixed structural shape is rapidly growing [17]. Their functionality is related to their dynamics, as proposed by Careri and collaborators [1], rather than to the fixed structure needed for the *lock-and-key* model.

In the same first 10 years of the XXI century, the description of the cell as an ensemble of networks has become widely accepted [18]. Protein function in cell organization has been associated with protein–protein interaction networks in the cell where most of the IDPs play the key role of "hub" proteins. The fluctuations of IDPs between multiple conformations as shown in Fig. 1 allow these proteins to bind to a wide range of different target proteins. IDPs constantly probe and sense signals from many partners in the complex cellular environment, i.e., by fluctuating between multiple conformations, they express the capability of many links. In these networks, the protein conformations can be described as nodes, and the potential barriers as links in "link-weighted" networks [19]. The network's dynamical state is determined by the landscape of the potential barriers that separate the states of quasi-stationary protein conformations. Therefore, the network topology with weighted links

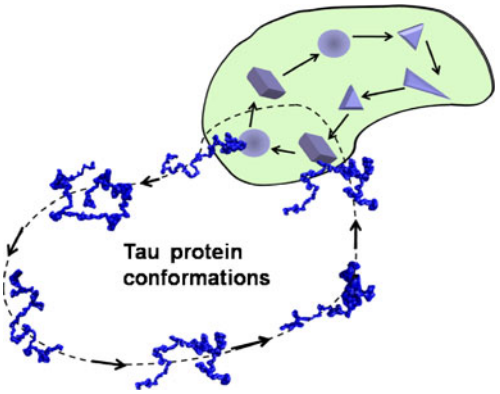

Fig. 1 Pictorial view of the intrinsic fluctuations of the first flexible protein: the tau protein (some of the multiple conformers of the tau protein are shown in the lower part of the figure connected by the *dashed line*) in its dynamical functional state. The biological function of the protein in the cell is determined by coherent biochemical reactions as proposed by G. Careri in the seventies. The picture shows a second fluctuating protein that fluctuates between different landscapes of multiple protein conformations (pictorially indicated by the different geometrical multiform shapes inside the *solid line* in the upper part of the figure) with complex association and dissociation molecular processes controlling the biochemical reactions between flexible proteins

based on the data of the complex energy landscape can be used to determine the fluctuations and correlations in the network dynamics [20].

Between intrinsic disordered proteins, much attention has been addressed to the cellular transcription factor "cAMP response element-binding" (CREB), a gene-regulatory protein involved in learning and memory, and CREB-binding protein (CBP). Several bonds form cooperatively within CREB and with CBP [21]. A well-known IDP protein is the tumor suppressor p53 [22], a hub in the multiple signaling networks implicated in human cancer. The key intrinsic dynamics of IDPs relating to their functionality has been shown in the case of the Sic1 protein [23], the cyclin-dependent kinase (CDK) inhibitor that interacts with its receptor Cdc4 [24]. The complex is a mixture of different conformations shifting around dynamically. The observation of this unusual binding mode between Sic1 and Cdc4 extends the understanding of protein interactions from predominantly static complexes to a new scenario of dynamic ensembles of intrinsically fluctuating quasi-stationary states.

Protein conformational fluctuations are relevant also for folded proteins [25–27] and today are considered an essential key feature for understanding protein function as proposed by Careri [2]. The investigation of the dynamics of rebinding of ligands and relaxation in the myoglobin [28] pocket has been a first case where it has been shown that the water solvent has a key role in slaving the protein fluctuations [29] in the protein energy landscape [30–32]. The protein fluctuations between conformers have been found to be slaved by fluctuations in the hydration shell measured by dielectric relaxation spectroscopy [33–35].

1.2 The case of tau protein

The dynamics of a protein is expected to depend on environmental conditions, for example on temperature and on solvent. Here, we have investigated the role of temperature and

solvent on the landscape of protein conformations spanned by the tau protein, one of the largest IDPs. Because of its intrinsically disordered character, tau in solution explores a large region of the conformational space, fluctuating between a large number of conformers. Tau is a microtubule-associated protein expressed primarily in neurons. It is found in the human central nervous system (CNS) in six isoforms, ranging from 352 to 441 amino acids. In its physiological state, tau promotes the growth and assembly of microtubules [36, 37]; but under pathological conditions, the same tau aggregates in paired helical filaments (PHFs). This phenomenon prevents the tau protein from carrying out its physiological stabilization role, and, together with several other factors, is associated with the degeneration of the microtubules and the death of neurons [38]. Tau entails no permanent secondary structures, and the aggregation in PHFs is supposed to start from a segment forming a transient β-structure. As the application to the monomeric tau protein of standard structural techniques, such as macromolecular crystallography or electron microscopy, is not possible, small-angle X-ray scattering provides a unique tool to obtain structural information on tau in solution and, more generally, on the whole IDP category [39–46].

In this work, we investigate the influence of temperature and solvent on the behavior of the longest CNS tau isoform, htau40 (441 residues, and molecular mass 45.8 kD [43]). For the former, we report SAXS measurements at 293 K and 318 K; as for the latter, we have performed a Molecular Dynamics computer simulation of tau in water or methanol solvent.

2 Materials and methods

2.1 Protein preparation

The htau40 protein was purchased from Sigma-Aldrich (St. Louis, MO). The protein powder was reconstituted in 50 mM MES (pH = 6.8), 100 mM NaCl, and 0.5 mM EGTA, desalted with standard procedures, and concentrated to a nominal protein concentration of 2 mg/ml in 0.1 M PBS (pH = 6.8). The solution was centrifuged for 10 min at 10,000 $\times g$ and the supernatant was filtered to eliminate aggregates. Protein quality was assayed by SDS-PAGE in 12% (w/v) polyacrylamide. The gels were stained with Coomassie brilliant blue R-250. The SDS-PAGE analysis revealed the occurrence of a major protein band with the expected size (approx. 45 kDa) and a 90% purity. As described in the following section, the protein was subjected to temperature variation in situ during the SAXS measurements. The temperature variation does not induce protein aggregation, as demonstrated by SDS-PAGE analysis at varying temperatures.

2.2 Small-angle X-ray scattering (SAXS) measurements

SAXS measurements [44, 45] have been acquired at the BioSAXS beamline ID 14–3 at ESRF (Grenoble, France) [46]. A pictorial view of the typical experimental geometry of a SAXS experiment is presented in Fig. 2. A volume of 50 μl of solution has been placed in a 1.8-mm-diameter quartz capillary with a few tens of microns wall thickness. Ten consecutive 2-s exposures were performed at constant temperature without observing any radiation damage. SAXS profiles have been acquired at the standard room temperature of 293 K; the temperature effect has been studied at 318 K after a temperature equilibration in the capillary sample holder. An exposure time of 3 s was used. Measurements at different temperatures have been acquired at different positions of the capillary sample holder, in

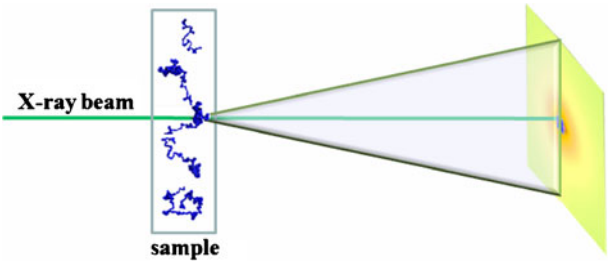

Fig. 2 Pictorial view of the small angle X-ray scattering experiment using high luminance synchrotron radiation X-ray beam to probe the low-resolution shape of the fluctuating tau protein conformations in the solvent

order to further exclude the presence of radiation damage. Solvent scattering was measured to allow for subtraction of the background scattering.

2.3 Ensemble optimization method

An explicit description of the structural ensemble of the tau protein can be obtained by the software RANCH, which produces an ensemble of conformers. The ensemble is based on a data bank of residue-specific (ψ, ϕ) values, derived from coil segments of globular proteins, and takes properly into account the coexistence of multiple conformations in solution. This ensemble is then optimized through the genetic algorithm GAJOE, which selects those conformers that, on average, best reproduce (using the program CRYSOL) the SAXS experimental curve.

2.4 Molecular dynamics simulation

A 3D structure of tau protein was produced following a procedure described in detail in [47]. First, an extended 3D structure was obtained from its primary sequence using the program VMD [48], and placed in a large box with periodic boundary conditions (box volume = 15,253 nm^3). A dynamic evolution *in vacuo* at 300 K was performed with the package GROMACS using the ffG53a6 force field [49], which led to a rapid compaction of the molecule, as monitored through its gyration radius. This evolution was stopped when the gyration radius reached its experimental average value $R_g = 6.57$ nm [50]; at this point the box was filled with about 5×10^5 water molecules (space water model) or with about 2.5×10^5 methanol molecules [51]; the energy of the system was then minimized, and a short equilibration was performed, first at constant temperature (modified Berendsen thermostat) and volume, then at constant temperature and pressure (Parrinello–Rahman pressure coupling). The conformation produced by this procedure was used as the starting point to simulate the dynamic evolution in time of the protein, using GROMACS with a time step of 2 fs.

3 Results

3.1 SAXS results

In Fig. 3, we report the Kratky plot of the tau protein measured at two different temperatures, 293 K and 318 K. In the Kratky plot, the scattered intensity profile I(s) is multiplied by the

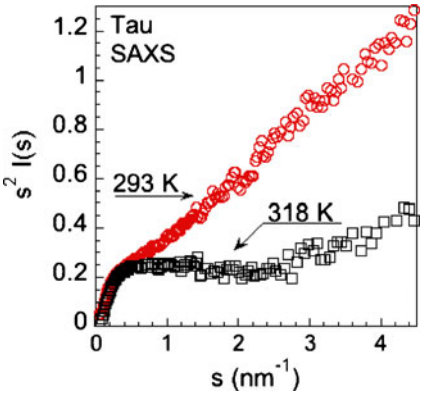

Fig. 3 The small angle X-ray diffraction intensity I(s) multiplied by the square of the momentum transfer s, called a Kratky plot, as a function of the momentum transfer s of the tau protein at two different temperatures

square of the scattering wave vector s^2 (i.e., $s^2I(s)$ versus s). The scattering profile for an unfolded state of a molecule falls off as 1/s at high values of the exchanged momentum s. The clearly defined surface of a folded protein leads to a scattering profile which falls off as $1/s^4$ [46]. Therefore, the occurrence of a well-defined peak in the Kratky plot indicates that the molecule is in a compact state.

The increase of $s^2I(s)$ at high value of momentum transfer s, observed in Fig. 3, points out that the tau protein has an expanded disordered state at both temperatures, in our experimental conditions. It can be also noted that the Kratky plot, in the intermediate s region, increases faster at 293 K than at 318 K.

3.2 EOM method

A pool of 10,000 representative backbone models of the tau protein were created using the program RANCH. The corresponding theoretical scattering intensity of the models was calculated by the software CRYSOL. The genetic algorithm GAJOE was then employed, at both experimental temperatures, to select from the set of theoretical scattering curves an ensemble that provided the best fit of the experimental SAXS data. In Fig. 4, we report

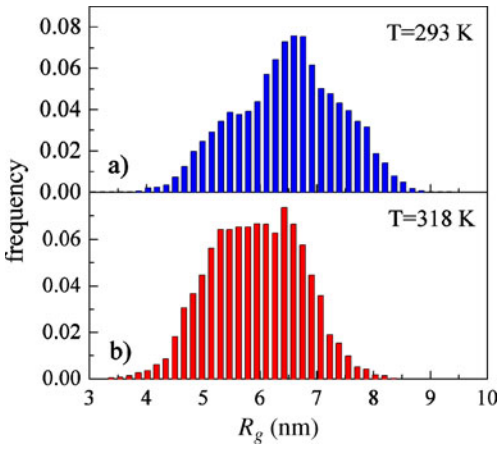

Fig. 4 The distribution of different conformations of the tau protein characterized by their radius of gyration at 293 K (**a**) and at 318 K (**b**)

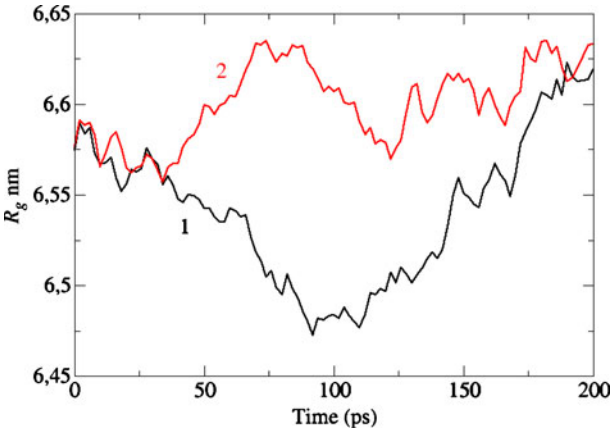

Fig. 5 Molecular dynamics calculation at $T = 300$ K of the conformations spanned in time by the tau protein, characterized by their gyration radius R_g in water (*curve* #1) and in methanol (*curve* #2)

the distribution of the tau conformations spanned by the tau protein characterized by their radius of gyration (R_g) at 293 K (panel a) and at 318 K (panel b). Given the multiple nature of the conformations of the molecule, the average R_g of the tau protein corresponds to the average computed over the selected ensemble of conformers. This average turns out to be $\langle R_g \rangle = 6.8 \pm 0.2$ nm at $T = 293$ K, and $\langle R_g \rangle = 5.9 \pm 0.3$ nm at $T = 318$ K. A close inspection of the selected distribution highlights that these values derive from an increased propensity of tau to populate more compact conformational states ($\langle R_g \rangle \approx 5.4$ nm) at the higher temperature, rather than extended states (peak at $\langle R_g \rangle \approx 6.8$ nm) at the lower one. This behavior is consistent with the information provided by the Kratky plots, which indicates an overall compaction of the tau protein with increasing temperature.

3.3 Molecular simulation results

We performed a MD simulation of a tau molecule embedded in water or methanol solvent, using the GROMACS package specified before, at $T = 300$ K. In Fig. 5, we report the value of the gyration radius R_g in water (curve #1) and in methanol (curve #2) during a segment of 200 ps; during these 200 ps the gyration radius oscillates in both cases around its

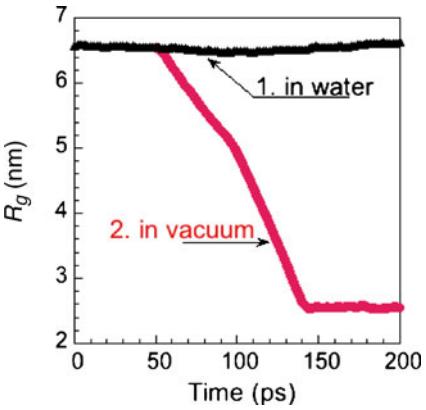

Fig. 6 Molecular dynamics calculation at $T = 300$ K. Gyration radius R_g of tau in water (*curve* #1). Removing the water and running the calculations *in vacuo* (*curve* #2), induces a rapid evolution of the protein toward collapsed conformations on a time scale of 100 ps

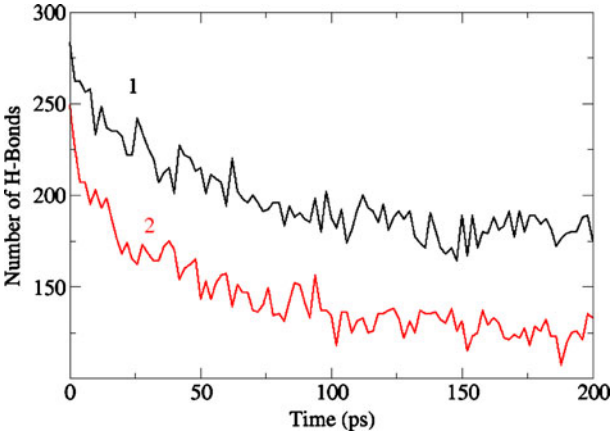

Fig. 7 Molecular dynamics calculation at $T = 300$ K. Number of intramolecular hydrogen bonds of tau in water (*curve* #1) and in methanol (*curve* #2)

experimental average value $R_g = 6.6$ nm [51]. If at time $t = 50$ ps of this segment one takes away the water molecules and lets the tau molecule evolve *in vacuo*, the latter collapses to a compact form ($R_g = 2.5$ nm) in less than 100 ps (Fig. 6, curve #2), and keeps this form afterwards. We have also measured, in the same time interval, the number of intramolecular hydrogen bonds in tau, and in Fig. 7 we report the results in water (curve #1) and in methanol (curve #2). Both curves show an initial decrease in the number of H-bonds, due to their replacement by H-bonds between tau and solvent; after 200 ps these numbers appear to be stabilized around 175 in water, and around 125 in methanol.

4 Discussion

The free energy landscape of disordered proteins is characterized by small potential barriers between conformers, and depends on solvent conditions [40]. We have shown that the tau protein dynamics in this landscape depends on the temperature, which affects the probability of the system to be found in different regions of the accessible phase space. We found at 318 K a large variation of the SAXS spectrum of tau from the spectrum at 293 K, corresponding to a compaction of the molecule as in the Sic1 protein [23]. This compaction suggests that it is related to the different time spent by the protein in different parts of the phase space region at the two temperatures. Figure 4 shows that the two distributions of the conformers, as monitored through the values of the gyration radius R_g, show an increase with temperature of the population of conformers characterized by smaller values of R_g, while at the lower temperature extended conformers are preferred. This interpretation is consistent with recent results, showing that the average radius of gyration in the intrinsically disordered protein prothymosin α decreases with increasing temperature [41].

As already mentioned, the tau protein is one of the largest totally disordered IDPs [39], and the solvent is expected to play an important role in the dynamical evolution of the conformational landscape of the protein. We have performed a computer simulation of tau in water and in methanol, which provides a hint as to the relation between the overall shape of tau and the solvent–protein interaction. It is interesting to notice that the two solvents behave quite differently in the replacement of intramolecular H-bonds, as shown in Fig. 7, but this

difference does not seem to be of relevance for the overall configuration of tau, the value of R_g after 200 ps being almost the same in both cases, as shown in Fig. 5. On the other hand, we have found that the absence of water effectively induces a dramatic change in the overall shape of tau, and highlights the critical role of the solvent in determining the configuration of the molecule. The case study described in the present work is just an example of a growing body of properties that characterize the relation between a biomolecule's function and its environment.

5 Conclusions

Protein dynamics is being accepted as the key feature of protein function, as proposed by Careri [2]. Both temperature and solvent can affect the dynamics of the protein, in different ways; therefore, we have separately tested their influence on the behavior of tau, an intrinsically disordered protein. Our experimental and simulation results point out that the tau protein undergoes a significant reduction in the average protein radius of gyration, both when the temperature is raised and when the solvent is removed. The larger temperature drives the molecule's trajectory preferentially in to phase space regions characterized by conformations with more compact shapes. On the other hand, the removal of the solvent changes completely the phase space landscape, inducing an even more drastic compaction of the molecule. Unveiling the complexity of protein dynamics between multiple quasi-stationary conformational states will be a hot topic in next years as the development of new time-resolved experiments join with space-resolved nanoscale microscopy to reveal space time correlation dynamics [52, 53]. Further investigations of coherence in living matter could be unveiled if quantum phenomena such as quantum criticality and shape resonances [54, 55] are found to underlie the mysterious dynamical functionality of intrinsically disordered proteins.

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