Physica E 42 (2010) 662–665

Contents lists available at ScienceDirect

Physica E

journal homepage: www.elsevier.com/locate/physe

Proteins, supercooled liquids, and glasses: A micro-review

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ARTICLE INFO

Available online 15 August 2009

Keywords:
Conformational substates
Energy landscape
Hierarchy
α and β fluctuations

ABSTRACT

Proteins are complex systems that connect biology, biophysics, chemistry, and physics, and even mathematics. They share similarities with supercooled liquids and glasses, such as frustration, the existence of an energy landscape, and at least two types of fluctuations. Proteins are, however, far more complex and have functions that are essential for life. The study of the physics of proteins is much younger than the corresponding study of glasses and thus glasses and supercooled liquids can provide suggestions of what to look for in proteins. The present micro-review presents concepts that are common to proteins, supercooled liquids, and glasses and omits details that can be found in the original papers.

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1. Frustrated structures

Proteins are the building blocks of life. They are involved in all essential functions of living systems [1,2]. For physicists they are mesoscopic systems that permit exiting investigations into their structure and dynamics. Proteins are built from 20 amino acids. Of the order of hundreds of amino acids are linked covalently to form a polypeptide chain. In the proper environment, the chain folds into the working protein that is often globular. The arrangement of the amino acids along the chain determines structure and function. Glasses and proteins are frustrated systems; they do not assume unique structures in which each atom is in a well-defined position. This property is often called disorder. The term applies to glasses. For proteins it is misleading. The different positions that atoms can assume are essential for function. The structure of a protein as seen by X-ray diffraction appears to be unique. This unique structure appears in textbooks and leads many students, teachers, and researchers to assume that proteins are like beautiful crystals in which each atom occupies a unique position. Reality is different. Proteins, like glasses and other complex systems, can assume a very large number of different structures or conformations.

Proteins and glasses, while having some similar properties, differ in some respects. The size of a glass is typically much larger than interatomic distances, whereas the size of a protein is only about one order of magnitude larger. Glasses are usually not affected by the structure of the environment; proteins are surrounded by a hydration shell, about two layers of water and are embedded in a bulk solvent. As will be discussed later, the hydration shell and the bulk solvent are essential for the protein dynamics.

2. Conformational substates

The concept that biomolecules can assume many different conformations is not new; it goes back a long time. Biophysicists like Linderstrom-Lang and Gregorio Weber suggested many years ago that a given protein had to be able to assume many conformations in order to work. This concept was not universally accepted, possibly because definitive evidence for inhomogeneity of proteins was lacking. The existence of many conformations was proven by flash photolysis experiments at cryogenic temperatures [3]. The experiments showed that the kinetics observed at low temperature could not be described by a single exponential of the form

$$N(t) = N(0)\exp\{-kt\}, \tag{1}$$

where k is a rate coefficient. Some books say that if one exponential does not work, use two. However, the cryogenic experiments could not be fitted with two or three exponentials; they required a continuum of rate coefficients,

$$N(t) = \int f(k)\exp\{-kt\}\,dk. \tag{2}$$

The cryogenic experiments thus proved that proteins can exist in a large number of different structures or conformations, called conformational substates. "Substates" and not "states" because proteins can have different states, for instance oxidized and reduced. Each of such states can exist in a very large number of substates.

3. The energy landscape

If indeed proteins have a large number of conformational substates, how can one characterize an ensemble of proteins? Here

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1386-9477/$ - see front matter Published by Elsevier B.V.
doi:10.1016/j.physe.2009.08.005

is where the energy landscape enters. Each individual protein can be described by a point in a high-dimensional conformation space, where each point is described by the coordinates of all atoms in the protein and the hydration shell. An ensemble of proteins is described by the structure of all the points in the conformation hyperspace. This structure is called "energy landscape". Conformational substates are valleys in the energy landscape. Only a computer can visualize the entire landscape. To at least get a crude insight one can draw a one-dimensional cross section through the full landscape. The cross section gives the energy of the glass or protein as a function of a conformation coordinate. The energy landscape concept applies to glasses and proteins, but the landscape is far more complex and organized in proteins.

4. The hierarchy of substates

Initially we assumed that the energy landscape (not yet so called) had no substructure and that the barriers between substates were all of the same order of magnitude. We also assumed that the energy barriers between conformational substates were internal protein properties. It turned out, however, that both assumptions were wrong. Consider first the barriers between substates. They are not approximately equal. Experiments showed that the transitions between substates fell into at least two classes with rates between substates in the two classes being very different [4].

Thus there are valleys within valleys in the energy landscape. In other words, the energy landscape has at least two tiers. Further experiments showed that the organization of the energy landscape is even more complex. Proteins can assume very different conformations with different functions [5,6]. Thus there are valleys within valleys within valleys. We are not yet done, however! The hierarchy of the energy landscape of proteins contains even more tiers as cryogenics experiments demonstrate [7]. The second assumption, that barriers between substates reside in the protein itself, is also wrong as will be discussed later.

Glasses and supercooled can also be described by an energy landscape [8], but there is less structure than in proteins.

5. Fluctuations

Fluctuations are essential for the function of proteins. Because fluctuation phenomena in glasses and supercooled liquids have been studied for much longer than in biomolecules, results well established in the former provide help for understanding biomolecular dynamics. Supercooled liquids and glasses show two prominent types of fluctuations, called α and β [9,10]. The two classes are characterized for instance by their temperature dependence. The rate coefficients $k_\alpha(T)$ of the α fluctuations usually do not follow a standard Arrhenius relation, but can be approximated by the Vogel-Tammann-Fulcher relation,

$$k_\alpha(T) = A_\alpha \exp\{DT/(T - T_0)\}. \quad (3)$$

Here A_α, D, and T_0 are experimentally determined coefficients. It is customary to characterize a system exhibiting α fluctuations by the glass transition temperature T_g where $k_\alpha(T_g) = 0.01\ s^{-1}$. The rate coefficient $k_\alpha(T)$ is related to the viscosity $\eta(T)$ by the Maxwell relation

$$k_\alpha(T) = G/\eta(T) \quad (4)$$

where G is the short-time shear modulus.

The β fluctuations can be described by a standard Arrhenius relation,

$$k_\beta(T) = A_\beta \exp\{H_\beta/RT\}. \quad (5)$$

In most supercooled liquids, the α and β fluctuations merge at a temperature well above T_g.

Recent work with proteins has shown that proteins also experience α and β fluctuations and that these are vital for the functioning of proteins. Because much of the information on fluctuations and their importance for protein dynamics has come from research with myoglobin, we briefly describe this protein.

6. Myoglobin

Myoglobin (Mb), the hydrogen atom of biology, is the main actor in the present story. Mb was the first protein whose structure was determined. It is a globular protein, about 3 nm in diameter. The protein is surrounded by a hydration shell of about two layers of water and it is embedded in a bulk solvent that can be characterized by its viscosity $\eta(T)$. Mb contains a small number of cavities that cannot be accidental defects, because they have been conserved for a very long time and appear in a wide variety of Mbs from different species. The largest cavity, called heme pocket, contains a heme group with an iron atom at its center. Four smaller cavities, called Xe 1–Xe 4, have also been observed in X-ray diffraction studies. They are called xenon pockets, because they have been observed in Mb that was exposed to xenon.

Forty years ago Mb was "understood". It was believed that it had only one main role, dioxygen storage, and that O_2 binding was a simple one-step process. Now, after forty years of research by many groups, we realize that ligand binding is a complex process, still not fully understood. The long path to the present understanding has not been in vain, however. The research by many groups has provided insight into protein dynamics in general and has yielded concepts that may be applicable to many or all biological processes. Three concepts have already been discussed in Sections 2–4, namely conformational substates, the energy landscape, and the hierarchy of substates. More concepts emerge from the study of the binding process of CO and O_2.

Consider CO, covalently bound to the heme iron in Mb (state A). The Fe–CO bond can be broken by a laser pulse and CO is then "free" in the heme pocket (state B). The CO then has three choices: It can rebind ($B \Rightarrow A$) with a rate coefficient k_{BA}, or move to the Xe pockets ($B \Rightarrow D$, k_{BD}), or exit into the solvent ($B \Rightarrow S$, $k_{\rm exit}$). Insight into protein dynamics is gained from studying these three processes as function of time and temperature. It is remarkable that the three different motions are controlled in three different ways so that their study yields information about different control mechanisms in biomolecules. In the following sections the three major processes will be sketched, starting with the "simplest" case, entry and exit of CO.

7. Gating and α slaving

The X-ray structure of Mb shows no channel or hole for exit and entry of a small ligand such as CO or O_2. Transit between the interior and exterior must therefore be enabled by fluctuations that open a gate [11,12]. Clues for the mechanism of gating came from experiments that showed that the rate coefficient, $k_{\rm exit}(T)$, for the exit of CO from Mb depends on the viscosity of the bulk solvent. Measurements in different solvents [13] further indicated that $k_{\rm exit}(T)$ follows approximately the