学术讨论

Irreversible Thermodynamic Theory for Protein Folding and Protein Thermodynamic Structures

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Abstract The interpretation of protein physical properties at the most fundamental mechanistic and thermodynamic level plays a key role in physical and molecular understanding of biology. The protein thermodynamic structure (potherse) is generated through irreversible thermodynamic possesses. Protein folding is a specific physical mechanism for the origin of natural order. It was shown that pothersal change is the basic (molecular) thermodynamic unit of any physiological reaction which acts as a molecular switch. The Weiss equation is re-derived using protein mechanics theory. Some protein physical properties are also discussed.

Key words protein folding, order, protein thermodynamic structure (potherse), protein thermodynamic conformation (pother), Weiss equation, chaperone

The physical properties and movements of macromolecules are fundamental scientific problems in our understanding of biology. A vast amount of experimental information has been obtained over past decades in the physical properties of macromolecules. The descriptions of protein molecule becomes more detailed by the day, yet our understanding of them at the most fundamental mechanistic and thermodynamic level remains poor. Many different scientific experiments have been carried out to verify different theories, but there is no fundamental biophysical theory that can interpret essential relationships between different scientific discipline; therefore, theoretical study is urgently needed in this field.

Most models of protein folding are empirically based and constructed on specific protein. There is not a real axiomatic theory in the field. Here, the pure scientific theory for protein based upon irreversible thermodynamic theory is described.

1 Basic concepts and principles of irreversible thermodynamic theory

1.1 Dynamic freedom of the thermodynamic system

When a thermodynamic system acted upon by a mechanical force, the system will exhibit mechanical movements which will eventually transform into thermodynamic changes of the system. Although there is no physical time separating them, we can artificially define a theoretical turning point in the transformation as the dynamic freedom of the thermodynamic system[1]. This physical measure is the key concept in our irreversible thermodynamic theory[1~3] which can be used to analyze protein folding and other physical movements.

A simple thermodynamic system can be assumed to have S independent thermodynamic states with a turning rate V, then

$$T = S/V \qquad (1)$$

Where T is the dynamic freedom of the system, S is the sample space of the thermodynamic system states and V represents the collision frequency.

All properties and features of the system can be recognized as a complex blending of mechanical and thermodynamic changes of the system.

1.2 Generations of sub-thermodynamic system within system

When a thermodynamic system changes irreversibly, the thermodynamic changes of the system can not follow the changes of the stimulus, the system can be naturally divided into many

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thermodynamic sub-systems with these sub-systems always in thermodynamic equilibrium. Thus the thermodynamic sub-systems are a natural fact of this physical process.

The physical movements of the system can be classified as fundamental and advanced physical movements. Thus the generation of the system is natural phenomenon.

1.3 System logic

Our theory assumes that every object represents a thermodynamic system. System logic must then be carefully studied since it differs fundamentally from the logic of particles.

First, advanced physical movements construct a new independent logic space with new logic generated in this space. Therefore, a logic limitation exists in the interpretation of advanced physical movements by fundamental physical laws. All feature of advanced physical movement can not be interpreted by fundamental movements. For example, the principle that one native protein sequence folds into one protein can not be fully interpreted by physical laws because biological rules control the processes.

Secondly, there is no basic concept of an elementary particle. An object can only be considered as a particle in some specific cases. As the infrastructure and constituents of a system affect the relationships between systems, the system logic is fundamentally different from the logic of particle. The linear relationships between constituents of systems are not equal to the linear relationships between particles. The logic relationships between systems depend on the measuring method. For example, we can find some amino acid residues that are directly involved in the expression of enzyme activity, but it is wrong to conclude that these amino acid residues determine the enzyme activity.

Thirdly, the development of natural order generates many independent system parameters. Some system properties can not be logically deduced from other. For example, protein folding, protein stability and protein function differ from each other and it is impossible to find any theoretical relationships between them.

This discussion of system logic is simple, but provides essential relationships between it and physical theory.

2 Protein folding

2.1 Physical principles of protein folding

There are three necessary prerequisites for protein folding to be carried out by way of irreversible thermodynamic mechanism. First, there is a thermodynamic pathway that leads to correct peptide folding. Secondly, the protein has a stable geometrical structure. Thirdly, there is a suitable environment that enables orderly protein folding processes. Therefore the protein folding pathway is fundamentally different from protein stability.

To reduce the mathematical difficulties and make it possible to theoretically analyze protein folding, we assume that: 1) all protein states generated during protein folding has the same energy; 2) the norm of possible thermodynamic states generated in protein folding is $N!$, where N is the number of variables; 3) each amino acid residue has two independent variables since it has two involving angles and the turning rate of protein thermodynamic conformations is 10^{10} turn/s (although it is variable).

To avoid heterogeneous protein conformation, the protein folding must seek its most stable thermodynamic state in a limited area. Most globular proteins can fold in 1 min, we can estimate from equation (1) that only in an area of up to 8 amino acid residues can it reach its lowest energy state by way of random collisions, if $T = 1$ min and $N = 2 \times$ the number of amino acid residues.

If an area folds through random collisions, this area must be smaller than 8 amino acid residues. An area in excess of 8 amino acid residues must have some physical factor that reduces the freedom of the thermodynamic system, or it has two or more independent thermodynamic movements.

The differences between thermodynamic principles and geometric principles produce a new problem. A thermodynamic change of a protein results in a greater geometric state ($N!$) than the number of geometrical parameters required to fix the geometric structure ($3N + 1$); therefore, the number of factors affecting protein folding is greater than the number of factors affecting structural shape. Therefore, gene mutants[4] exist that only affect protein folding and have no effect on protein stability.

When the protein folding according to

irreversible thermodynamic principles is finished, the protein reorganizes its structure according to geometrical principles.

Generally speaking, protein folding can be interpreted as the following. In a suitable environment, every independent irreversible thermodynamic movement as a peptide unfolds in time and space, forms some intermediate potherse, which can be further organized into an advanced potherse. Due to differences generated from geometrical structures between thermodynamic structure, some proteins may undergo a protein conformational collapse and be finally transformed into a native protein. Figure 1 illustrates the general features of protein folding.

Therefore protein can not adopt its lowest energy state, since the energy does not logically control protein folding.

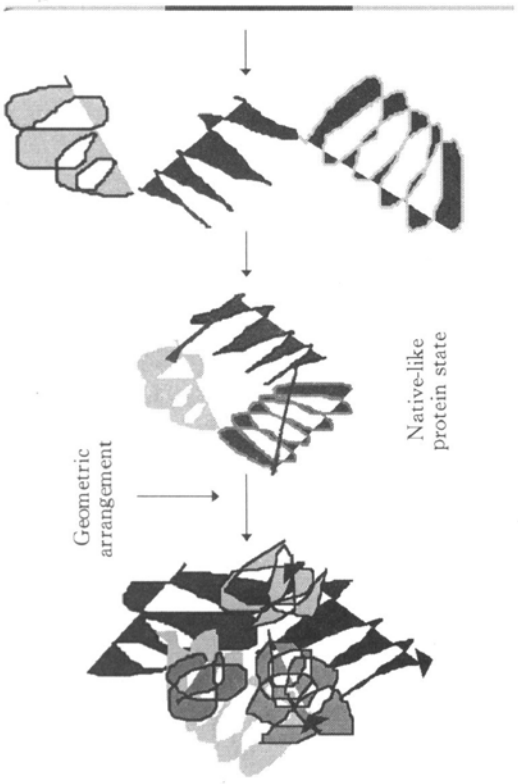

Fig. 1 protein folding principle

First, the polypeptide folds into a proteir-like intermediate state, which is the end of the thermodynamic protein folding pathway. This proteir-like intermediate state can further collapse into a more tight and stable state (or native protein) according to geometric principles which produces many derived potherses.

2.2 Protein folding stability

Although there is only a little experimental evidence, the concept of protein folding stability is indeed useful in our understanding of protein folding. Since the protein folding is a partially mechanical and partially thermodynamic pathway, the concept of protein folding stability is required to interpret some natural phenomenon. From the studies of landscape theory[5], T_f/T_g reflects some properties of protein folding stability, where T_f represents the folding temperature, T_g represents the glass temperature.

2.3 Biological rules of protein folding

Physical principles provide the necessary prerequisites for protein folding, but they do not provide sufficient prerequisites for protein folding and they do not account for biological functions. Some biological rules must be added[1].

Principle 1. The native protein sequence is the product of evolution. Proteins that could not fold into one fixed state was eliminated in evolution. It is well known that some protein sequences can not fold and some protein sequences may theoretically fold into two or more states. Therefore the Anfisen[6] law of one protein sequence folding into one protein is a biological rule, not a physical law.

Principle 2. Generally, protein stability and protein folding stability mirror the protein physiological functional requirements.

Principle 3. Protein folding is essentially independent of other proteins and can not be logically affected by other factors. If not, the independence of protein folding is lost and we can not study it scientifically and logically.

3 Protein thermodynamic structure

3.1 Protein thermodynamic structure (potherse)

The above analysis reveals that a protein is not uniform thermodynamic system but a complex of thermodynamic subsystems. Therefore the relationships between different thermodynamic sub-systems must be considered. This consideration produces the concept of protein thermodynamic structure.

For reasons of language, the protein thermodynamic structure is renamed as potherse. Potherse is an independent thermodynamic system within the protein related to the thermodynamic and mechanical parameters. Respectively, protein thermodynamic conformation is renamed as pother. According to equilibrium thermodynamic[1,2], a potherse constructs a sample space of pother. The pothersal change involves the most basic thermodynamic and molecular

unit of a protein.

Enzyme catalyzed reactions involves three potherses, corresponding to K_m, K_p and V_m (ES, EP and transition state). Therefore the enzyme active site differs from enzyme binding site. According to equation 1 and the time duration of a enzyme catalyzed reaction (10^{-4} to 10^{-7} s), it can be estimated that the active site only directly involves 4 ~ 6 free amino acid resides.

The potherse number of a protein varies as the protein reorganizes its thermodynamic structure in different environments. Therefore we must consider a protein as a complex and dynamics structure, rather than a fixed structure. However, any physical measurement will find one potherse that relates to our interesting subject.

Since the pothersal change involves the basic thermodynamic unit of any physiological reaction, the protein and enzyme molecular function can not be further divided into properties of their constituents or amino acid residues. This interpretation does not exclude the possibility of finding some relationship between protein sequence and function, but it is impossible to find linear relationships between protein sequence and protein function. This conclusion has been confirmed by the following phenomena: genetics studies of enzymes have shown that some gene mutants and their apparent effect on enzyme activity can be suppressed by another gene mutant. This phenomenon also occurs in protein folding where some mutated proteins can not fold by themself in some cases and their effect on protein folding can be suppressed by another gene mutant[4].

One potherse only relates to a very smaller number of independent amino acid residues (up to 8). This result agrees with the size of apron very well. Therefore one amino acid substitution in a protein sequence can greatly affect protein properties largely in some cases.

3.2 Three types of potherse

There are three types of potherse, each corresponding to protein folding intermediate, protein stability-relative potherse and derived (function-relative) potherse. They differ from each other conceptually.

In most cases, the stability-relative potherse does not express itself as an independent thermodynamic system but is integrated into other potherse. Therefore we should study protein stability in more extreme conditions because only in such case will it behave as a thermodynamic system.

Derived potherse are generated at later times in protein folding while of arranging protein geometric structure. Our theory shown that most protein functions are related to derived potherse. Therefore, enzyme stability differs from its functional stability. It can be predicted that for any protein, a protein sequence can be found which is more stable and can fully express its activity at low temperature.

3.3 Multiple dual natures of protein

Since proteins exhibit thermodynamic structures and geometric structures, proteins have multiple dual natures, such as dual natures of solid and fluid, dual natures of micromolecule and macromaterial, dual mechanical and thermodynamic changes and dual mechanical and thermodynamic properties. This property enables us to analyze some protein properties using mechanical and thermodynamic theory. With this feature, the protein can link all the biophysical movements and physiological changes *in vivo* and play a central role in signal transduction.

3.4 Pothersal change and molecular switch

Because pothersal change is a transition between thermodynamic states, the transformation may or may not occur (none and all rule). The transformation of a potherses constructs another potherse. Theoretically, all pothersal changes can be recognized as molecular switches.

The pother and potherse exhibit different behaviors. The potheral change can be speed up by many physical factors, such as temperature and mechanical force. However, if the effect is small, it can not induce the potherse change, but if the effect becomes larger, the pothersal change may be induced. Because of the multiple dual natures of proteins, almost every physical and chemical factor can induce pothersal change.

3.5 Rederivation of Weiss equation

The concept of protein structural change induced by mechanical force was advanced in our laboratory in 1991[7]. Recently, this view has been confirmed by many other laboratories[8]. However, the most detailed experimental study of protein mechanics takes place in neural conduction where the Weiss

equation describes the excitability of nerve cells which has not been previously analyzed by protein mechanics theory.

According to protein theory, the open and closed state of a channel protein represents different potherse, the excitability of a nerve cell responding to a stimulus represents the mechanics characteristics of channel pothersal change induced by electrical force which can be analysed by protein theory. The protein conformational change in an electrical field (or electrical force) can be described as:

$$dC = kV$$

Where k is the tensile strain constant, dC represents the protein thermodynamic conformational parameter change induced by the electrical field and V is the potential.

For the initial rate of protein conformational change toward protein structural change:

$$dC = g(V - V_0)\,dt$$

Where g is a constant and V_0 is the minimum potential required for static protein thermodynamic structural changes.

Then

$$\int_{C_0}^{C_0+\Delta C} dC = \int_0^T g(V - V_0)\,dt$$

$$\Delta C = gVT - gV_0T$$

$$V = V_0 + \Delta C/(gT)$$

Where C is the minimal protein conformational change between two different potherse, C_0 is the protein conformational parameter (for the closed state) and $(C + \Delta C)$ represents another protein conformational parameter of the potherse (open state).

The Weiss equation is: $V = a + b/t$

Therefore: $a = V_0 \quad b = \Delta C/g$

Where a is the rheobase and b/a represents the chronaxie.

This analysis confirms that the Weiss equation expresses the universal mechanism for protein thermodynamic structural change responding to stimulus (mechanical force). Figure 2 shows the theoretical interpretation of the pothersal change.

Many scientists have previously utilized the polarization concept to describe the response of protein to an electrical field but this is not suitable. Polarization does not mirror any structural features of the protein, so it is more a philosophical concept, rather than a scientific concept.

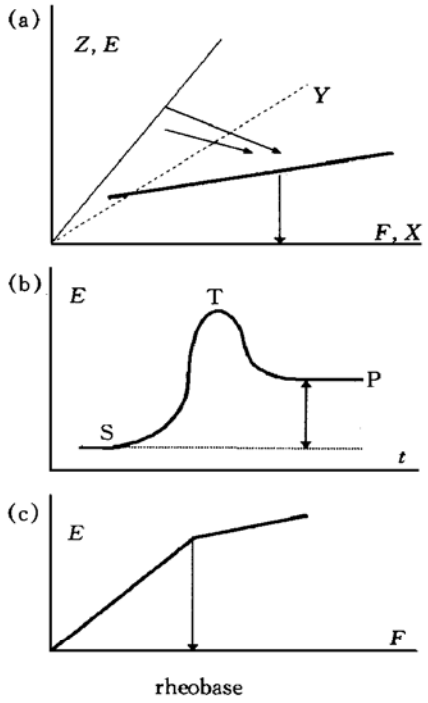

Fig. 2 potherse change characteristics

Where, E represents energy and F represents force. (a) When a protein is stimulated by a mechanical force, it first deformed but remains in its thermodynamic state until it suddenly transforms into another pothersal state. (b) The pothersal change occurs in solution by thermodynamic change with three potherses (S, T, P) involved in this transformation. Where, S and P represent two different potherse, T represents transition potherse. (c) If the difference between T and P can be ignored, the question becomes very simple. The mechanical characteristics of the pothersal change can be described by the Weiss equation: $V = a + b/t$, where a is the rheobase that represents the minimum strength of the stimulus required for pothersal change and b/a represents the chronaxie.

4 Discussion

4.1 Function of the molecular chaperone

Since thermodynamic change in a solution can not provide enough energy for some pothersal changes, some pothersal changes must be catalyzed by enzymes. Previous studies have shown that this function is partially carried out by molecular chaperones, so it is only subsidiary function of the molecular chaperon that affects protein folding.

1) Most molecular chaperons are heat-induced proteins. In this case, protein folding is not urgently required, but protein complex reorganization is urgently required for the physiological regulation of the stress response (signal transduction activities).

2) Molecular chaperons can promote the protein degradation, protein complex dissociation, modulate

the activity of some protein complexes, and assist protein translocation and secretion. These functions have no any biological relationship to protein folding.

3) Experimental results have also shown that molecular chaperones can unfold native proteins[9,10]. These results are not consistent with the concept that the chaperone determines protein folding, but promotes protein thermodynamics conformational change.

4) Although the existence of molecular chaperones does not contradict irreversible thermodynamic theory, it is not the absolute logical prerequisite for protein folding.

According to our theory, molecular chaperones may assist protein folding through two mechanisms, by suppressing protein aggregation in protein folding and by promoting pothersal change during protein folding (or unfold misfolded protein structures). It does not logically determine protein folding.

4.2 Biophysical mechanism of anaesthetics

Our biosignal theory[2,3] indicates that one signal corresponds to one potherse, the conduction of a biosignal is the spreading of the activity of signal transduction systems. Therefore some biophysical features of signal conduction can be analysed by protein theory. All physical and chemical factors that can affect the activity of signal conduction can be theoretically classified as follows:

Group 1: high temperature, general anaesthetics[11] or small organic molecules which can increase protein fluidity (or the rate of potheral change). This group can increase the rate of signal transmission at low concentration (or at low quantity), and decrease it at high concentration by decreasing the protein conformational change coupling or inducing the dissociation of the protein complex.

Group 2: low temperature, larger organic molecules, and high pressure, such as cholesterol which can decrease the membrane protein flexibility directly or indirectly (by mechanical interaction between membrane and protein) which then decreases the rate of signal transduction. It is well known that high pressure can reverse the effects of general anaesthetics. Lower temperature inhibits the potheral changes and thus can induce anaesthesia states.

Group 3: nervous transmitters and agonists bind to specific protein and this binding induces the pothersal change and initiates the signal transduction activity.

Group 4: Local anaesthetic and ideal competent antagonists can bind to directly protein and which inhibits (or helpful) pothersal changes.

Since proteins exhibit dual natures, proteins show some physical features of emulation. The general anaesthetics can exert their effects as assistant surfactants to modify the protein mechanical and thermodynamic properties. This hypothesis requires further confirmation.

1) Generally, it is unnecessary to differentiate the effect of general anaesthetics applied to proteins or membranes. The fluidity of membranes and flexibility of proteins describe the same physical properties and influence each other *in vivo*. General anaesthetics can alter the protein conformation and enzyme activity[11].

2) The diameter of protein particles is about 10 nm. The surface tension between benzene and water is about 35mN/m. Since protein surface pressure is similar to that between water and benzene, the surface pressure of protein particles is about 2.02×10^7 Pa. Physical chemists have shown assistant surfactants can greatly decrease the dynamic surface pressure of colloids, which explains why high pressure can reverse the effects of general anaesthetics.

3) Although high temperature and general anaesthetics all increase protein fluidity, temperature in the range of 26 ℃ to 42 ℃, has little or no effect on protein surface pressure, so temperatures and general anaesthetics induce anaesthesia in different ways. Small molecules have anaesthetic potency while larger molecule can reverse the effect of general anaesthetics. This phenomenon corresponds well to numerous physical chemistry studies of assistant surfactant.

4) General anaesthetics have great power to dissociate the protein-polystyrene complex and thus can interfere with the hydrophobic interaction of molecules (unpublished results).

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蛋白质折迭的不可逆热力学理论及蛋白质动态热力学结构

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摘要 在机械力学及热力学基础上阐述蛋白质的各种物理性质是从物理学角度理解生物学的基础性工作. 详细讨论了蛋白质折迭的不可逆热力学理论, 蛋白质动态热力学结构理论. 理论推断蛋白质动态热力学变化是一切生物学状态变化的基本热力学状态单位并作为分子生物学变化的分子开关. 利用此理论解释了蛋白质的物理学性质及麻醉药的生物物理学机制.

关键词 蛋白质动态热力学结构, 折迭, 蛋白质理论
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