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Protein Flexibility as a Biosignal

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ABSTRACT: Dynamic properties of a protein are crucial for all protein functions, and those of signaling proteins are closely related to the biological function of living beings. The protein flexibility signal concept can be used to analyze this relationship. Protein flexibility controls the rate of protein conformational change and influences protein function. The modification of protein flexibility results in a change of protein activity. The logical nature of protein flexibility cannot be explained by applying the principles of protein three-dimensional structure theory or conformation concept. Signaling proteins show high protein flexibility. Many properties of signaling can be traced back to the dynamic natures of signaling protein. The action mechanism of volatile anesthetics and universal cellular reactions are related to flexibility in the change of signaling proteins. We conclude that protein dynamics is an enzyme-enhanced process, called dynamicase.

KEY WORDS: protein flexibility, protein structure-function relationship, signaling, hydrophobicity, temperature

I. INTRODUCTION

For a given protein, the logical relationship between protein dynamics and protein function has been well documented both experimentally[1–5] and theoretically.[6] The correlation between living temperatures of a species and thermal stability of their proteins has also been well established.[1] To date, there are no developed views about the correlation between general biological functions (phenomena) and protein dynamics.[7] In view of experimental science, it is very difficult to identify function-related proteins among the many protein candidates of a cell. Fortunately, we now have enough field and theoretical data to address such questions. This article explores basic principles and experimental data about the concept of protein flexibility signaling, as well as some fundamental problems of biology, such as action mechanism for volatile anesthetics[8] and the universal cellular reaction (UCR),[9] on the basis of this hypothesis.

The protein model of the biosignal has been developed on the basis of protein thermodynamics structure theory.[6,7] It states that one type of biosignal at the molecular level corresponds to one potherse, a subsystem of thermodynamics within a protein, or concerted motions of a protein, which are always related to one type of protein conformational state. Therefore, all properties of a biosignal can be described by the concepts of protein science, and all features of proteins can act as biosignals under specific conditions.

Although comprehensive understanding of the dynamic properties of protein requires the complete study of protein dynamics, the protein flexibility concept describes the overall profile of dynamics properties of a protein and has been conveniently and widely used in the field of biochemistry.[1,10] As it represents the fundamental property of singling protein, it acts as a biosignal. This recognition has improved our understanding of biosignal theory and subsequent discussions on this topic.

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II. GENERIC PROPERTIES OF PROTEIN FLEXIBILITY

A protein is not a dead structure expressed in a three-dimensional protein structure. In solution, a protein shows many different conformational states and fluctuates

among these states.[6,11] The dynamic nature of a protein is understandable but cannot be analyzed by applying the principles of three-dimensional protein structure theory. In biochemistry, the concept of protein flexibility is widely used to describe the dynamic nature of protein conformational change. To date, there is no unified definition for this term because it can be judged by many different methods. Conveniently, two different methods have been widely accepted in biochemistry: the first is the variation (or deviation) of protein activity (structure) in response to a stimulus, such as temperature and urea,[1,10] and the second is the relative rate of protein conformational change.[12]

According to protein thermodynamics structure theory, there is a clear relationship between protein dynamics and protein conformation (Fig. 1). As illustrated in the figure, the effects of diversified factors and substances on protein flexibility can be analyzed by using the principles represented. Temperature can directly change the flexibility of a protein by accelerating or decreasing the rate (velocity) of internal motion (pother), which further results in a change in protein conformation. In addition, urea and smaller organic substances have been shown to greatly impact the interaction between different pothers (hydrophobic interaction), and thus alter the flexibility of a protein concomitantly with the increase of protein volume.[13,14] Hydrostatic pressure exerts protein conformation action by reducing protein volume and thus influencing protein flexibility indirectly. Consistent with the above analysis, hydrostatic pressure can reverse the effect of volatile anesthetics on anesthesia induction[15–17] and has a mild effect on enzyme activity.[16,17] Consequently, it can only modify parameters of anesthesia induced by venous anesthetics, which has little impact on protein volume.[18] Because our discussion is limited to the generic properties of a protein, detailed mathematical descriptions are beyond the scope of this review and have been reported elsewhere (e.g., Weber and Drickamer[17]).

The efficiency of protein conformational coupling among different sites of a protein can be greatly influenced by a change in protein flexibility.[2,9,19,20] An increase in protein flexibility results in the conformational change of a protein and in protein denaturation, concomitant with exposure of hydrophobic surface of a protein and unspecific protein aggregation.[10,21–23] The generic relationship among enzyme activity and protein flexibility in shown in Fig. 2.[10,24,25]

One area that is often ignored is the irreversible nature of protein dynamics at di-

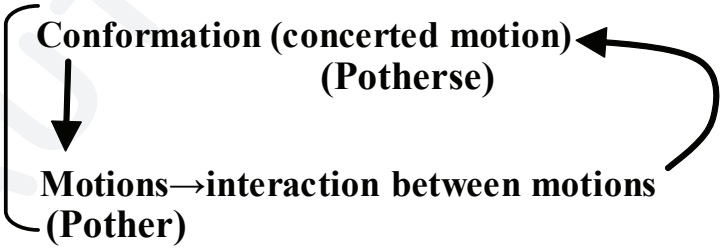

FIGURE 1. The logic cycle relationship between protein conformation and internal motion. The change of pother (internal motion of the main chain of a protein) can induce the change of concerted motions and thus induce the change of protein conformation. As a structural constraint, protein conformation controls the range and amplitude of the internal motion of a protein. One type of conformation is generated from one type of concerted motions of a protein. The interaction between side chains of a protein cannot induce protein global conformational change unless they couple to the motion of the main chains of a protein. Thus, the global conformational change of a protein is induced by the change of pother.

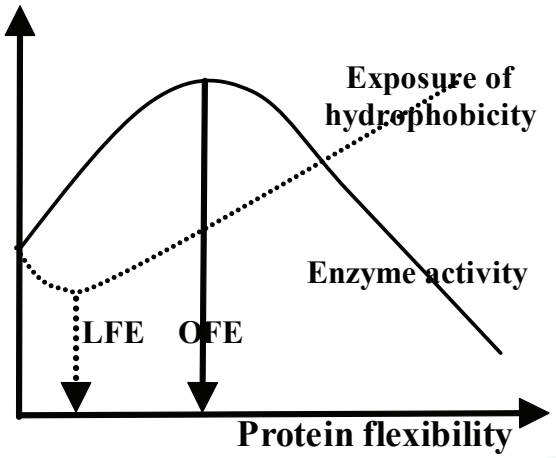

FIGURE 2. The generic relationship between protein activity and protein flexibility. LEF represents protein flexibility for the lowest exposure of hydrophobicity. OPE represents optimum flexibility for enzyme activity. In this figure, protein flexibility refers to urea concentration (resistance of enzyme activity to urea). The shifting of the origin of coordinates can change the profile of the figure and the first phase can disappear in some cases. Below optimum flexibility, protein activity increases with increases in protein flexibility. Above optimum protein flexibility, protein activity decreases with increases in protein flexibility; thus, the protein loses its function and begins to denature. The exposure of the hydrophobicity of a protein to solution is also illustrated in this figure. (Although protein flexibility can be calculated precisely under some conditions, it is unnecessary for the scope of this report.)

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versified levels and their relationship.[26] All proteins and DNA show hierarchical structures, and advanced structures are sensitive to mechanical stimulus.[27,28] At the equilibrium state, the relationship between all types of internal motions at diversified levels of hierarchical structure can be described by the laws of equilibrium thermodynamics. However, this may not true when many types of thermal processes change irreversibly. A stimulus applied to an advanced structure of a protein can hardly influence structure at a fundamental level. Thus, the motion and change of the cell skeleton are separate from the protein dynamics of its components, as shown in Fig. 3.

A. Cooperation and Counteraction Mechanism of Protein Dynamics in Signal Transduction

Signaling proteins can respond to many different environmental factors, and complex interactions between their effects occur in vivo within a signaling protein. Two extremities for these complex interactions are cooperation (synergetic) and counteraction (reversal effect), which can be studied experimentally. The best-known example is the pressure reversal phenomenon of anesthesia induced by volatile anesthetics.[15,18]

Kosmotropes and chaotropes have opposite effects on water structure and protein flexibility by interfering with the hydrogen bonding network between water molecules and weakening their hydrophobic effects.[28–30] Chaotropes, which increase protein flexibility, can compensate for the stress effects of kosmotropes or low temperatures. On the other hand, kosmotropes can reduce the effects of chaotropes or high temperatures.[31]

One could predict that heat and urea stress share similar signal transduction because they both increase protein flexibility. This hypothesis has been supported by data from the literature.[32–35] For example, it has shown that urea can strengthen the effect of

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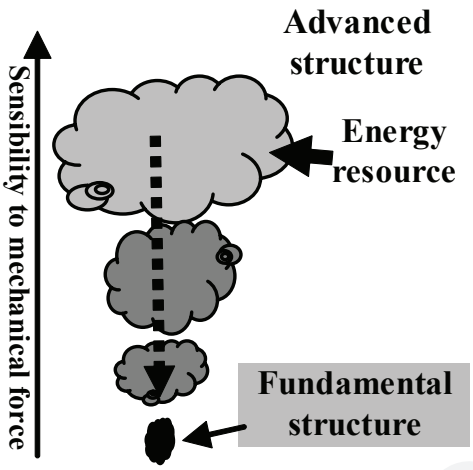

FIGURE 3. The irreversible nature of protein dynamics. The size of different structures (secondary, tertiary, and quaternary) of a protein differs largely and advanced structures are more sensitive to mechanical stimuli. Thus, the energy of a stimulus applied to an advanced structure will slowly, or cannot, transmit to the fundamental structure (motion) of a protein. In contrast, a change in the thermal motion of a protein by the elevation of temperature can be immediately transferred into diversified levels of biological structure.

heat on some types of signal transduction, and its effect can be suppressed by high concentrations of salt and other substances.[33–35]

The cooperation of urea, temperature, and anesthetics in anesthesia induction is evident for fish.[8] In vitro testing has indicated that a potentiating effect of temperature on the γ-aminobutyric acid (GABA) receptor (or nicotinic acetylcholine receptor) could be strengthened by volatile anesthetics.[36,37] However, unlike fish, the cooperation between temperature and volatile anesthesia is not obvious in anesthesia induction of animals.[38] All of these results are consistent with the conclusion that the N-methyl-D-aspartic acid (NMDA) and GABA receptors are not full anesthesia targets of volatile anesthetics.[8]

B. Flexibility Signal of NMDA and GABA Receptors

The NMDA and GABA receptors play an important role in the regulation of nerve cell excitability and have opposite effects on cell excitability. The NMDA receptor enhances the excitability of a nerve cell, whereas the GABA receptor suppresses it.[8,39] Thus, the nature of their flexibility is different. The relationship between the protein function and flexibility of two proteins is shown in Fig. 4.

The nature of protein flexibility of these two receptors matches their physiological function. For instance, the excitability of a nerve cell is high in cases of higher protein flexibility (elevated temperature); therefore, the requirement is decreased for NMDA receptor activity and increased for GABA receptor activity.

Opposing behaviors of NMDA and GABA receptor activity on stimuli have been shown in many volatile anesthetics[38] such as ethanol,[39] nitrous oxide,[40,41] xenon,[41,42] and urea.[8] Barbital, a GABA receptor antagonist, can also antagonize the effect of Katamine on NMDA receptors in fish anesthesia (unpublished data). These data indicate that the dynamic properties of NMDA receptors differ from those of GABA receptors, and also show that the binding site of volatile anesthetics is same as that of venous anesthetics. Thus, volatile anesthetics, like venous anesthetics,

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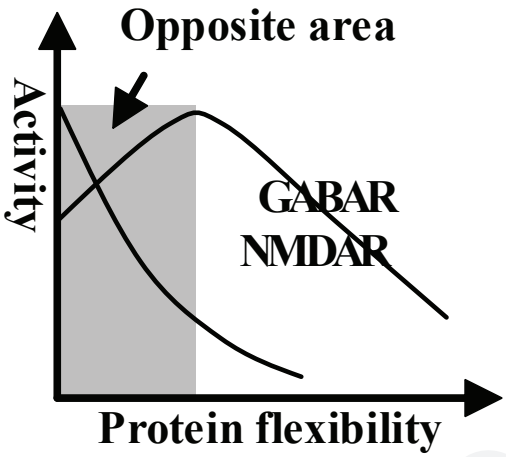

FIGURE 4. Relationship of protein flexibility and activity of GABA and NMDA receptors. In the section of first phase of flexibility GBBA receptor, the GABA and NMDA receptors show the opposing nature of flexibility. In this figure, the protein flexibility represents urea concentration. (Modified from Wang et al.[8])

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alter the local protein flexibility of a receptor at the antagonist (venous anesthetics) binding site (or coupling potherse),[6] decreasing conformational coupling efficiency between different parts of a receptor.[3,6] Additional research has also supported this conclusion.[36,37]

Figure 5 shows the correlation between the dynamic properties of NMDA and heat tolerance of fish. In this case, the protein flexibility of NMDA could be calculated as being reciprocal of protein activity.

Near 29°C, the NMDA receptor of Beijing winter grass carp will completely lose its function as protein flexibility increases, which induces a coma state in the fish. Thus, we concluded that the heat endurance of fish is controlled by protein flexibility of the NMDA receptor.[8]

C. Na+ and K+ Channel Flexibility in Neural Conduction

Many proteins, particularly the Na+ and K+ channels, are involved in neural conduction. The flexibility of these proteins has great impact on the nature of neural conduction.

At higher temperatures, the protein conformational rate increases and the velocity of neural conduction also increases. Volatile anesthetics and temperatures can both increase protein flexibility, but volatile anesthetics decrease the velocity of neural conduction.[43,44] The underlying cause is the increased protein flexibility at different parts of a protein, which causes anesthetics to suppress the excitability of nerve cells.[8,39]

The protein flexibility of Na+ and K+ channels can be judged by the rise and fall times of neural conduction (Q10 value), which is proportional to the conformational change rate of a channel protein. The protein flexibility of Na+ and K+ channels are represented in Fig. 6.

The environments of tested squid species can be ranked from coldest to warmest as follows: *Loligo opalescens* > *L. pealei* > *L. plei* > *Sepioteuthis sepioidea.*[45] Interestingly, protein flexibility of both Na+ and K+ channels among these species can follow the same sequence, but with low to high protein flexibility. This indicates that a low level of protein flexibility is needed when the species live at elevated temperatures. At the highest temperature, high protein flexibility results in the activation of the K+ channel and a loss of potassium for a cell.[46,47] These data indicate

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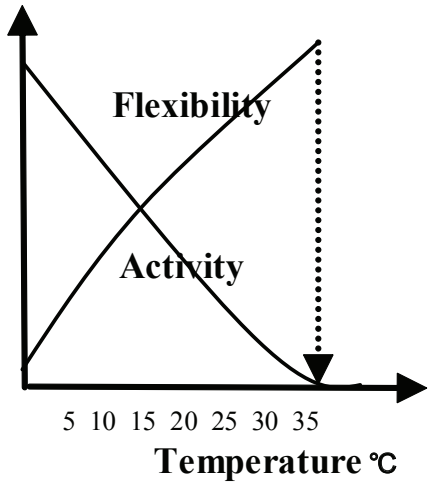

FIGURE 5. Protein flexibility of NMDA receptor of Beijing winter grass carp. Protein flexibility represents the variation of protein function in response to temperature, and could be simply calculated as the reciprocal of protein activity. (Modified from Wang et al.[8])

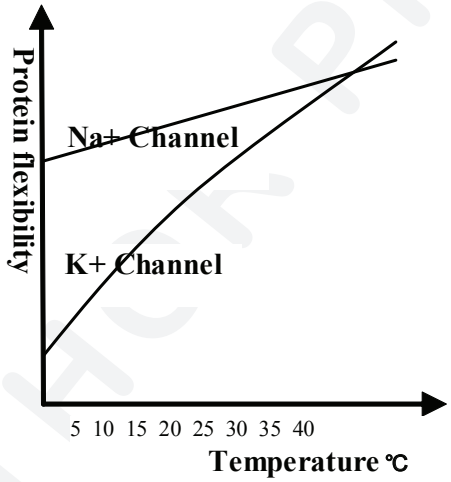

FIGURE 6. Protein flexibility of Na+ and delayed rectifier K+ channels. Protein flexibility is shown for the *Loligo plei* species and represents the relative rate of conformational change of a channel. (Modified from Mitsuiye et al.[43])

that the protein flexibility of the K+ channel (or K+ channel complex in vivo) controls the stability of neural conduction as well as the K+ hormesis of a cell.

Rosenthal and Bezanilla noted that the neural conduction will fail when the rise and fall times of a neural impulse are nearly the same[45]; however, their study did not provide an explanation for this finding. In our opinion, it represents the theoretical limit (or threshold) for the balance of protein flexibility between the K+ and Na channels, which reflects the complex regulating mechanisms of a nerve cell.

Surprisingly, the molecular and biophysical mechanisms of the loss of conduction ability of neural impulses at high temperatures and diversified conditions are largely

unexplored, and remain an area for future research.

D. Action Mechanism for Anesthesia Induction

Debate about the action mechanism of volatile general anesthetics persists. The membrane hypothesis, which states that the target of volatile anesthetics are the lipids of cell membranes, has attracted much attention in the past century.[39] However, this hypothesis has been rejected by most scientists recently because of research by Franks and Lieb.[48]

Theoretical analysis has demonstrated that an increase in protein flexibility induced by volatile general anesthetics underlies the fundamental action mechanisms of volatile anesthetics.[26] This prediction has been confirmed experimentally,[8] in which research showed that urea, a substance to increase protein flexibility, demonstrated weak potency of anesthetics and the ability to cooperate with many anesthetics in anesthesia induction. In addition, many surfactants have also shown anesthetic potency.[49] Figure 7 shows the mechanism of action of volatile anesthetics. In agreement with this conclusion is that numerous classes of general anesthetics inhibit etomidate binding to γ-aminobutyric acid type A ($GABA_A$) receptors.[50] Data indicate that numerous anesthetics bind to common areas of $GABA_A$ receptors. Structural analysis has indicated that the S4-S5 linker couples voltage sensing and activation of pacemaker channels[51–54] and that protein dynamics plays a key role in receptor activity.[53–55]

The protein flexibility hypothesis explains the mechanism of action of volatile anesthetics. Because volatile anesthetics bind to the hydrophobic area of a protein in unspecific ways,[50] they also obey the Meyer-Overton rule.[39,56,57] Only smaller organic molecules that act as an assistant surfactant have the ability to increase protein flexibility, and this capability decreases with increased molecular weight. Thus, the cutoff phenomenon of volatile anesthetics can be naturally interpreted.[58,59] Pressure can decrease protein flexibility and thus reverse the effects of volatile general anesthetics in some cases.[15–18] Dynamic regulation of receptor activity has become a global trend.[60–62]

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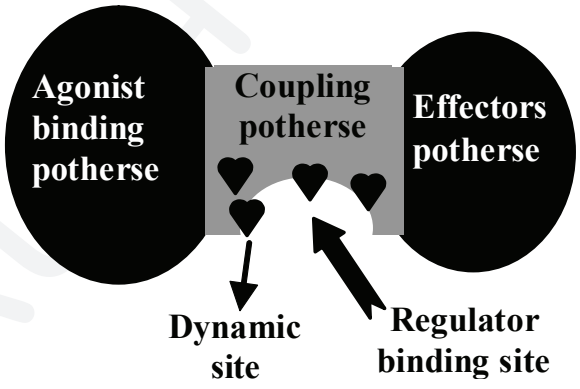

FIGURE 7. General protein thermodynamics structure of a receptor and working mechanism for volatile anesthetics. The regulator (volatile anesthetics acts protein dynamics regulator in this case) can modulate the flexibility of coupling potherse of a receptor at the dynamics site located near the binding site of the regulator. The regulator can also alter the efficiency of protein conformational coupling, and thus can modify receptor activity. The volatile anesthetics can also bind to other areas of a receptor with variable effects. However, binding to coupling potherse of a receptor represents a general and common mechanism for its effect. In addition, the coupling potherse is more flexible and shows high sensibility.

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The full target of volatile anesthetics is currently unknown.[8] Data have indicated that volatile anesthetics decrease mechanical coupling between receptors and actin skeleton and it may be an actin-associated protein.[63]

We have learned that increased flexibility of NMDA induced by elevated temperatures results in a coma state, rather than in anesthesia. How can we reconcile these different views? One explanation is that the loss of NMDA receptor activity is so rapid when temperature is elevated that an anesthesia state cannot be experimentally detected from a coma state. Another explanation is that the protein flexibility hypothesis cannot account for all natures of action mechanisms of volatile anesthetics, and other unknown events may be involved in the anesthesia induction of volatile anesthetics.

E. Protein Flexibility and Universal Cellular Reaction

The change of protein dynamics is fundamental for all protein functions and its effect can be seen in all types of biological structures. Thus, a fundamental response of cellular structures to changes of protein flexibility must lie therein. The UCR was first described by Nasonov and was recently reviewed by Matveev.[9,64,65] The UCR indicates that there is a standard response of biological structures to a wide variety of external stressors, such as noxious stimuli, heat, mechanical stress, hydrostatic pressure, electric currents, general anesthetics, altered pH and tonicity of the medium, heavy metal ions, hypoxia, and sound irradiation (200–7000 Hz, 94 dB). Figure 8 illustrates the general profile of the UCR. Matveev has proposed that the UCR is induced by a change of the hydrophobic phase volume of cellular proteins.[9] We further point out that a change of hydrophobic phase volume results from the change of protein flexibility.

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The biphasic phase response of the UCR, which can be explained naturally by the concept of protein flexibility (see Figs. 1 and 6), is the same and shows a correlation between protein activity and flexibility. Powerful evidence for our hypothesis indicates that the UCR can be evoked by sound irradiation and heat, which can influence protein structure indirectly by way of increasing protein dynamics. General anesthetics, altered pH, and heavy metals can modify the local flexibility of a protein at binding sites. Consistent with this hypothesis, it has been shown that nearly all types of stress can induce the expression of chaperones[32–35,66–69] or share common signal transduction pathways in vivo.

Alexandrov indicated that there is correspondence between protein flexibility levels and their biological role because signaling proteins involved in allosteric regulation show the highest flexibility, which is controlled by natural selection.[1] Normally, most enzymes show higher stability than that required for the survival of living beings. The relationship between enzyme conformational flexibility and temperature of living beings is poor from a physiological standpoint, but is obvious from an evolution standpoint.[1,2,70] Taken together, this recognition of the concept of a protein flexibility signal and a possible mechanism of the URC was proposed as follows.

Phase 1 of a cell represents a resting or sedative state. Chemical and biophysical stimuli with low intensity can greatly impair activity of the signaling proteins that show the highest protein flexibility, and they have little impact on metabolic proteins and enzymes (or metabolic reactions). This results in the global suppression of the signaling activities of a cell (inhibition of growth) and the metabolic system of a cell remains stable, concomitant with the increase in membrane potential and resistance to the stimulus (by physiological adaptation mechanism). The cellular state of anesthetized animals can be also described

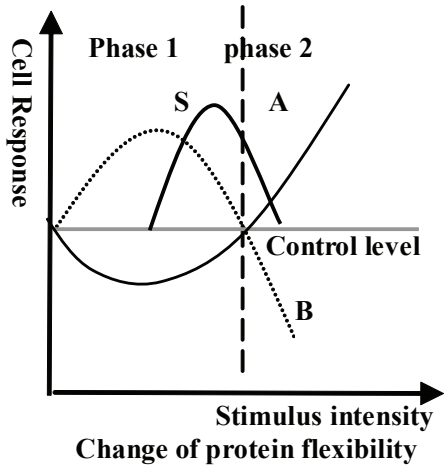

FIGURE 8. Universal cellular response. The cellular responses to stimuli show two typical profiles: type A (viscosity, turbidity, and vital dye binding capacity) and type B (membrane potential and resistance to the stimulus). The activity of fish (behavior) under "urea stress" and "heat stress" can also be described by the type A response (ref 8, the threshold is 0.37 mol urea, unpublished data). The letter "S" represents the signaling similarity between the UCR and anesthetics response. (Modified from Matveev9 with permission.)

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as such. Similarly to the URC, anesthesia is also a universal phenomenon for all biological species, including *Escherichia coli*. [69,71] Aanesthesia is generally recognized as a whole animal phenomenon; however, a cell shows similar phenomena regardless of whether it can be called anesthesia.

Phase 2 of a cell is called the excited state or agitated phase. When protein dynamics is greatly enhanced by higher-intensity stimuli, many types of signal transduction activities are triggered and the stability of metabolic system of a cell could be gradually lost. Many types of harmful production cannot be eliminated, concomitantly with reorganization of the protein complex (including the cell skeleton) on a larger scale. The hydrophobic surface of proteins may be exposed, and appearance of aggregation which are vital to dye binding. The nascent polypeptides (unfolded protein) are much more sensitive to environmental stress, and inclusion may be produced. It has been observed that inclusion generated at higher temperatures is more stable.[72] This will result in an increase of the turbidity and viscosity of a cell, depolarization of the membrane, and decreased resistance to the stimulus.

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F. In Vivo Protein Dynamics Is an Enzyme-Enhanced Process

Most biological processes are catalyzed by enzymes. We have many reasons to posit that the dynamics of protein, DNA, and RNA are enzyme-enhanced processes. These enzymes, termed dynamicase, should meet the following criteria: 1) they should regulate the dynamics of protein or other macromolecules, and 2) they should consume extra energy such as adenosine-5'-triphosphate (ATP). At present, proteins of two families, the DNA helicase[73] and chaperone families,[74–78] are worthy of being labeled as dynamicase.

By binding to the hydrophobic surface of a protein complex (or aggregation), the energy of conformational change of dynamicase generated in ATP hydrolysis can be transferred into its binding protein and regulate its dynamics features, resulting in the dissociation of a protein complex or the disruption of aggregation. Consequently, the free unfolded

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polypeptides may further fold into an active state.[77–79] We suggest that the chaperone family has more functions in vivo, which require further study .

By other mechanisms, chaperonin GroES can drive protein folding without the help of ATP hydrolysis.[80] In this case, it behaves as a common chaperone without dynamicase activity. Foldases—including peptidyl proline isomerase, disulfide isomerase, and chaperones without ATPase activity—can also regulate the dynamics properties of polypeptides at some sites by binding to and releasing them, thereby assisting in protein folding.[81,82] The chaperone activity of disulfide isomerase does not result from its isomerase activity.[83] Because they regulate the dynamics state of polypeptides at particular sites and cannot enhance the dynamics levels, their effect varies largely for different polypeptides. In our view, they are not dynamicase. To function well, the chaperone activity is often structured into a protein (or protein complex) that has another function, such as protease.[84]

G. Prolyl Cis-Trans Isomerization as a Dynamic Regulator of Protein

Loop or unstructured areas contribute to protein flexibility to a large extent. The protein flexibility of the loop area of a protein is largely influenced by species of residues and protein sequences.[85–88] Proline is unique in the realm of amino acids in its ability to adopt more cis conformation in the backbone of a protein, particularly in unstructured areas.[87–91] The isomerization between prolyl cis and trans conformations is catalyzed by peptidyl prolyl cis-trans isomerase and alters the dynamic nature of a protein, which results in the change of protein activity[92,93] or protein folding ability.[82,94] Some scientists have called this type of isomerization a molecular switch of backbone dynamics.[95]

The prolyl cis-trans isomerization is influenced by the gross flexibility of a protein or by other factors such as phosphorylation,[93] the time scale of which represents the dynamic nature of a protein that is logically related to protein function.[96,97] In cases with high protein flexibility, prolyl cis-trans isomerization regulates the conformational change of a protein[98] or protein folding.[99] In cases with low flexibility, the prolyl cis-trans isomerization cannot be processed and acts as a molecular switch for protein evolution.[100,101] In addition, the transition between cis and trans formation at residues 98 and 99 of orchid lectin has been found to control its quaternary structure.[102] This finding indicates that this transition can dramatically influence global protein flexibility and conformation. Therefore, the cis-trans isomerization, whether proline or nonproline, acts as a regulator for protein flexibility or dynamics.

CONCLUSION

The correlation between the dynamics properties of cellular signaling proteins and general biological phenomena is evident. The in vivo measurement of protein flexibility is of particular importance in resolving such questions of biology and medical science.[8] The development of new methods and their application are needed.

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