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Denaturants of Proteins as a Platform for Antiviral Medicines

Qinyi Zhao*

Medical Institute, China Rehabilitation Research Center (CRRC), Beijing PR, China.

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ABSTRACT

In the presence of protein denaturants at low concentrations, the physiological homeostasis of the host's body is not greatly disturbed, and the viral proteins with low stability could be deactivated, the virulence of a virus could be greatly decreased. Thus, the protein denaturants could act antiviral medicines. This opinion provides a new direction for the development of antiviral medicines, such as anti-Coronavirus. The theoretical foundation and related evidences for this model are reviewed.

Keywords: Protein denaturants, Temperature, Protein conformation, Virus, Medicines, Coronavirus

INTRODUCTION

It is a well-established opinion that the functional denaturation (inactivation) of viral proteins can greatly decrease the virulence of a virus and this principle has been utilized to produce live-attenuated vaccine [1,2].

A new concept of pharmacology is that all drugs take their role in biology or medicine by influencing the thermodynamic state of proteins or enzymes, or in detail, changing the functioning of a protein by adjusting the probability of active conformations of it [3-6]. In biochemistry, the effect of protein denaturants and temperature on protein deactivation or denaturation has been well established. Thus, protein denaturants could act drug under some cases. It has been proposed that an increasement of protein flexibility induced by volatile general anesthetics, in nature which are protein denaturants, underlies fundamental mechanism for the production of anesthesia [7].

All these indicate that protein denaturants could act medicine for treatment of viral infection. Here we will discuss its theory and evidences for it.

Molecular responding of a protein to protein denaturants

At high concentrations, the protein denaturants can induce protein denaturation, for ethanol, the working concentration is in range 50-80%, and 75% ethanol is selected for disinfectant. At low concentration, the protein denaturants cannot induce the protein denaturation, but it can alter thermodynamic balance between active to inactive conformation of the proteins and adjust the probability of active conformation of proteins, such as NMDA (N-methyl-D-aspartic acid) and GABA (γ-aminobutyric acid) receptors [3,7]. For animal and human, the NMDA receptor is the major excitatory receptor and GABA receptor is the major inhibitory receptor *in vivo*.

The functioning nature of a protein in the presence of protein denaturants could be analyzed on the basis of protein activity curves along concentration gradients of denaturant **(Figure 1)**.

Two types of active conformation curve (similar to activity curve) of proteins can be found for all proteins, and which is determined by the stability of active and inactive conformations of the protein [3,5]. One is expressed in dotted line; the GABA receptor belongs to this type; the activity of this type of proteins is firstly increased at low concentration of protein denaturant, and then decreased at high concentration. The features of another type of protein is expressed as solid line; the activity of the protein decreases as the concentration of protein denaturant increases. The NMDA receptor and temperature-sensitive protein belong to this group. The functioning features of these receptors match well up to physiology. At high temperature or in the presence of protein denaturant, the protein flexibility and excitability of a cell is increased; in order to maintaining the physiological balance, the body needs more inhibitory activity (or GABA receptor) and little excitatory activity (NMDA receptor).

Corresponding author: Qinyi Zhao, China Rehabilitation Research Center (CRRC), Jiaowenbeilu No 10, Fengtai District, Beijing, China, Tel: 0086-10-68789113; E-mail: qinyizhao@gmail.com; qinyi_zhao@163.com

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If the stability of a virus protein is low, its conformation will be sensitive to protein denaturant and temperature. The prot--ein denaturant will greatly decrease the virulence of such virus. With the help of the immunity of the body, the disease will be cured rapidly. The principle is same as that of live-attenuated vaccine. For viruses, such as Coronavirus, which are unstable and sensitive to temperature, it could be expected some protein denaturants act effective cure for these diseases [8].

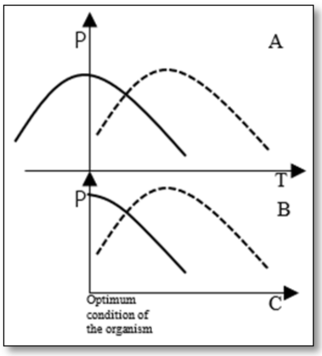

Figure 1. Diagram of two types of active conformation curve of proteins. T is temperature, C is concentration of protein denaturant. P is probability of active conformation of a protein and it also expresses the normalized activity of a protein (validated for receptors). The Coordinate origin represents the optimum condition of the body. The active conformation curves of GABA receptor (dotted line) and NMDA receptor (black line) along gradient of temperature are shown in **Figure 1A**. The active conformation curves of GABA and NMDA receptor along gradient of protein denaturant are shown in **Figure 1B**. Note: some parts of conformation curve of a protein in **Figure 1A** cannot be exhibited in **Figure 1B**.

Physiological responses to the protein denaturants

The introduction of protein denaturants into body could results in numerous changes of physiology for protein denaturants can alter functioning of all proteins. Some physiological alteration can be permitted. If the physiological alterations have great impact on the general functioning of the body, it could be observed as by-effects and toxicity.

At present, the effect of phenol (as contaminant of soil), volatile anesthetics, ethanol (food) on the cell biology has been extensively studied. The detailed properties vary from substance to substance, but the general profile is same e.g [9-16]. The general features of behaviors of the host in the presence of such substances can be expressed in **(Figure 2)**.

In **Figure 2**, the general physiological states of a cell in the presence of protein denaturant were drawn.

In the low concentrations of protein denaturant, a cell can remain its ability of growing. In this range, many types of signaling activity (functioning of proteins) has been altered, but all these alterations will not result in fatal damage to the essential functioning of a cell.

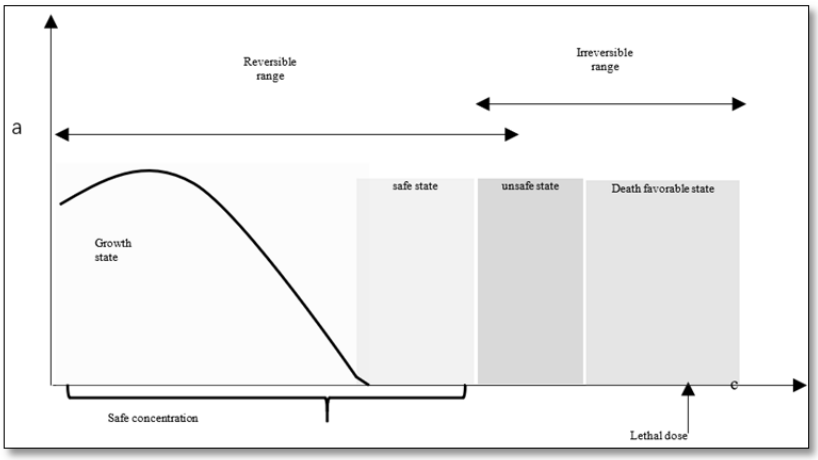

Figure 2. General profile of cell physiological states in the presence of protein denaturant. The a is growth rate, c is the concentration of protein denaturant. In reversible range, all physiological changes of an organism are reversible. In irreversible range, some physiological changes cannot revert into its original state when the protein denaturant is removed.

When the concentration of protein denaturant is increased, the cell will into a new state (safe state). In this range, the growth ability of cells is lost, dysfunctions of the cell appear, but the cell remains alive. The anesthesia is a representative state for these physiological states.

In unsafe state, although the cell remains alive, some functions of a cell (or organism) may be damaged forever.
When the concentration of protein denaturant increases further, the physiological homeostasis of the cell will be destroyed gradually, and cells will go to dying at different

times. The concept of lethal dose has been utilized to describe this property. If the protein denaturants are used disinfectant, the concentration of it would be much higher than that of lethal dose. For example, the phenol acts bacteriostatic at concentrations of 0.1%-1%.

Figure 2 also tells us that the protein denaturant shows great impact on physiological state of human at concentrations much lower than that when it acts disinfectant.

Thus, for anyone of protein denaturants, the safe concentrations can be found, and in this range, the protein denaturant has great impact on functioning of some specific proteins and stress signaling activities of a cell can be produced, but not on physiological homeostasis of the host's body [17]. In other words, it is potential to act medicine in this range of concentrations of protein denaturants.

Sensibilities of different proteins to denaturants

The sensibility of a protein to denaturant is logically related to its thermal stability. If a protein shows low thermal stability, its activity will be very sensitive to protein denaturant; if the stability of a protein is high, its activity will be insensitive to protein denaturant [5]. Normally, the regulatory receptors are sensitive to protein denaturants and their activities could be influenced by low concentration of protein denaturant. The constitutive enzymes have high thermal stability and the deactivation of these enzymes occurs at high concentration of protein denaturants and this phenomenon has nothing to do with model discussed in this manuscript. But some regulatory enzymes and virus protein are unstable, and this feature has been exploited by our method. The principles are shown in **Figure 3**.

In **Figure 3**, it can be seen that at applied concentrations of protein denaturant, the activity of most constitutive enzymes of host cell will not be altered. If stability of virus proteins (brown lines) is low, their activities will be decreased. The activities of some regulatory receptor or enzymes are also influenced, and some of these changes could be felt by nervous system of our body, then people can feel pain and etc.

Accompanied with deactivation of protein functions is the protein conformational change and alteration of protein thermal states. In view of structural biology, it includes protein conformational change within a protein molecule, the dissociation and reorganization of protein subunits, and etc.

Compared with protein stability, the stability of protein folding is rather low. The disturbance of protein folding will result in the aggregation of polypeptide [18-21]. Normally, the stability of protein folding is related to protein stability. Or in other word, a protein, which has low stability, will show low stability of protein folding. Perhaps the protein folding stability is tightly related to the antivirus effect of protein denaturant. As there is no enough experimental data, it will not be discussed here. If protein denaturants have value in clinical medicine, it must satisfy following criteria.

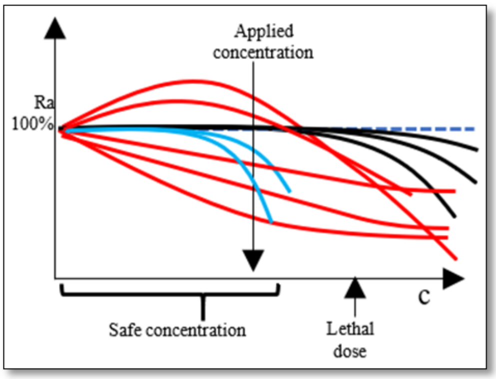

Figure 3. Sensibility of different proteins to protein denaturants.

The Ra represents normalized (or relative) activity of a protein, c is the concentration of protein denaturant. Five red lines represent activity curves of different regulatory proteins (enzymes). Three black lines represent activity curves of different constitutive proteins (enzymes). The brown lines represent activity curves of a protein (enzyme) with low thermal stability.

The effective concentration of a protein denaturant on protein deformation should be lower than concentration at lethal dose. If the toxicity of protein denaturants arises up from its ability of protein denaturation, the rightness of this opinion will be guaranteed. It can be approved by fact that the growth of a cell is inhibited at rather low concentration of protein denaturant [22].

The sensibility of different proteins to a protein denaturant should be different. Fortunately, the common knowledge of biochemistry tells us that is true.

Related biological phenomena and evidences

Recently, Dr Yuehua Li (Wuhan, China) has being used phenol to treat Coronavirus and it has induced serious debate in China. All Chinese scientists believe that his method is safe, but validity of his method cannot be approved by pharmacology [23]. He injects 0.5 ml solution (1 ml phenol and 2% Lidocaine 5 ml in 100 ml physiological saline) at 4 acupoints of the body. Our theory has provided theoretical foundation for his method. As Coronavirus is temperature sensitive, one or more proteins of Coronavirus was partly deactivated in the presence of phenol and thus the virulence of virus is decreased. In addition, the immunity of the body was activated in the presence of phenol (as Immune adjuvant) [24]. When the activity of Coronavirus proteins is decreased and immunity activity of the body is activated, the patient will recover from illness soon. Agreeing with this opinion, phenol acts inhibitor for many enzymes [25-27].

Based on the current available data, it is suggested that the intravenous injection of 0.5ml phenol in 100 ml physiological saline, or oral administration is helpful to treat Coronavirus and reduces the probability of patient to become critically ill. The method of Yuehua Li is also practically. In addition, the elevation of human temperature from 37 to 40°C for a time (see, 12 h) may be a good method to reduce the virulence of some virus. The bat is the natural host for many viruses, such as Coronavirus, Ebola virus. However, these viruses do not cause the disease in bat. The main reason is that the temperature of bat is high, and virulence of a virus is greatly decreased [28].

Safety of protein denaturants

When the principle has been established and effect of medicine has been approved, the next job is to assess the safety of medicine in vivo. For a special substance, the safety should be studied experimentally. But some results indicated that at least some protein denaturants can be safely utilized in vivo.

The fish can endure 0.3 mol urea for a longtime (at least one day), the ethanol concentration may be up to 500 mg/100 ml in blood (temulence concentration), or about 0.11 mol. It indicated that protein denaturants work at concentration higher than that of common drugs. The volatile general anesthetics, which are protein denaturants, has been widely utilized in clinic. Although the liver toxicity has been observed for ethanol and volatile anesthetics, the safety of these medicines is enough in view of clinical medicine [29-31].

The acute toxicity or LD50 of SDS (sodium dodecyl sulfate) is 1.2 g/kg by the oral route in mammals [32]. The long-term toxicity of SDS in rat has been reported [33]. At high concentration (5%) and of longtime administration, the SDS can result in the weakness of rats, but it is enough safe in view of clinical medicine. The toxicity of some surfactants has also been tested [34-37]. It indicated that living body can endure high concentration of some materials.

The concentration of volatile anesthetics in water is about 10 mmol in water [38]. It is higher than that of most medicines (in range of 1-100 µmol).

For human, the lethal dose of phenol is about 354 mg/kg (about 6 mmol, or 0.035%) [23]. If the toxicity of a protein denaturant is not related to its ability of protein denaturing, the toxicity of that protein denaturant will appear at rather low concentration. Fortunately, this phenomenon can be easily judged experimentally.

According to common knowledge of biochemistry, all protein denaturant, such as urea, phenol, volatile anesthetics (organic solvent with small weight), can freely enter a cell and cross the blood brain barrier of the body. The equilibrium state, in which the concentration of it at diversified parts of the body are same, can be rapidly reached. Some protein denaturant, such as volatile anesthetics, SDS, has impact on the physical state of cell membrane [39,40]. However, this effect can be ignored in low concentration of SDS, and thus it will not be discussed here. The protein denaturants can be routinely removed from the body by filtration in the kidney or evaporation in the lungs (phenol and volatile anesthetics).

Interaction between protein denaturant and specific medicines

When a protein conformational state is influenced by protein denaturants or temperature, the effect of medicines which target on this protein would be influenced. This phenomenon is called drug interaction. The underlying mechanism is that both substances, specific drug and protein denaturants, influence the thermal state or conformational state of a protein [4-6]. The interaction between volatile anesthetics (or urea) and other medicines has been reported [7,41,42]. Then it could be predicted that effect of specific medicine for virus treatment could be enhanced in the presence of protein denaturants.

Many substances are known protein denaturants, which include urea, SDS, metformin (biguanide), vanillin and other active ingredient of perfume and spice, organics solvents with small molecular weight, such as ethanol, chloroform, phenol, volatile anesthetics. In common sense, the plant polyphenol is not considered as protein denaturant, but polyphenol is indeed protein denaturants and it can inhibit enzyme activity at low concentration [43]. The plant polyphenols are active ingredient of many Chinese herbal medicines [44,45]. Most protein denaturants, such as derivative of phenol, Chlorophenol, have not been carefully studied. The effect of other protein denaturants, such as chloroform and volatile anesthetics, in virus treatment should also be studied in future.

As it has been discussed aforementioned, the interaction among polyphenols and protein denaturants in treatment of viral infection could be stressed in future [44,45]. In future, the standard methods could be established for treatment of viral infection although it may not be specific as traditional drugs.

Conclusions and Discussion

Thus, it could be concluded that the protein denaturants provide a new platform for the development of medicines to treat viral infection. But several problems must be resolved before it could be applied into practice. Although all protein denaturants are potential for medical application, the protein denaturants cannot be applied in clinic directly. As the effect of a protein denaturant in virus treatment is dependent on the sensibility of the virus to denaturant, the first work of us is to measure the sensibility of virus to protein denaturation and most sensitive denaturant could be firstly selected.

Second is the safety of protein denaturants. Although the toxicity of some protein denaturants has been well documented, most of them have not been carefully studied. Third is the species of protein denaturants. Till now, only typical protein denaturants have been studied. The none-typical protein denaturants should be studied in future.

It is reported that the Ebola virus is sensitive to Sodium deoxycholate, phenol, it indicates that this method could be applied to the treatment of infection of Ebola virus [46].

The Congo red is a none-typical protein denaturant, it is prone to bind to denatured protein and amyloid, and then it can shift the thermal balance of native and unfolded state of a protein [47,48]. It has been reported that Congo red have effect in treatment of some diseases [49,50].

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