

Vibration Characteristics of Misfolded Proteins and Their Consequences

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Abstract The functioning of native protein has been discussed briefly. Disruption in native protein with misfolding in its structure due to infection or otherwise has also been discussed. The physical characteristics of prion- a variety of protein responsible for number of both degenerative and non-degenerative diseases have been detailed here from the view point of protein vibration due to external stimuli. Attempts have been taken to focus on the effects of charge groups on the surface of both folded and misfolded protein. Suggestion for application of magnetic materials which may be able to create potential barrier in controlling the chain process of infectious prions and other similar proteins have also been discussed basing on the frequencies of vibration of protein and also on the vibration energy thus evaluated.

Keywords Folded Protein, Misfolded Protein, Protein Aggregation, Prion Protein, Amyloid Disease, EEG Rhythms

1. Brief Note on Protein Function

Modern understanding of how proteins function emerges since last 200 years of biochemical studies. Biochemical studies i.e. biochemistry deals with chemical processes in living organisms. It reveals from different experimental facts that most of the chemical reaction is pertaining to cells and their structural components are mediated by Proteins. In other words, it may be held that proteins are proper components for cell function. Proteins as Hermann Staudinger held are organized polymers, giant molecules made of small molecular constituents linked each other by chemical bonds in chain. These are very essential components of living body. While stable proteins render various services to living organism, unstable or infectious proteins carry factors to living organism in the form of attacking with a number of diseases. Before going to this content, it requires to review some basic components of

protein formation and its function.

From experimental evidences, it reveals that DNA is the vehicle of genetic information and it contains the information to make proteins. Scientists of the day are of the opinion that genetic information is transformed from a language of four letters (nucleotides) in DNA to a language system of twenty (amino acids) in proteins. The process of converting the information contained in nucleotides to amino acid using the genetic code is called translation. Ultimately translation helps in converting single dimension of the genetic code into three dimensional protein structures which is usually expressed in folded form. Thus all the information needed for a protein to fold into three dimensional conformations is contained in the amino acid sequence. This means the Physiochemical properties of the amino acid sequence help the proteins to fold into their correct minimal energy configuration. Proteins fold because of the fact that amino acids interact locally. This limits the conformational space that a protein has to explore and to follow a funnel like energy landscape. All these help the proteins to fold. In 1972, Anfinsen [1] held the view that native (natural) conformation of a protein occurs due to the fact that the particular shape is thermodynamically stable in the inter cellular environment. This means protein achieves the lowest energy state possible.

In a properly folded protein, hydrophobic amino acid residues are found together but shielding each other from water molecules while hydrophilic amino acid residues are exposed on the surface of the protein interacting with the water of cytoplasm. Big amino acids are found to make nooks and crannies for small ones –ultimately resulting in light folding of protein. This light folding of proteins minimizes the overall free energy of the protein.

Now the question is –what happens to a denatured protein? When the primary bonds that hold the protein with three dimensional structures are disrupted, the protein unfolds. However, after restoring the natural cellular conditions, Anfinsen observed that “enzyme’s amino acid structure refolded spontaneously into its original form”.

Again, the question arises what happens to a protein

when it becomes toxic. It is an established fact that protein functions in its native conformation and is known as alpha helix. It is a right handed spiral coil [2]. But an extensive conformational change occurs when a protein becomes toxic. Then the protein acquires a motif known as beta sheet. However, beta sheet conformation also exists in many functional native proteins. The transition from alpha helix to beta sheet is characteristic of amyloid deposits. Exposition of hydrophobic amino acid residues is found during abnormal conformational transition from alpha helix to beta sheet. This promotes protein aggregation.

2. Misfolded Proteins and Concerned Diseases

It is an established fact that misfolded protein results when a protein follows the wrong way in its folding process. This may be happened spontaneously. Most of the time, only native conformation of protein is produced in cell. Sometimes, a random event occurs during this processing of protein and one of these molecules follow the wrong path resulting into a toxic configuration. Remarkably, this toxic configuration is often able to interact with other native configuration of the same protein and catalyzes this native one into toxic state. This may continue as a chain and hence toxic activity is amplified. Thus the toxicity leads to a catastrophic effect that kills the cell or impairs its function. One of these classes of proteins is known as prion. A number of diseases both degenerative and non degenerative are due to misfolding of proteins. Some of these are [3] listed below:

Table 1. Names of some diseases along with major aggregating proteins

Proteopathy	Major aggregating protein
Alzheimer's disease	Amyloid β peptide (A β); Tau protein
Prion diseases (multiple)	Prion Protein
Parkinson's disease and other synucleinopathies (multiple)	α -Synuclein
Huntington's disease	Protein with tandem glutamine expansions
AH (heavy chain) amyloidosis	Immunoglobulin heavy chain
AA (secondary) amyloidosis	Amyloid A protein
Type II diabetes	Islet amyloid polypeptide
Sickle cell disease	Hemoglobin

Accumulation of misfolded proteins can cause neurodegenerative diseases commonly known as amyloid diseases. Very common of these are Alzheimer's diseases. These affect the adult population over sixty five years old. Parkinson's and Huntington's diseases have similar amyloid origins. This disease can be sporadic (occurring without any family history) or inherited. Whatever reasons may be behind the disease, some genetic factors leading to mutations are found to be associated with this disease. This means a single copy of a defective gene may develop the

disease in an individual. The genetics can be even more complicated in Alzheimer's disease and for other less common neurodegenerative disease. This is due to the fact that "different mutations of the same gene and combinations of these mutations may affect disease risk" [4].

It may also be held that protein aggregation diseases are not exclusive to CNS (Central Nervous System). These may also appear in peripheral tissues. They are called amyloidogenic diseases. These include type 2 diabetes, some form of atherosclerosis, hemodialysis related disorders, short chain amyloidosis and many others.

On the other hand, prions are responsible for a number of infectious diseases. These include Scrapie, Transmissible Spongiform encephalopathies (TSEs), bovine spongiform encephalopathy (mad cow disease), which can cause Creutzfeldt – Jakob disease in human and Kuru. Kuru was identified as infective in neurodegenerative disease in Fore tribe of the eastern highland of Papua New Guinea. Gajdusek et al [5] first showed that Kuru could be infective in Chimpanzees after inter cerebral inoculation with brain suspension from Kuru patients. As an example, the present chapter is concerned with misfolded prions- responsible for a number of amyloid diseases.

3. Methodology

3.1. Vibration Characteristic of Native and Misfolded Proteins

The characteristic and functions of some misfolded proteins responsible for both degenerative and infectious diseases have been explained by a good number of scholars including some Nobel laureates in physiology and medicines [6]. An attempt is taken here to examine these differently. In a paper [7], the authors explained the vibration characteristics of some native proteins responsible for recalling with the application of four basic types of ECG rhythms having amplitudes of 20 to 100 and 15 to 350 microvolt's and frequencies in the range of 0.5 to 30 cycles per second.

The authors [7] showed that the recalling of the learnt event depends on the number of frequencies of vibration in protein.

The problem of protein vibration responsible for memory was considered [7] in the light of the solution of the standard form of Schrodinger Equation.

Inserting the potential energy function $v = \frac{1}{2} kr^2$ in one dimensional Schrodinger Equation

$$\frac{-\hbar^2}{8\pi^2m} \frac{d^2\psi}{dr^2} + v(r)\psi = \epsilon\psi \quad (1)$$

which turns to

$$\frac{-\hbar^2}{8\pi^2m} \frac{d^2\psi}{dr^2} + \frac{1}{2}kr^2\psi = \epsilon\psi \quad (2)$$

The energies of the allowed vibration states derived from

the solutions of the Schrodinger's Equation are

$$E_{vib} = \left(v + \frac{1}{2}\right) \sqrt{\frac{k}{m}} \quad v = 0, 1, 2 \quad (3)$$

While the expression for vibration frequency stands as

$$\nu_{vib} = \frac{1}{2\pi} \left(v + \frac{1}{2}\right) \sqrt{\frac{k}{m}} \quad (4)$$

The vibrations occurring in protein depend on molecular weights of the concerning proteins available in particular portions of the brain. Thus, frequent vibrations, as the authors held, are created in light proteins of small molecular weight while slow vibrations are created in proteins of middle range and high molecular weights.

The simple expression for vibration energy is

$$E_{vib} = \frac{h}{2\pi} \sqrt{\frac{k}{m}} \quad (5)$$

Since the frequency of vibration as revealed from Eqn. (4) is inversely proportional to the square root of a protein's weight, the number of vibration will be less in middle range and high molecular weights in comparison to proteins of small molecular weights. However, energy spacing will be small in proteins of small molecular weight while reverse will be in case of middle range and high molecular weights. Thus the vibrations of three categories of proteins

pertaining to small, medium and high molecular weights are diagrammatically described in **Fig. 1**.

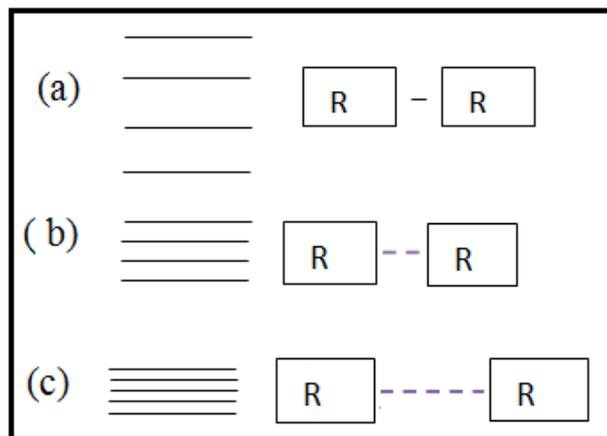


Figure 1. Vibrations of Protein shown in figure (a) high molecular weight protein, (b) medium range molecular weight protein, (c) low molecular weight protein.

Basing on these approaches the authors calculated vibration frequencies of some memory related proteins, the ID names and their corresponding molecular weight are shown in **Table 1**.

Table 2. ID names of some proteins and their molecular mass in S.I. Units.

ID Name of the Protein	Molecular mass in S.I. Units
CD40L/CD154	m= 16.9 Kilodalton
BCPM I	m= 19.98 Kilodalton
CSP 24	m= 24 Kilodalton (Phospo protein)
32 P04	m= 32 Kilodalton (Phospo protein)
Mspi 42	m= 42 Kilodalton (merzoite surface protein)
Cam-k	m= 54 Kilodalton
TAFI/DYT 3	m= 250 Kilodalton

Table 3. Vibration frequencies (m^{-1}) of some memory related proteins with molecular weights.

External electrical stimuli in microvolt	m= 16.9	m= 19.98	m= 24	m= 32	m= 42	m= 54	m= 250
20	0.71	0.67	0.60	0.52	0.46	0.40	0.18
25	0.81	0.74	0.68	0.58	0.51	0.45	0.25
50	1.14	1.05	0.96	0.83	0.72	0.63	0.29
100	1.60	1.49	1.34	1.16	1.01	0.90	0.41
150	1.93	1.83	1.63	1.42	1.24	1.06	0.50
250	2.23	2.37	2.15	1.86	1.62	1.43	0.66
350	3.03	2.67	2.55	2.20	1.92	1.69	0.79

From Table - 3 it reveals that frequencies of vibration in protein are in decreasing order with respect to ascending mass of proteins for constant amplitude of external electrical stimuli.

As an example, let us consider our approaches of protein vibration in the context of misfolded protein – responsible for a number of amyloid diseases.

Prions become infectious due to its conformational misfolding. For example, PrP^{sc} [8] may be registered as a conformationally misfolded form of a normal cell surface prion protein called PrP^c. Minute amount of PrP^{sc} replicates by conversion of host PrP^c during the time between infection and the appearance of the clinical symptoms, which in turn generates large amount of PrP^{sc} aggregates in the brain of the diseased individual. A good number of scholars [8] are now conducting experiments to reproduce this event in vitro. They have reported a process involving the cyclic application of misfolded protein which converts large amount of PrP^c rapidly into a protease resistant PrP^{sc} like form. This is found to be in parity to polymerase chain reaction when PrP^c is disrupted due to continued formation of PrP^{sc}. They noticed that after cyclic application, more than 97% of PrP^c present in the sample converts to PrP^{sc}. They also claimed the application of this method in diagnosing the presence of undetectable prion infectious agent in tissues and biological fluids.

Scholars [8] explained this basic principle in case of neurodegenerative diseases like Alzheimer, Parkinson and Transmissible Spongiform Encephalopathy (TSE) which are characterized by abnormal protein deposits. By using light scattering and non-denaturing gel electrophoresis, it revealed that infectivity and converting activity of PrP^c content packed markedly in PrP^c particles having molecular

masses in the range of (300-600) KD and of diameter 100 nm on average. However, it was also held by the scholars that non-fibrillar particles of PrP^c molecules of masses (14-28) KD and of (17-27) nm diameter may be considered as the effective starter (initiators) of TSE. This means non-fibrillar particles of PrP^c molecules of masses (14-28) KD when get infectious, turn to PrP^{sc} of masses approx. (300-600) KD and of diameter on average 100nm. This is due to the fact that infectious protein gets increased. Since the density of protein remains almost constant 1.37gm/cm³ for protein of mass < 20KD and for mass > 20KD, the density of protein remains constant at 1.5/gm⁻¹cm⁻³, the mass of infectious protein will eventually increase and volume will also get increased. This is in parity with above statement held by the scholars [8]. The whole process of infection may be considered as the phenomenon of genetic intervention of protein to protein.

4. Results and Discussion

Under the above crucial junctures, the main thrust of this paper is to examine the physical characteristics of PrP^c conversion into PrP^{sc} which is responsible for a number of degenerative diseases (including neuro) and TSE diseases from the view point of our hypothetical approach of protein vibration. Vibration frequencies and energies of prions with molecular masses (14-28) KD taking as native protein and of masses (300-600)KD taking as infectious (misfolded) protein have been calculated by using Eqn. (4) and Eqn. (5) with the same initial energy as used for some native proteins [7]. These are tabled in Table No (4) and Table No (5)

Table 4. Vibration frequencies (m⁻¹) of some prion proteins

External electrical stimuli in micro volt	Initiation form of prions		Infectious prions	
	m = 14KD	m = 28KD	m = 300KD	m = 600KD
20	0.79	0.56	0.16	0.12
25	0.88	0.62	0.18	0.13
50	1.25	0.88	0.26	0.18
100	1.78	1.25	0.38	0.26
150	2.18	1.54	0.46	0.33
250	2.80	1.99	0.60	0.42
350	3.34	2.35	0.72	0.50

Table 5. Vibration energy of some prions

Initial energy x10 ⁻²² joule(μv)	Final energy in the form of 10 ⁻²² joule			
	Initiation form of prions		Infectious prions	
	m = 14KD	m = 28KD	m = 300KD	m = 600KD
11(20)	0.04	0.03	0.01	0.01
13.75(25)	0.05	0.03	0.01	0.01
27.50(50)	0.07	0.05	0.02	0.02
55.00(100)	0.10	0.07	0.02	0.02
82.50(150)	0.13	0.09	0.02	0.02
137.50(250)	0.16	0.11	0.04	0.03
192.50(350)	0.20	0.14	0.04	0.03

The nature of vibration of infectious prions and native ones will be those as shown in Fig. 1(a), 1(b) and 1(c).

From Fig. 1 (a) it reveals that the misfolded protein aggregates in such a way that it does not respond to any external stimuli which means it remains almost inert with respect to external stimuli. This is the fact that Alzheimer's and Parkinson's patients cannot recall any incident even after few minutes of the incident occurs.

The frequencies of infectious prions are expected to take part in accelerating the chemical reaction concerned with infection processes. In this context; we may consider the role of charge distribution pattern of protein. It is an established fact that most of the proteins contain charged amino acids. Catabolic functioning or bindings of individual charges in active site has been identified by a number of Scholars. But till date it is not clear about assigning function to charge beyond this active region. This raises the question-whether manipulation of these charges can change the properties of protein. Irine Gitlin and others [9] held the view that it is possible to examine the role of charged residence on the surface of proteins of active region with the help of a combined method of "Capillary Electrophoresis and charged Ladder." These charge residues are found in the entire surface of native proteins. However, charge groups are also present in interior.

For conduction or induction we are to consider the passive depolarization of the membrane which helps in increasing sodium permeability and also in increasing the flow of sodium ions across the membrane. We are also to consider active depolarization of the membrane generating action potential and also local responses.

While charges are found to be distributed regularly on the surface and interior of native proteins, charges are likely to be distributed randomly on the surface of infectious (misfolded) proteins. Whatever, may be pattern of charge distribution, the magnitude of charges may be evaluated from the standard formula [10] like.

$$v = \frac{q}{4\pi\epsilon r} \quad (6)$$

$$q = 4\pi\epsilon r v$$

where v = measure of action potential

r = distance or dimension

ϵ = dielectric constant

Electrical energy concerning the above may be evaluated with the application of Eqn. (6)

$$E = \int_0^{ze} q dq$$

With $q = \frac{e}{4\pi\epsilon r}$ for an ion in a dielectric with no interfering ions. This becomes

$$E = \frac{1}{r} \int_{q=0}^{ze} \frac{q}{4\pi\epsilon} dq$$

$$= \frac{z^2}{2r} \left[\frac{e^2}{4\pi\epsilon} \right] \quad (7)$$

In a paper [11] Stigter and Dill derived a relation between

electrical free energy, net charge number, interaction factor and others as $g_e = kTWZ^2$ (8) for native and denatured protein where

g_e = electrical free energy

Z = net electric charge

T = temperature

k = Boltzman constant

W = interaction factor taken empirically from titration curve.

Now, one can determine the magnitude of electrical energy theoretically with the help of Eqn. (7) and also basing on practical activity concerning titration curve by using Eqn. (8). Whatever may be the magnitude of electrical energy, one could take into account the fact that electrical energy generated in proteins should always be greater than the vibration energy as evaluated above.

5. Conclusions

It is a fact that no successful treatment for any of the amyloid diseases is available at present. But the increasing knowledge of amyloid accumulation particularly in aged population is getting attention of the researchers. With expanding use of RNA interference (RNA) technology, therapeutic inhibition of precursor protein synthesis is within the reach of the scholars. Drugs like Chaperones are being tested. Attempts to prevent protein hyper phosphorylation with specific inhibitors are also in progress. In this regard, Scientists have more options to find common structures for the design of specific chemical inhibitors of aggregation. Vaccines against aggregates are also being developed [12].

The basic principle of all these pharmacological treatments is to minimize the misfolding of proteins and till to - date it is found that molecular chaperones help them in finding their native functional conformation. In 1987 John Ellis [13] named chaperone as a peer protein, which helps other protein to fold or to assemble into native ones or attempts to repair the protein complex. These are some of the standard therapeutic measures for treatment of amyloid diseases emerged out of misfolding or infectious proteins.

But the present paper focuses on alternative approach concerning the physical characteristics of amyloid diseases. Some approaches of physical treatment are proposed here when protein is misfolded. At this stage, volume gets extended and charged group spread over the whole of the surface. As a result, electric field also gets extended. The extended electric field should not be allowed to cross the potential barrier with the application of some kind of physical medicine. This means electric energy basing either on action potential (AP) generated due to external stimuli or electrostatic force or due to charge group in native protein should be always greater than the vibration energy as proposed thereof ($E_{AP} > E_v$).

On the other hand, it is also proposed that magnetic

materials like iron or cobalt may generate magnetic field which will in turn help in contracting the charged group of amino acids of misfolded (infectious) protein. This means infectious protein will not be allowed to undergo further misfolding pathway and hence disease may come under control. Our approach may be compatible with the present mode of treating Cancer by Chemotherapy – One of the main objectives of which is not to allow the infectious protein to follow the misfolding pathway further and at the same time it attempts to destroy the infections cells too.

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