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Review

Understanding protein folding from globular to amyloid state Aggregation: Darker side of protein

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ABSTRACT

Folding and unfolding are crucial ways of modulating biological activity and targeting proteins to different cellular locations. In the living system, protein folding occurs in a very crowded environment, often assisted with helper proteins. In some cases this pathway can go off beam and the protein can either misfold or aggregate or form structures of elongated-unbranched morphology known as amyloid fibrils. Protein folding is not just an academic matter. Recombinant biotechnology and pharmaceutical industries are some of the fields where both theoretical and practical knowledge of protein folding is required. Misfolded protein and amyloid fibrils that escape the cellular quality control check are the basic reason of a number of increasingly widespread neurodegenerative diseases such as Alzheimer's and variant Creutzfeldt-Jakob *etc.* Thus, protein folding study also emerges as an interesting area in the field of biomedical research. This review deals with basic concepts related to protein folding and misfolding forming toxic aggregates and amyloid fibrils as well as disease associated with them. A more practical approach will be revealed to the early diagnosis of aggregation-prone diseases and amyloid states and their balanced therapeutics.

Contents

Abbreviations: ANS, 8-anilino-1-naphthalene-sulphonic acid; MG, molten globule.

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1. Introduction

Protein folding is a spontaneous process under suitable physiological conditions and is determined mainly by its amino acid sequence. Understanding this complex process will therefore provide a unique insight into the way in which evolutionary selection has influenced the properties of a molecular system for functional advantage. The tendency of a polypeptide chain with proper primary structure to fold into its native form, without external help, completes the vital link in the series leading to the expression of genetic information. Once the native form is achieved, the intrinsically flexible, irregular polymer chain folds into a more compact form with the specific structure required for its biological activity. Folding of protein into their condensed three dimensional form is the most basic and universal example of self-assembly. Although it has long been known that the amino acid sequence in one way or the other dictates the biological active conformation of protein, but the tools required to detect the intermediate states along the folding pathway are accessible in the past two decades [1]. These tools are revealing a tightly regulated pathway where multiple factors guide nascent polypeptide to select the correct shape from a large number of possibilities. Revealing the process through which protein folding takes place is one of the grand challenge of modern science [1]. There are strict quality-control checks that come into play if the folding process fails, ensuring that the misfolded products are targeted for destruction before they cause any harm [2]. Those that escape this cellular scrutiny are prone to forming aggregates that can damage or kill cells through various mechanisms.

Previously our lab has published a review article describing some of the consequences of misfolding and aggregation particularly in the context of neurodegenerative and nonneurodegenerative conformational diseases [2]. However, this present review article deals with the advances in the field of protein folding like structural and morphological analysis of amyloids, mechanism of aggregates formation, use of computers for the analysis of primary sequence of protein, advances in protein folding and aggregation and mechanism of aggregate toxicity. In our previous review more emphasis was given on disease and this review provides detailed mechanism about protein folding and misfolding leading to aggregation and amyloid formation. This article provides a more practical approach to the early diagnosis of protein misfolding and aggregation-prone diseases and their balanced therapeutics.

2. History of protein folding

The protein folding problem was clearly recognized more than a half century ago in the works carried out by Anson and Mirsky [3], who observed that denaturation is a reversible process. Mirsky and Pauling further hypothesized that native protein has a characteristic structure that is abolished upon denaturation [4]. These observations end up ultimately in the experimental work carried out by Anfinsen and co-workers, who showed that reduced and denatured ribonuclease will renature spontaneously *in vitro* (Fig. 1), with full restoration of enzymatic activity and return of its four native disulfide bridges [5]. He showed that a globular protein is

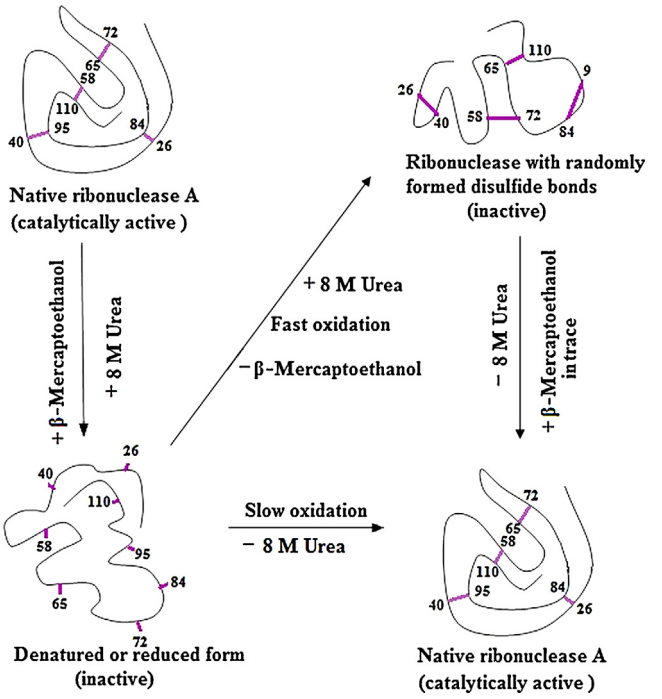

Fig. 1. Anfinsen's experiment on the folding behaviour of ribonuclease *in vitro*. This experiment revealed that protein folds spontaneously and reversibly into their native conformation.

capable of spontaneous folding *in vitro* which totally depends on its amino acid sequence in the given environment [5]. This experiment showed that if a protein chain is not modified to a larger extent after initial *in vivo* folding, then, it renature spontaneously and restores its activity and specificity after solvent normalization. This renaturation process requires a careful selection of experimental conditions; otherwise, aggregation can prevent the protein chains from folding. This observation has led to the present day view that protein tertiary structure is dictated by amino acid sequence, although molecular chaperones may influence the folding kinetics in some cases [6]. Moreover, it was established that the protein chain synthesized chemically, without any cell or ribosome, and placed in the proper ambient conditions, folds into a biologically active protein [7]. Protein folding *in vitro* is the simplest case of pure self-organization. Here no biological molecule is involved for normal folding of polypeptide chain. Further, in 1968, Cyrus Levinthal concluded that random searches are not an effective way to find the correct folded state of a protein [8]. This contradiction later got famous as "Levinthal's paradox". Since then, computational and theoretical advances have aimed to shed some light on the protein folding problem and have complemented experiments by elucidating some of the folding mechanisms at atomic detail. For answering "Levinthal's paradox", he himself proposed that protein itself cannot fold on the basis of random search and there must be some explicit pathway for proper folding of protein. This ultimately results in the establishment of a number of models describing the process of protein folding. Small protein molecules having buried