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Protein folding: Requirement for simulations on the basis of sequential growth of polypeptides

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ABSTRACT

To solve the problem of protein folding, denaturation-renaturation experiments on native proteins and a variety of theoretical-computational simulations of full-length polypeptide chains have usually been used as convenient *in vitro* models for the past several decades. However, there is a lot of irrefutable evidence that the information contained in the primary structure of a protein about its spatial structure is realized in the cells during the residue-by-residue elongation on the ribosome from the N- to the C-terminus. On this basis therefore, simulations of the folding and formation of the native spatial structure of proteins will be of requirement.

KEYWORDS

Protein folding;
Cotranslational folding;
Residual structure;
Protein elasticity.

INTRODUCTION

The protein folding problem, i.e. how a protein molecule acquires the native spatial structure on the basis of genetically determined primary amino acid sequence following its synthesis on the ribosome is recognized a major unsolved problem of biochemistry, cellular and physical-chemical biology[1,2]. To solve the problem, experimental studies of denaturation and renaturation of native proteins and a variety of theoretical-computational simulations of polypeptide chains have usually been used as convenient *in vitro* models for the past several decades. These traditional *in vitro* approaches present protein folding as a spontaneous process during which the formation of the native spatial structure of proteins occurs from an unfolded random coil state[1-3]. I.e., unfolded full-length polypeptide chains are considered the object for study to explore the mechanism of protein folding following their synthesis on the ribosome. This approach has been legitimated based on the results of the classical denaturation-renaturation experiments on small single-domain proteins[4], and especially on bovine pancreatic ribonuclease[5-7], obtained by the early 1970's. According to the results, proteins unfold and adopt a random coil state under strongly denaturing conditions with no residual ordered structure being present, and moreover some denatured proteins restore their initial state spontaneously after the removal of the

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denaturing action[4-7].

As early as in the classical denaturation experiments, a slight perceptible indication of regular structures was detected in some proteins under strongly denaturing conditions[4]. Since then evidence had been accumulated in a great body of new precise experiments that the denatured state of proteins is far from a random chain. At present, it is doubtless that almost all proteins, including those examined in the classical denaturation experiments, do not unfold fully and attain a random coil state under any, even strongly denaturing conditions, but contain a considerable amount of residual folded structures[8]. This fact casts doubt on the basis of evidence of the utilization of unfolded random coil polypeptides in studies of the protein folding process. If the same fact is taken into account, the capacity of some denatured proteins for spontaneous renaturation has a plain explanation, too, as a manifestation of the physical properties of proteins *per se* (most likely, the property of rubber-like elasticity in particular) caused by the residual folded structure present[9]. The residual structural elements might really be initiating centers of renaturation where the regeneration of the initial native spatial structure of protein begins, because a native protein is used initially in the denaturation-renaturation experiments.

Since the early 1960's, abundant convincing evidence has accumulated that proteins fold cotranslationally during the synthesis on the ribosome or during the translocation of nascent polypeptides into the cellular compartments across the membranes. First, there is a large number of observations, both *in vivo* and on model systems, that polypeptides synthesized on the ribosome exhibit the enzymatic activity, bind conformational antibodies, cofactors or ligands specific for the corresponding mature proteins, and form correct intra- and interchain disulfide bonds and protease-resistant compact structures[9], (for references). Second, such processes in cells as the binding of the signal recognition particles to the N-terminal signal sequences and of chaperones to the specific sequences of most of newly synthesizing polypeptides in the cytoplasm, as well as the splitting of the signal sequences of nascent polypeptides by the signal peptidases in the cellular compartments occur when these sequences are properly folded during the synthesis and translocation from the ribosome or during the translocation into the compartments across the membranes[10]. And finally, numerous data are available on the rates of synthesis of polypeptide chains on the ribosome in the cells and the formation of secondary structure elements (α-helices, β-sheets, and turns) and compact states in unfolded polypeptides[9], (for references). If these data are compared, one become clear that the elongation of a polypeptide chain on the ribosome by each of its individual amino-acid residue occurs much more slowly (the minimal time required is no less than 10^{-2}s) than the secondary structure elements and a compact structure are formed in an unfolded polypeptide (10^{-7}-10^{-4}s and 10^{-5}-10^{-3}s, respectively). Consequently, during the elongation of a polypeptide chain by each of its residue on the ribosome either the secondary and compact structures should be formed in the cytoplasm of the N-terminal part of the chain that has emerged from the ribosome. This is a sound deductive reasoning for the sequential folding of proteins during the residue-by-residue elongation on the ribosome from the N- to the C-terminus, which also allows one to propose a tentative folding model of proteins[9].

Indubitably, all of the information needed to establish the native secondary and spatial structures of proteins is resided in their genetically determined primary linear amino acid sequence[5,7]. The problem of protein folding is to clarify how this information is realized to determine the native structure of a protein molecule following its synthesis on the ribosome, and to predict the native structure from the known amino acid sequence on this basis. An inference of this minireview is that the utilization of full-length polypeptide chains and their unfolded states evidently seems hypothetical to solve the problem of protein folding. Meanwhile, there is a lot of irrefutable evidence that the information contained in the primary structure of a protein about its spatial structure is realized in the cells during the synthesis on the ribosome, and numerous data available are a sound argument for the sequential folding of polypeptides gradually during the residue-by-residue elongation from the N- to the C-terminus. Thus, there are many reasons and prerequisites for the simulations of both the folding and formation of the native conformation of proteins on the basis of the conception of gradual formation of the native structure during the residue-by-resi-

due elongation of polypeptide chain.

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