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Hypothesis

The 'molten globule' state is involved in the translocation of proteins across membranes?

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Strong evidence exists that the translocation of proteins across a variety of membranes involves a non-native or denatured conformational states. On the other hand a compact state having secondary but not rigid tertiary structure and called the 'molten globule' state has been identified as being stable under mild denaturing conditions. A similar state has been shown to accumulate on the folding pathway of globular proteins. These states are compact though sufficiently expanded to include water, and they are internally mobile. It is proposed that these molten globule states may be suitable candidates for protein translocation across biological membranes.

Protein folding; Molten globule state; Protein translocation; Membrane translation; Deraturation

1. INTRODUCTION

It is now well established that proteins can be translocated across membranes by a post-translational process. This has been shown in some cases even for the endoplasmic reticulum of eukaryotes (for which co-translational translocation was originally proposed [1,2]) as well as for bacterial, mitochondrial, glyoxysomal and chloroplast membranes (for references see [2,3]). This raised the 'seemingly impossible problem of how a fully folded soluble protein can pass the permeability barrier of a lipid bilayer' [3].

We shall review in this paper some of the evidence that protein translocation demands a non-native (denatured) state of the protein molecule. We shall suggest also that the involvement of a denatured state in translocation does not imply that the proteins are completely or essentially [4] unfolded. We shall argue that the involvement of a compact but flexible 'molten globule' state is consistent with the observed phenomena.

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2. DENATURATION IS REQUIRED FOR TRANSLOCATION

There are three types of evidence pointing to the fact that denaturation is an obligatory prerequisite for transfer of proteins across membranes.

2.1. *Stabilization inhibits translocation*

Fusion of a mitochondrial signal peptide to dihydrofolate reductase (DHFR) allows import of this enzyme into mitochondria. Translocation is however inhibited by methotrexate or other folate analogs which, when bound, stabilize DHFR against proteolytic digestion [5]. Moreover the destabilization of this protein by prior urea denaturation, point mutation or membrane bind-

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ing accelerates dramatically its import into mitochondria [6–9]. Cytochrome *c* and the precursors of cytochrome c_1 and b_2 are imported into mitochondria in their apo forms which have no rigid tertiary structure, while the binding of haem to cytochrome *c* inhibits its translocation [10]. The wild-type precursor of maltose-binding protein of *E. coli*, which is sensitive to proteolysis, is exported rapidly and completely in vivo while a mutant that is resistant to proteolysis is exported only slowly and incompletely [11].

2.2. *Non-native proteins are translocated*

The interruption of biosynthesis of dihydrofolate reductase (fused with a mitochondrial N-terminal import signal) produces a protein which lacks its C-terminal residues. This protein is sensitive to proteases and cannot bind methotrexate, i.e. it is incompletely folded. It is translocated through a mitochondrial membrane even without added ATP [12]. The precursor of β-lactamase immediately after biosynthesis is not completely folded, being bound to a protein Gro/EL which binds denatured proteins and can be translocated [13]. When the protein subsequently folds it is no longer bound by Gro/EL or susceptible to translocation.

2.3. *Proteins transiently bound to membranes have non-native structure*

Precursors of F_1-ATPase β-subunits and of cytochrome c_1 are translocated across the mitochondrial membranes and can be trapped at the state where the N-terminus penetrates the matrix while much of the protein remains on the outer surface. This part of the protein is susceptible to proteolysis [14]. Similarly, during secretion of β-lactamase into the periplasm of *E. coli*, proteins that are transiently bound to the periplasmic side of the inner membrane are sensitive to proteolysis. On release into the periplasm the molecules are transformed into an active protease-resistant structure [15].

The translocation of dihydrofolate reductase fused with a mitochondrial import signal [5] has been dissected into two stages the second of which is ATP dependent. In the first step attachment to the membrane, which can be inhibited by methotrexate, occurs in an at least partly unfolded form (as it is sensitive to proteolysis) [5,6].

In addition to this direct evidence that denaturation is required for translocation there is also some more indirect evidence.

2.4. *Denaturation facilitates binding to native and model membranes*

The major portion of the diphtheria toxin molecule can be transformed at low pH to a denatured state [16,17] which retains a large proportion of the native secondary structure [16]. In this state the protein has an increased surface hydrophobicity [16,18] and is bound to detergent micelles [16,19]. Bovine α-lactalbumin binds to phospholipid vesicles at low pH (i.e. in the denatured state) with part of the molecule inserted into a lipid bilayer [20]. Recently Harter et al. [21] reported that many proteins (including the water soluble domain of hemagglutinin, ovalbumin, carbonic anhydrase, etc.) bind to liposomes at acid pH.

2.5. *Non-matured proteins are translocated*

Proteins are translocated before their leader sequences are cleaved off. The existence of leader sequences may delay the formation of protein native structure [22]. The last statement receives indirect support from the fact that protein disulphide isomerase (which catalyses the formation of the active protein with the correct disulphide bonds) is located at the *trans* side of the endoplasmic membrane [23]. It is well known that many proteins cannot achieve their native structures without the formation of native disulphide bonds.

3. THE MOLTEN GLOBULE STATE AS THE DENATURED STATE REQUIRED FOR TRANSLOCATION

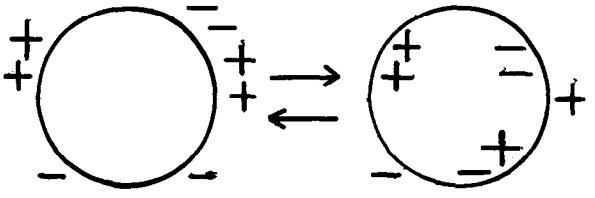
3.1. *Nature of molten globule states*

Denatured proteins possess the completely unordered structure of a statistical coil only under certain extreme conditions. More usually denatured proteins can be described as being 'partly unfolded' [24]. The most interesting examples of such 'partly unfolded' states are the 'molten globule' states which have been observed for a number of proteins [25–33] at low pH, high temperatures, etc. These states can be slightly [25–27,34] or more [35] expanded as compared with the native state but all of them are much more compact than the statistical coil and possess native-

like secondary structure. However they lack rigid tertiary structure as indicated by circular dichroism of aromatic groups (fluorescence and circular dichroism) and NMR spectra. Especially important is the fact that molten globule states are mobile and contain water inside the globule. The increased mobility of the molten globule state has been confirmed by NMR [27,36] and deuterium exchange [25–28]. The existence of water inside the molten globule (suggested by the increase of its volume) has been predicted theoretically [37] and confirmed by the measurements of its partial specific volume and compressibility (Kharakoz, D.P. and Bychkova, V.E., unpublished). Molten globule states have been described also as *kinetic intermediates* which accumulate during folding of the protein from the completely unfolded state [32,36,38,39]. Their properties are similar to those of the equilibrium molten globule states. There are examples (see e.g. [33,40]) that even proteins which do not exhibit the *equilibrium* molten globule state still fold through a molten globule as a kinetical intermediate.

3.2. *The molten globule as a translocating species*

Based on the above findings concerning translocation and the near native conformational states of proteins, the following hypothesis is proposed. The combination of mobility and of internal water capable of solvating polar groups allows the possibility of equilibria of the following type occurring within the molten globule state:



charges exposed charges sheltered

The protein molecule in the molten globule state can thereby accommodate both to polar and to non-polar environments. This may permit proteins in this state to interact with lipid-water interfaces more readily than in the native state thus explaining the increased membrane binding at low pH [20,21]. Moreover the second species, with a less polar surface would be translocated through the bilayer with a small free energy of activation. It is proposed therefore that a protein in the molten globule state may readily be translocated across a membrane.

4. CONCLUSION

It is quite possible that non-native protein conformations are involved not only in transmembrane translocation but also in other events within a living cell. (i) A nascent protein chain may have a non-native stage which can be stabilized by binding to Gro/EL protein [13]. (ii) Heat-shock and other stress proteins bind non-active protein subunits preventing them from aggregating until the quaternary structure is formed [41–45]. The same proteins (as well as Gro/EL in procaryotes) can be involved in transmembrane translocation of proteins [46,47]. (iii) Protein degradation, at acid pH [48,49] or in the ubiquitin system [49,50], may demand the presence or formation of non-native states. It is possible that in some of these cases also the non-native state of the protein may be the molten globule state.

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