35 Mathematical modelling of posttranslational protein translocation

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Introduction

Many proteins are transported posttranslationally across the endoplasmic reticulum (ER) membrane. Studies in yeast have shown that posttranslational translocation requires a seven-component membrane protein complex, the Sec complex, as well as a soluble lumenal protein, called BiP. The Sec complex binds to the signal sequence of the translocation substrate, but the subsequent movement of the polypeptide through the channel requires the additional presence of BiP which is a member of the Hsp70 family of ATPases. BiP may provide the driving force for posttranslational translocation by acting as a molecular ratchet [1]. Using purified components, it was shown that multiple BiP molecules associate with the translocation substrate prepro-α-factor during its translocation through the channel. Binding required the prior interaction of BiP with the J-domain of Sec63p. Once bound to the substrate, BiP minimized passive backwards movement of the polypeptide through the channel. A Brownian ratchet, in which forward movement is caused by passive diffusion, seems to be sufficient to achieve translocation [1]. These data did not exclude, however, that BiP also actively promotes forward movements, for example, by "pulling" on the incoming polypeptide chain, in addition to serving as a molecular ratchet.

Several attempts have been made previously to describe the ratcheting process in mathematical terms. The first model did not specify the nature of the ratcheting molecules and assumed a steady state situation in which a polypeptide chain moves with a constant rate through the channel [2]. This model was recently extended for the case of mitochondrial protein import [3].

The recent progress on the ER system offers new possibilities for mathematical

modelling. Several parameters of the ATPase cycle of BiP have been determined experimentally [4], and actual translocation rates, without the complicating effect of the preceding binding step, have been measured in a soluble system that contains only purified components [1]. In addition, the number of BiP molecules associated with fully translocated chains and the rate of backsliding of a polypeptide chain through the channel have been determined [1]. All these data correspond to non-steady state conditions and the previous mathematical models are therefore not applicable.

Model assumptions and equations

We consider protein translocation as a stochastic process consisting of various elementary steps. Specifically the following assumptions are made: (a) the substrate is described as a chain C of L discrete and equal segments which move stepwise with respect to the membrane; (b) the chain is initially bound to the cytoplasmic side of the channel through its signal sequence and is not permitted to dissociate into the cytoplasm; (c) within the channel a diffusion of the chain C occurs but subjected to a constant free energy gradient; (d) each segment can be occupied by a BiP molecule but BiP binding occurs only at a segment next to the membrane, (e) BiP molecules may dissociate either immediately after binding or at a later stage of the translocation process, and f) substrate molecules released from the channel at the lumenal side cannot rebind to the channel.

During translocation a protein molecule may attain different states σ_i which are specified by the number l of segments already translocated to the lumen and by the segments occupied by BiP molecules. The class of all states may be subdivided into two subclasses, first, the states σ_B corresponding to chains bound to the channel and second, the states σ_R where the whole chain has been released to the lumen. This subdivision is illustrated in the following Scheme for two states each of length $L = 10$:

σ_B : $\underline{0}\,\underline{0}\,\underline{0} \mid \underline{1}\,\underline{0}\,\underline{1}\,\underline{0}\,\underline{0}\,\underline{0}\,\underline{1}$	σ_R : $\underline{1}\,\underline{1}\,\underline{1}\,\underline{0}\,\underline{1}\,\underline{0}\,\underline{0}\,\underline{1}\,\underline{0}\,\underline{0}$
bound state	released state
$l = 7$ segments translocated	$l = L = 10$ segments translocated.

In this scheme the vertical line represents the position of the membrane, the "1" denote segments occupied by a BiP molecule and the "0" empty segments. Note that segments on the left side of the membrane are always empty. The totel number S of states is $S = 3 \cdot 2^L - 1$ with $S_B = 2^{L+1} - 1$ states in subclass σ_B and $S_R = 2^L$ states in subclass σ_R.

We describe the time dependent change of the probability $P_i(t)$ for finding at

time t the protein in state σ_i by a master equation:

$$\frac{dP_i(t)}{dt} = \sum_{j=1}^{S} w_{ij} P_j(t) - \sum_{j=1}^{S} w_{ji} P_i(t) \tag{35.1}$$

where w_{ij} denotes the rate for transition from state σ_j to state σ_i. For specifying these rates we take into account that, under the given model assumptions, there are five different types of elementary processes affecting the probabilities P_i: (i) inward chain movement, (ii) outward chain movement, (iii) binding of a BiP molecule to the translocation substrate, (iv) irreversible dissociation of BiP molecules, and (v) release of the translocated chain into the lumen of the ER. In eq. 35.1 the transition rates w_{ij} are unequal to zero if and only if state σ_i can be reached from state σ_j by one of the transitions mentioned above. The w_{ij} are expressed in terms of the following rate constants s_+ (1), s_- (2), u (3), z (4), and r (5) depending on the type of the transition (numbers given in brackets). The ratio s_+/s_- reflects the free energy difference $\Delta F = -RT \cdot \ln(s_+/s_-)$ associated with one diffusion step. ΔF may result, for example, by conformational entropy contributions and energy differences between folded and unfolded chains.

Summation over all states with a given value of l yields the probability $p_L(l,t)$ for finding chains where l segments are on the lumenal side. Similarly, an appropriate summation yields the probability $p_N(n,t)$ for finding a chain with n bound BiP molecules.

The differential equation system (eq. 35.1) was solved numerically by using a 4^{th} order Runge-Kutta algorithm. As the number of states increases strongly with the number of segments we treated only cases with $L \leq 10$.

Results

The model is tested first by simulating backsliding experiments. In these experiments, a substrate with a bulky chemical group at its C-terminus is first imported into proteoliposomes containing lumenal BiP and ATP, resulting in a stalled molecule with the bulky group abutting the cytoplasmic end of the channel. Addition of protease results in a characteristic fragment that corresponds to the piece of the polypeptide chain inside the vesicles. When ATP is depleted, the polypeptide chain slides backwards, resulting in the loss of the characteristic fragment.

To model this experiment, we assumed that at the beginning all substrate molecules have no segment exposed to the cytoplasm and that no further BiP binding occurs ($u = 0$). With $z = 0.2\ \text{min}^{-1}$ and $s_+ = s_- = 8.13\ \text{min}^{-1}$, the experimental data for backsliding can be reasonably well described. The best z-value is slightly lower than the one determined in experiments for the dissociation of BiP-ADP from a synthetic peptide ($0.5\ \text{min}^{-1}$) [4]. Our results show that the rate of backsliding is essentially determined by the rate at which BiP molecules dissociate from the substrate.

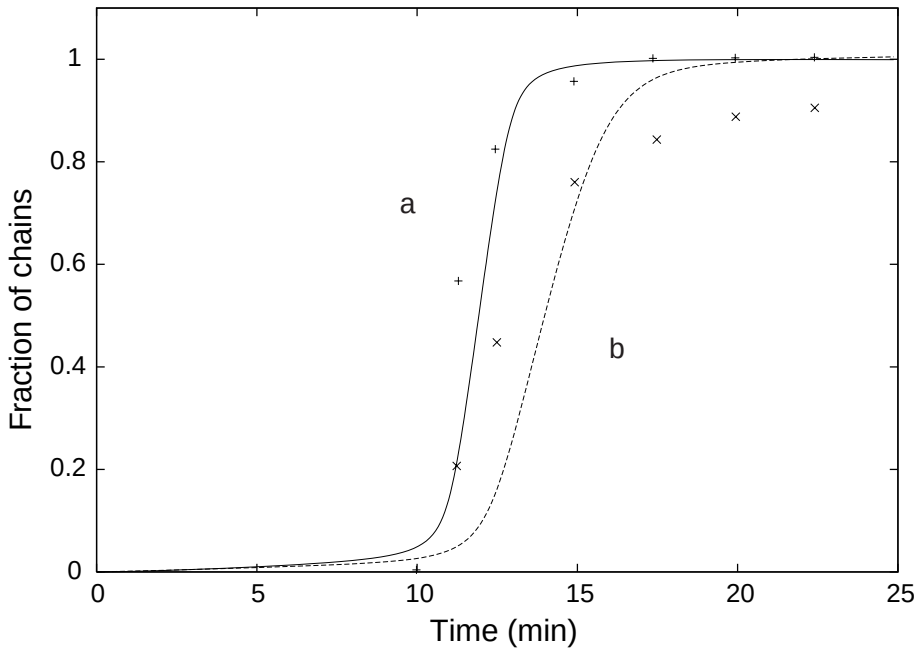

Fig. 35.1 Fraction of translocated protein substrates as function of time (for explanation see text); Experimental data from [1]. The time axis is rescaled by a factor $5/8$ to allow for $L = 10$ instead of $L = 16$

We next tested whether the model can describe data in which the translocation of a substrate through the channel was followed in a soluble system. In these experiments, a soluble complex of substrate and channel was first generated, and active translocation was started by the addition of BiP and ATP. Molecules that were no longer associated with the channel were scored as fully translocated. For modelling, we used as the starting condition that for $t = 0$ all substrate molecules have no segment on the lumenal side of the membrane. BiP and ATP are added at $t = 10$ min. Curve (a) in Fig. 35.1 represents the total fraction of translocated proteins as a function of time (experimental data: +) and curve (b) that fraction of proteins which is translocated and carrying no BiP molecules (experimental data: $\times$). It is seen that the simulations are in a fairly good agreement with the experimental data from [1]. In these simulations it is assumed that $s_+ = r = 6.0\ \text{min}^{-1}$, $s_- = 11.0\ \text{min}^{-1}$, $u = 50\ \text{min}^{-1}$, and $z = 1.0\ \text{min}^{-1}$. Modelling of the forward translocation reaction demonstrates that the substrate, when on the cytoplasmic side, must undergo significant unfolding or unstripping before it can slide through the channel. In addition, the estimated sliding rate constants indicate contact of the chain with the pore of the channel. Finally, our date show that polypeptide chains appear in the ER lumen in a synchronous manner, and that only short time is required for dissociation of all BiP molecules, allowing the substrate to continue with folding and modification reactions.

References

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