BIOCHEMICAL SOCIETY SUMMER STUDENTSHIP REPORT 20007

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Aim of the project

Bacteria post-translational translocation of secretary proteins employs the membrane protein complex SecYEG and a cytosolic ATPase SecA. SecA drives the pre-protein across the SecYEG channel within the membrane by coupling it to ATP hydrolysis (1). In this project, we aimed to gain a further understanding of the regulation of SecA during protein translocation.

Description of work and Assessment of results

Over-expression and purification:

SecA was over-expressed and purified in preparation for a kinetic analysis. To over-express SecA protein, *E. coli* BL21 host cells containing plasmid pT7SecA2 were grown at 37°C. After an induction by IPTG, cells were harvested, and lysed. Centrifugation removes cell debris, while the supernatant, containing SecA was loaded onto a Ni column. Further purification involved ion exchange and gel filtration chromatography. Finally, SecA was concentrated and stored at -80 °C.

Analysis of the pre-steady state ATP turnover:

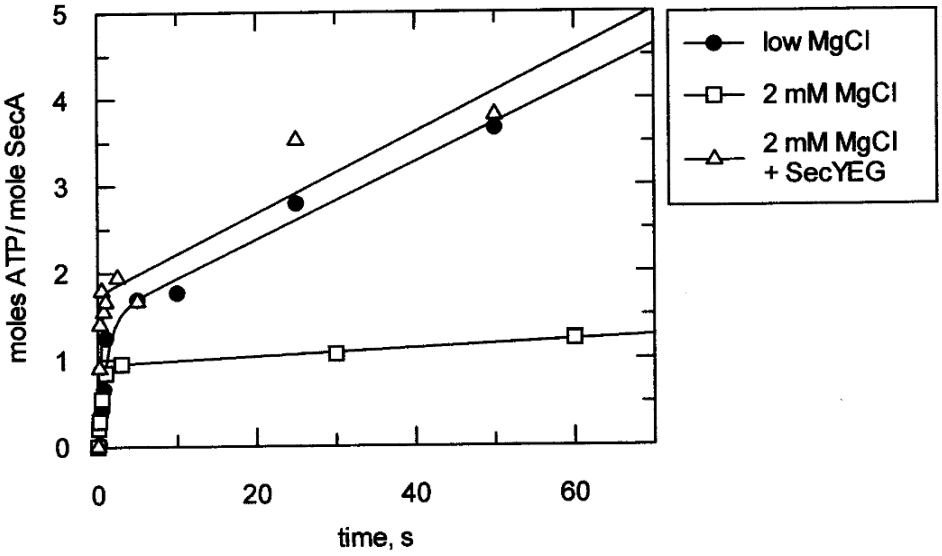
It has been shown that magnesium has an effect on the steady state ATPase activity of SecA (2). With a low concentration of Mg, SecA shows an increase in ATPase rate by 30 times compared with high concentrations of magnesium. A further addition of SecYEG gives another stimulation of the ATPase activity (3). Quenched flow experiments were performed with the aim to examine this effect on enzyme ATP hydrolysis in the pre-steady state.

The results are shown in Figure1. Experiments were compared with low magnesium, high magnesium and high magnesium plus SecYEG. We concluded that high magnesium slows the steady state rate of ATP hydrolysis by a factor of 10. However, addition of SecYEG can reverse it back to a faster rate. The rate in the pre-steady state changed only by a factor of two, which might be less significant.

Analysis of the signal sequence-SecYEG interaction and its effect on protein translocation:

Translocation assays have also been performed to study the interaction of a signal sequence peptide LamB with the SecYEG channel. Peptides of various concentrations were incubated in the presence of proOmpA, SecA, ATP and proteoliposome containing SecYEG. Translocation was conducted at 37°C for 15 minutes. It was followed by a treatment of protease, which digested the substrates left outside the proteoliposome but not those translocated. Western blot and immuno-fluorescence were then carried out to visualise the amount of proOmpA translocated.

The results (Figure 2) show that increasing the concentration of LamB results in a corresponding decrease in the amount of proOmpA translocated. It indicated that the peptide acts as a competitor to the signal sequence of proOmpA, therefore binding to the substrate-binding site of SecYEG. This is important information as the peptide will now be employed in a series of additional biophysical experiments.



Parameter	Value	Std. Error
Initial value	0.9377	0.0312
Rate constant	1.9434	0.1766
m	0.0047	0.0008

Parameter	Value	Std. Error
Initial value	1.4797	0.1686
Rate constant	0.9030	0.2267
m	0.0448	0.0058

Parameter	Value	Std. Error
Initial value	1.7400	0.1747
Rate constant	4.5480	1.7342
m	0.0469	0.0072

Figure 1. ATPase activity of SecA with low/ high concentration of $MgCl_2$
m =steady state rate constant
Rate constant= pre-steady state rate constant

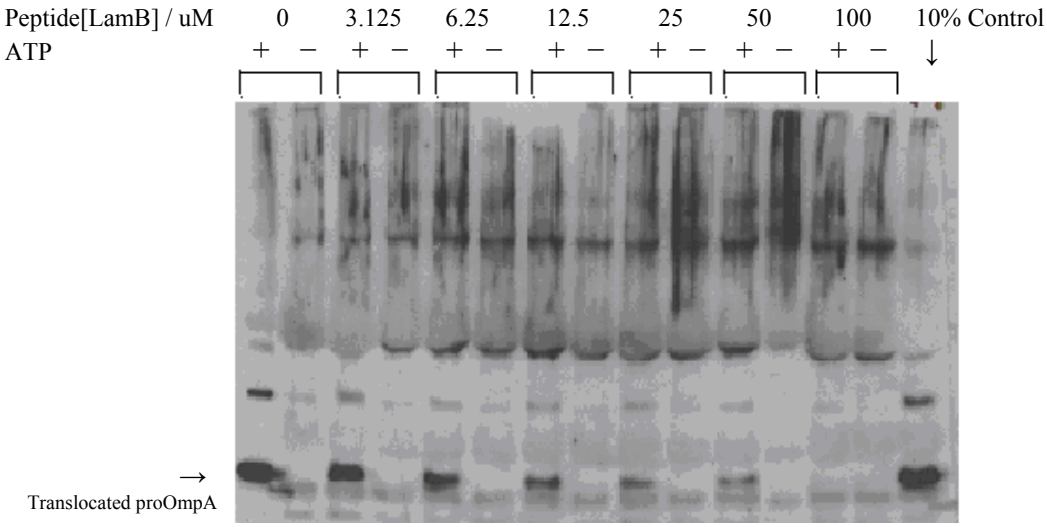


Figure 2. Translocation assay showing the effect of a competitive signal sequence peptide concentration

Outcomes of studentship

During this studentship, I learned how to purify a specific protein and how to carry out ATPase and protein translocation assays. It also gave me a chance to use techniques like western blot and immuno- fluorescence.

Future directions in which the project should be taken

SecA is known to have an IRA region (intermolecular regulator of ATP hydrolysis) that regulates its ATPase activity. Mutation of this domain will result in a high ATPase activity (4). After seeing the effect of magnesium on SecA from our experimental data, we wonder whether there is a link between the allosteric Mg-binding domain and the IRA domain.

Also, from the translocation experiment, the signal sequence peptide LamB is shown to bind to the physiological substrate-binding site of SecYEG by competing with proOmpA. Therefore, it can now be used in studies aimed at determining the structure of SecYEG bound to a signal sequence peptide.

Departures from the original proposal

As in the original plan, this project was focussed on understanding of protein translocation. But this summer, we also studied the motor for translocation SecA, rather than focussing on the SecYEG complex only. Regulation of SecA's activity plays an important role in controlling protein translocation.

Values of studentship to the student

This project provided not only another opportunity to develop my laboratory techniques, it gave me an insight how scientists target and solve scientific problems. Furthermore, I became more confident working in a laboratory after this studentship. It will be very beneficial for my practical project in the final year.

I would like to thank my supervisor Dr. Ian Collinson and Dr. Alice Robson for all of their guidance and patience during this project.

References

(1) Alice Robson & Ian Collinson (2006) *EMBO reports 7*, 1099-1103
(2) Gold, V., Robson, A., Clarke, A., and Collinson, I. (2007) *J. Biol. Chem.* 282, 17424-17432
(3) Gold, Robson & Collinson, Unpublished result
(4) Karamanou, S., Vrontou, E., Sianidis, G., Baud, C., Roos, T., Kuhn, A., Politou, S., Economou, A. (1999) *Mol. Microbiol.*34, 1133-1145