

# Building a Computer Simulation of Threonine Synthesis in *Escherichia coli*.

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## ABSTRACT

We have developed a computer simulation of the threonine synthesis pathway in *E. coli tir8* based on kinetic functions developed from measurements on the pathway enzymes under near-physiological conditions. An important lesson learnt, relevant to plans to build *in silico* models of bacterial metabolism, is that existing literature data may contain some relevant kinetic constants, but it generally lacks usable kinetic functions for this purpose. The model successfully simulates the main features of the time courses of threonine synthesis we observed in a cell-free extract, without alteration of the experimentally determined parameters. It was then used to predict the steady-state of the pathway under intracellular conditions. Flux control coefficients were calculated and show that the control of flux is shared between the first three enzymes: aspartate kinase, aspartate semialdehyde dehydrogenase and homoserine dehydrogenase, with no single activity dominating the control. When the model pathway was embedded in a larger model that simulated the variable demands for threonine at different growth rates, it reproduced accumulation of free threonine at low growth rates, as observed in this strain, and can deliver the threonine requirements required for high growth rates. We conclude that the *in vitro* kinetics appear compatible with the *in vivo* metabolism. At low growth rates, the control of threonine flux remains largely in the pathway enzymes, but switches to the demand for threonine at high growth rates.

## 1. INTRODUCTION

The threonine synthesis pathway of *E. coli* is an interesting target for computer modelling for a number of reasons. A practical motivation is the industrial interest in commercial production of threonine by fermentation, though here *Corynebacterium glutamicum* strains are also important. However, another reason is that kinetic studies of the enzymes of the *E. coli* pathway date back over 50 years,

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so it is of interest to assess the utility of this literature for modelling purposes. Finally, since the biochemistry and physiology of laboratory strains of *E. coli* have been extensively studied, and the complete genome sequence determined, it is a natural candidate in the attempt to build an *in silico* model of a complete organism. Hence the problems and progress in our project to model threonine synthesis contain lessons for more ambitious and extensive projects to come.

The model described here is the culmination of a combined experimental and theoretical study of a threonine-producing mutant *E. coli tir8* that lacks threonine repression of the pathway enzymes, and contains higher levels of aspartate kinase than wild-type strains. The details of the experimental work can be found in [5, 9].

## 2. MODEL CONSTRUCTION

The extent of the system modelled is shown in Fig. 1. As an example of the issues encountered, consider the case of aspartate kinase I. (The issues relating to other enzymes of the pathway are discussed in our paper [5].) The threonine inhibited isoenzyme of aspartate kinase has been studied for many years, e.g. [1, 3, 8], though mainly in connection with various aspects of its mechanism, and under conditions where the reaction could be considered unidirectional. It was therefore known that the enzyme mechanism is random-order two substrate, that threonine inhibition is cooperative, partial and competitive. However, no complete equation for this enzyme has ever been published that simultaneously accounts for the action of the two substrates, the two products and the effector. It was particularly important that product effects be incorporated because the equilibrium constant favours the substrates [3], even though the reaction is universally represented in textbooks as if it were irreversible in the threonine direction. (The on-line database ECO-CYC does represent this reaction correctly, whereas KEGG does not.) In addition, we also directly measured a significant product inhibition by ADP. Taking all these factors into account, we proposed the equation shown in Fig. 2 to unify all the effects. This is necessarily a simplification, in that it does not contain a mechanistically correct equation

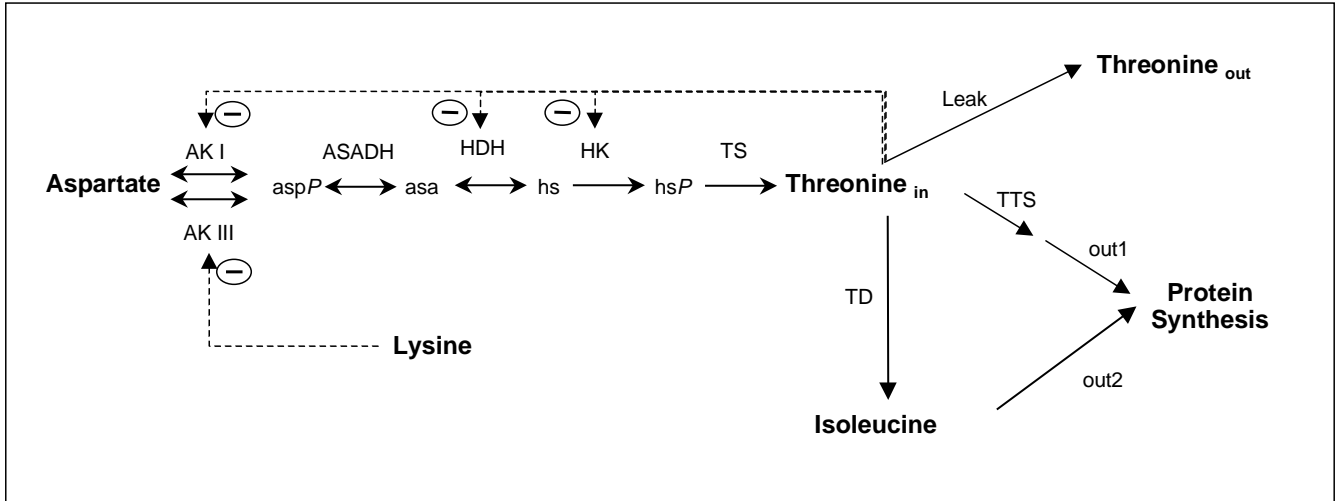


Figure 1: The model of threonine metabolism. The scheme shows the full model of threonine synthesis and utilization. Subsets of this scheme were used for simulations of cell-free synthesis and some *in vivo* simulations. ‘out1’ and ‘out2’ refer to the incorporation of threonine and isoleucine into protein. Other abbreviations: AK, aspartate kinase (EC 2.7.2.4); ASADH, aspartate semialdehyde dehydrogenase (EC 1.2.1.11); HDH, homoserine dehydrogenase (EC 1.1.1.3); HK, homoserine kinase (EC 2.7.1.39); TS, threonine synthetase (EC 4.2.3.1); TD, threonine deaminase (EC 4.2.1.16); TTS, threonyl-tRNA synthetase (EC 6.1.1.3).

$$v = \frac{V_f \left( asp.ATP - \frac{aspP.ADP}{K_{eq}} \right)}{\left( K_{asp} \frac{1 + \left( \frac{thr}{K_{ithr}} \right)^{n_h}}{1 + \left( \frac{thr}{\alpha K_{ithr}} \right)^{n_h}} + aspP \frac{K_{asp}}{K_{aspp}} + asp \right) \left( K_{ATP} \left( 1 + \frac{ADP}{K_{ADP}} \right) + ATP \right)}$$

Figure 2: The kinetic function used for aspartate kinase I. The function is an approximation to a reversible two-substrate equation with product inhibition and partially-competitive, cooperative inhibition by threonine.

for a 2-substrate enzyme, but such equations are designed to be used for *in vitro* enzyme mechanism studies, whereas *in vivo* the range of substrate and product concentrations to which the enzyme is exposed are likely to be much narrower. Therefore, there is not a requirement for the equation to be accurate for all combinations of substrate and product concentrations. In addition, reported studies were not at *in vivo* pH values and temperatures. We therefore made kinetic measurements under conditions closer to *in vivo*, and adjusted the parameters of the equation to match the experimental results best in the relevant part of the substrate concentration range.

Similar strategies were followed to produce kinetic equations for the other steps, for very similar reasons. The equations, with their parameters and their justification, can be found in [5].

The resulting set of kinetic functions was included in the model, which was implemented in the biochemical simulation package SCAMP [11, 10]. This allows both dynamic simulations, and solutions for steady states with automated calculation of elasticities and control coefficients as well as other derived parameters. A specimen command file for SCAMP is available from the authors’ web site (<http://mudshark.brookes.ac.uk>). The model has also been implemented

in JARNAC. The set of differential equations compiled by SCAMP has been summarized in [4].

### 3. MODEL VALIDATION

The model was tested by its ability to reproduce the experimental results obtained by incubating cell-free extracts with aspartate, ATP and NADPH, as shown in the example in Fig. 3. The experiments are described in more detail in [9] and further examples of fits are given in [4]. For these simulated fits, the model included the reactions in Fig. 1 from aspartate to internal threonine. In addition, the time courses of ATP and NADPH were simulated, and endogenous consumption of these metabolites by the cell-free extract was also included in the simulation. Only three parameters were altered from their experimentally determined values to achieve the fit between experiment and simulation. The main alteration was to the value of the equilibrium constant of aspartate semialdehyde dehydrogenase, which affected the amount of threonine formed by the end of a 90 min incubation, when the first two reactions of the pathway are close to equilibrium. We set this to a slightly higher value to match the experimental results, though the new value was still below that previously published [2]. We also found that the rate of endogenous ATP consumption by the cell-free extract, measured in the absence of threonine syn-

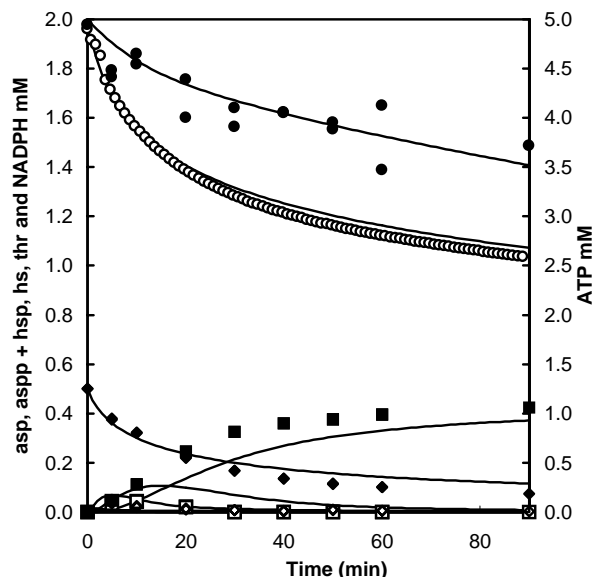


Figure 3: Threonine synthesis in a cell-free system. Initial conditions were 0.5 mM aspartate ( $\blacklozenge$ ), 2 mM NADPH ( $\circ$ ) and 5 mM ATP ( $\bullet$ ). Other metabolites: threonine ( $\blacksquare$ ); aspartyl phosphate + homoserine phosphate ( $\square$ ). The lines are the simulated results.

thesis, was higher than seemed to be occurring in the system when threonine was being formed, and we lowered the rate constant for this reaction accordingly.

#### 4. PREDICTED FLUX CONTROL COEFFICIENTS IN THE *IN VIVO* PATHWAY

The basic model used for the cell-free synthesis simulations was used to predict the steady state and flux control coefficients of the pathway *in vivo* assuming that the metabolite concentrations of ATP, ADP, NADPH, NADP, aspartate, lysine and threonine were constant at the values measured in the *E. coli* strain used for the experiments [4]. The predicted steady state flux under these conditions was in the range estimated to be needed by the bacterium to meet its threonine requirements, suggesting that the *in vitro* kinetics are adequately applicable to *in vivo* conditions. The aspartate kinase reaction is found to be close to equilibrium in most circumstances, as expected given its equilibrium constant, but in contrast to its usual representation, as mentioned previously.

The calculated flux control distribution is sensitive to the concentrations of these external metabolites. An example is shown in Fig. 4 for the dependence on aspartate, but this is typical of all the results for the other external metabolites in that, in all cases, the control is shared to varying degrees between the first three enzymes. An interesting aspect of Fig. 4 is that the pathway flux responds to aspartate in a hyperbolic manner even though the flux control coefficient of aspartate kinase is not high enough for it to be classed as rate-limiting.

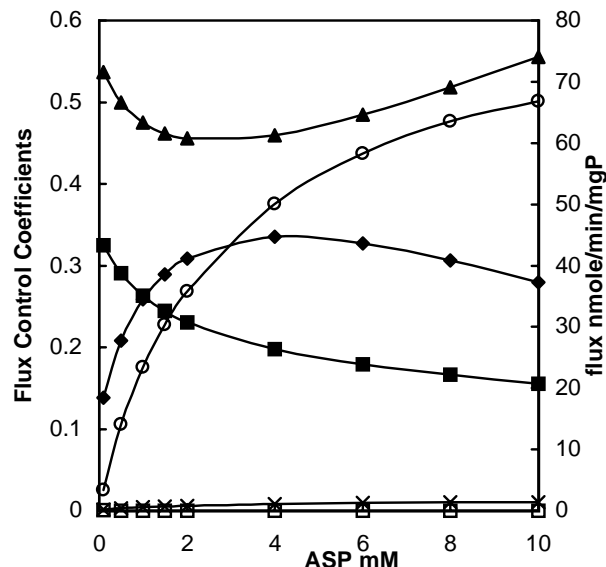


Figure 4: Flux control coefficients of the threonine synthesis pathway. Control coefficients of: aspartate kinase,  $\blacklozenge$ ; aspartate semialdehyde dehydrogenase,  $\blacksquare$ ; homoserine dehydrogenase,  $\blacktriangle$ ; homoserine kinase,  $\triangle$ ; threonine synthase,  $\square$ . The flux is also shown:  $\circ$ . The measured intracellular aspartate concentration was 1.3 mM.

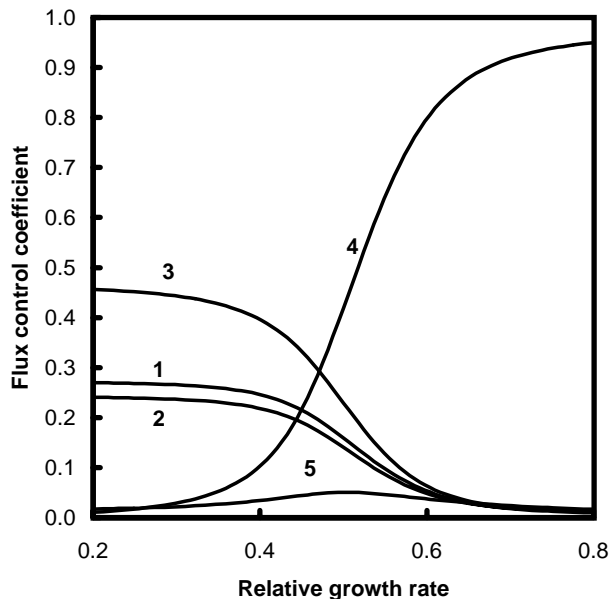
#### 5. EFFECT OF THREONINE DEMAND ON THE FLUX CONTROL DISTRIBUTION AND THREONINE EXCRETION.

The full model of Fig. 1 was extended by addition of approximated demand functions based on growth-dependent withdrawal of threonine and isoleucine for protein synthesis, and threonine loss through the cell wall at a comparable rate to that measured in *Corynebacterium glutamicum* [7]. At low growth rates, the flux through threonine synthesis exceeds the endogenous requirement and intracellular threonine rises so that the excess is lost by diffusion into the medium. This reproduces the qualitative characteristics of *tir8*, which produces free threonine when it enters stationary phase.

The variation of the flux control coefficients of the threonine pathway as a function of the demand reactions are shown in Fig. 5. The relative values of the control coefficients in the synthesis pathway do not change significantly, but they are collectively most significant under conditions of low intracellular demand for threonine and decrease as the demand increases. Intracellular demand for threonine comes to have the dominant control at high demand levels.

#### 6. CONCLUSION

The properties of our model of threonine synthesis suggest that it does adequately represent the properties of the *in vivo* pathway in *E. coli*. However, even though there was an existing literature on the kinetics of the enzymes of the pathway, it did not yield the kinetic functions needed for our purposes, nor the values of the kinetic parameters at pH values and temperatures appropriate to *in vivo* conditions. On a larger scale, this could prove a distinct limitation in the de-



**Figure 5: Flux control coefficients of the threonine synthesis pathway computed at different growth rates. The full model of Fig. 1 was simulated. Flux control coefficients of: curve 1, total aspartate kinase; curve 2, aspartate semialdehyde dehydrogenase; 3, homoserine dehydrogenase; 4, all protein synthesis steps from threonine (TTS, TD, out1 and out2 in Fig 1); 5, threonine leak from the cells.**

velopment of large models of bacterial metabolism. In other studies undertaken by one of us (DAF), it has proved possible to develop appropriate kinetic functions by fitting the data presented in original papers, after it has been reconstructed from the published graphs (e.g. [6, 12]). This was not an option in the case of the threonine pathway because many of the existing experiments were performed under inappropriate conditions for our needs. However, where it is possible, it does demonstrate that it is often the original data from the literature archive that is needed, and not necessarily the authors' analyses at the time.

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