Free Energy Driven Error Correction in Macromolecular Biosyntheses. A Theoretical Approach *

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When the intrinsic selectivity of enzymes is insufficient to separate the product formation flows of structurally similar substrates then error correction is necessary. To achieve error correction the enzyme uses a branched kinetic pathway. The main branch leads to product formation but there also exist side branches where the erroneous substrates are discarded. It was recognized by Hopfield [1] and Ninio [2] that the discard branches must be energetically driven in order to be effective. Hopfield [1] conjectured that the wasteful hydrolysis of nucleoside triphosphates without product formation in the aminoacylation of transfer RNA, at the ribosomes as well as in DNA replication reflected such energetically driven error correcting reactions. Kurland [3] could identify the displacement from equilibrium of the nucleoside triphosphates with their hydrolytic products as the prerequisite for error correction.

Theory. In other words, it is the free energy difference between, say, a mol of ATP and a mol of AMP and PP_i that makes it possible to discard an erroneously acylated transfer RNA molecule with a decreased error of the reaction in consequence. From this observation it is conceptually a small step to more general statements.[4] First, the accuracy of enzymatic selections depends on the intrinsic selectivity of enzymes in combination with the free energy which is dissipated when going from substrate to product as well as over the discard branches. Second, optimal solutions to the problem of accuracy can be found. They correspond to unique and predictable kinetic structures of enzymatic pathways where the free energy losses for a given error level are minimal. Third, such minimal theoretical values of the free energy losses for given error levels can be compared with free energy losses in *in vivo* systems. The outcome of the comparison will tell us how much of the dissipated free energy in protein synthesis that is necessary for its accuracy.[4,5,6]

Results. When the ratio D of cognate product formation flow over the non-cognate product formation flow approaches the intrinsic selectivity d of an enzymatic selection then the dissipative losses of the reaction go to infinity. Without error correction the ratio D cannot excede d and approaching this limit becomes very costly for the organism.

By introducing error correction in one step the upper accuracy limit is given by d^2 instead of d. For the selection of amino acids by aminoacyl-tRNA synthetases estimates of d indicate that the intrinsic selectivity of the enzymes in several cases does not excede a factor 100 whence the overall selectivity of proteinsynthesis is in the range of 10^4, *i.e.* d^2. However, the suggestion of Hopfield [1] that one proofreading step solves this apparent paradox leads to another conceptual contradiction. This contradiction stems from a general property of all error correcting mechanisms, namely that when they operate in the vicinity of their upper accuracy limits they dissipate large amounts of free energy. In particular such a mode of operation necessarily leads to large excess losses of nucleoside triphosphates associated with the correct product formation flow. This prediction is rejected by experimental observation.[7]

However, by postulating multistep error correction, where a number of proofreading steps are cascaded with all discard branches driven by a single nucleoside triphosphate, we have demonstrated a possible way out of the dilemma. Such multistep mechanisms not only shift the upper accuracy limit to substantially higher values but also turn out to improve the economy of the reaction in a dramatic way. Such an improvement in the efficiency is obtained when the mechanism is allowed to operate in a "relaxed way" far below its upper accuracy limit. The determination of such upper limits in error correcting systems has been done. One limit is related to the number n of steps, eqn. (1). When the number n becomes large another

$$D < d^{n+1} \tag{1}$$

limitation becomes pertinent, namely the displacement from equilibrium γ of the nucleoside triphosphate with its hydrolytic products, eqn. (2).

$$D < d\gamma \tag{2}$$

The theory was taken one step further to allow not only qualitative but also quantitative predictions of the kinetic properties of enzymatic selections. Such predictions have been based on a postulate of minimal dissipative losses for a given error level.

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With the intrinsic selectivity *d* as our single *ad hoc* parameter we have shown how an in this way optimized error correcting enzyme is associated with several symmetry properties and also stringent demands on its thermodynamic driving forces.

First, minimal free energy losses are associated with an equipartitioning of the fractional loss flows over the discard steps.

Second, the rate constants of the enzymatic pathway are organized so that thay differ with the same factor from discard step to discard step.

Third, the required displacement of the nucleoside triphosphate varies in proportion to the required discrimination ratio *D*.

Furthermore our treatment rejects the common notion that the two GTP molecules used per elongation cycle in proteinsynthesis are necessarily required for the accuracy of codon translation. In contrast the theory shows that protein synthesis dissipates free energy to an extent which dramatically excedes the requirements of optimized schemes. This immediately brings the role of the two GTP molecules in the elongation cycle back into focus as an important – and unresolved – question.

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