FEBS Letters 587 (2013) 2868–2875

journal homepage: www.FEBSLetters.org

A generic rate equation for catalysed, template-directed polymerisation

Jan-Hendrik S. Hofmeyr *, Olona P.C. Gqwaka, Johann M. Rohwer

Dept. of Biochemistry, University of Stellenbosch, Private Bag X1, 7602 Stellenbosch, South Africa

ARTICLE INFO

Article history:
Received 28 May 2013
Accepted 4 July 2013
Available online 12 July 2013

Edited by Athel Cornish-Bowden

Keywords:
Template-directed polymerisation
Generic rate equation

ABSTRACT

Biosynthetic networks link to growth and reproduction processes through template-directed synthesis of macromolecules such as polynucleotides and polypeptides. No rate equation exists that captures this link in a way that it can effectively be incorporated into a single computational model of the overall process. This paper describes the derivation of such a generic steady-state rate equation for catalysed, template-directed polymerisation reactions with varying monomer stoichiometry and varying chain length. The derivation is based on a classical Michaelis–Menten mechanism with template binding and an arbitrary number of chain elongation steps that produce a polymer composed of an arbitrary number of monomer types. The rate equation only requires the identity of the first dimer in the polymer sequence; for the remainder only the monomer composition needs be known. Further simplification of a term in the denominator yielded an equation requiring no positional information at all, only the monomer composition of the polymer; this equation still gave an excellent estimate of the reaction rate provided that either the monomer concentrations are at least half-saturating, or the polymer is very long.

1. Introduction

Metabolism has conventionally been studied using a reductionistic approach in which metabolic pathways have been regarded as isolated modules; due to the complexity of metabolic organisation this has of course been necessary for the identification of the individual reactions and their substrates, products and cofactors. However, to gain an understanding of the integrated nature of metabolism it is necessary to consider the coupling of metabolic pathways with each other, especially between intermediary metabolism as a whole with processes such as the synthesis of proteins, polynucleotides and complex lipids.

A particularly important aspect of cellular regulation is how the synthesis of a biopolymer is integrated with the pathways that supply its individual monomers. Fig. 1 depicts such a (hypothetical) situation for the synthesis of a polymer from five different monomer types, each of which is synthesised by its own biosynthetic pathway. To construct a computational model of such a system one needs a general rate equation that can account for a catalysed, template-directed polymerisation process that can produce, from a specified number of monomer types, a polymer with a given length and monomer composition. Such a rate equation must be able to handle conditions in which there is a varying demand for the monomers that constitute the biopolymer.

There have of course been many modelling studies of the kinetics of ribosomal polypeptide synthesis [1–11] or of the synthesis of polynucleotides such as DNA or RNA [12–15]. However, these studies all attempted to model the details of the complicated mechanistic processes that characterise the synthesis of a particular polymer. As is the case with classical enzyme kinetics, the aim of these studies was to understand mechanism, and not to model the integration of these processes with the biosynthesis of the monomers. The type of rate equation derived in this paper is of a different nature, namely that of a single rate equation that summarises the whole biopolymerisation process and allows for varying monomer stoichiometry and polymer length.

2. Derivation of a generic rate equation

2.1. Binding of template to a Michaelis–Menten enzyme

The derivation of a generic rate equation for a catalysed, template-directed polymerisation reaction is built up gradually, starting with the simple mechanism in Fig. 2, which is a classical irreversible uni–uni Michaelis–Menten mechanism [16,17] in which the enzyme E first binds to a molecule T that eventually will be the template that directs the sequence in which monomers bind and are ligated. A substrate S subsequently binds to ET forming an enzyme–template–substrate complex ETS. S is converted to product P, which is then released from ET. The reason for considering this mechanism before introducing the actual polymerisation process is that it suggests a way of handling thecomplexities

* Corresponding author. Fax: +27 218085863.
E-mail address: jhsh@sun.ac.za (J.-H.S. Hofmeyr).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.07.011

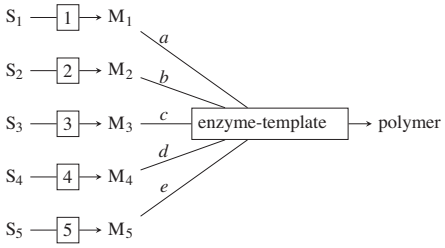

Fig. 1. Scheme of a hypothetical template-directed polymerisation system consisting of five biosynthetic blocks that each produces a monomer that is consumed with the indicated stoichiometry (*a* to *e*) to yield a polymer product with monomer composition $(M_1)_a(M_2)_b(M_3)_c(M_4)_d(M_5)_e$.

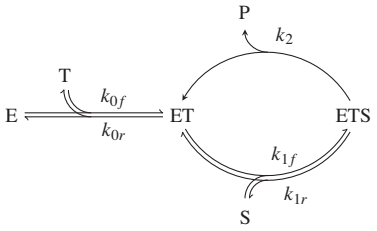

Fig. 2. A classical irreversible Michaelis–Menten mechanism [16,17] in which enzyme E first binds to a template molecule T. ET and ETS are the intermediate complexes. The double arrows denote reversible binding steps, while the single-headed arrow denotes the irreversible, combined catalytic and product release step. k_{0f}, k_{0r}, k_{1f}, k_{1r}, and k_2 are rate constants.

introduced by binding of template to enzyme. As a point of departure the binding step is allowed to fully equilibrate without fixing any of the enzyme or template forms. The rate of the production of P is

$$v = \frac{d[P]}{dt} = k_2[ETS] \tag{1}$$

The conservation equations for enzyme and template forms are

$$[E]_t = [E] + [ET] + [ETS] \tag{2}$$

where $[E]_t$ denotes the total concentration of the enzyme, and

$$[T]_t = [T] + [ET] + [ETS] \tag{3}$$

where $[T]_t$ denotes the total concentration of template T.

The dissociation constant for the enzyme–template complex is

$$K_0 = \frac{k_{0r}}{k_{0f}} = \frac{[E][T]}{[ET]} \tag{4}$$

and the Michaelis constant for S is

$$K_M = \frac{k_{1r} + k_2}{k_{1f}} = \frac{[ET][S]}{[ETS]} \tag{5}$$

Solving Eqs. (2)–(5) with Maxima [18] yields the following analytical expression for the steady-state concentration of [ETS]:

$$[ETS] = \frac{\sigma[(K_0 + [T]_t\Upsilon)(K_0 + [T]_t\Upsilon - X) + [E]_t\Upsilon(K_0 - [T]_t\Upsilon)]}{\Upsilon^3([T]_t - [E]_t) + \Upsilon^2(K_0 - X)} \tag{6}$$

where $\sigma = [S]/K_M$, $\Upsilon = 1 + \sigma$ and

$$X = \sqrt{([T]_t - [E]_t)^2\Upsilon^2 + 2K_0([T]_t + [E]_t)\Upsilon + K_0^2} \tag{7}$$

This expression is too complex to be of any practical use. Assuming that the concentration of free enzyme, [E], is constant removes the conservation Eq. (2) for $[E]_t$ from the equation system, so that only Eqs. (3)–(5) have to be solved simultaneously. This may seem too restrictive an assumption, but it would be justifiable if there were much less template than enzyme, i.e., $[T]_t \ll [E]_t$, which would imply that $[E] \approx [E]_t$. However, for what follows it is only necessary to assume that [E] is constant.

Solving for ETS and inserting into Eq. (1) yields

$$v = k_2[ETS] = \frac{k_2[T]_t\sigma}{1 + \frac{K_0}{[E]} + \sigma} \tag{8}$$

If $[E]_t \ll [T]_t$, [T] can be considered to be constant, leaving [E] variable. This would remove conservation Eq. (3) for $[T]_t$ from the equation system and lead to the expression:

$$v = k_2[ETS] = \frac{k_2[E]_t\sigma}{1 + \frac{K_0}{[T]} + \sigma} \tag{9}$$

In the rest of this paper [E] will be considered to be constant, but in all the derived rate equations [E] and $[T]_t$ can be replaced by [T] and $[E]_t$ respectively as in Eqs. (8) and (9).

These rate equations contain an additional positive term (K_0/[E] or K_0/[T]) in the denominator, as compared to the usual irreversible Michaelis–Menten equation in the absence of binding of T. Later in the paper conditions will be described under which these terms can be ignored.

2.2. Polymer formation through elongation

The next step towards a generic rate equation for template-directed polymerisation is to extend the reaction scheme in Fig. 2 by incorporating a template-directed polymerisation process consisting of an initial dimerisation step followed by one elongation step (Fig. 3). Monomers M_1 and M_2 bind sequentially to the enzyme–template complex (ET) and are then coupled. A third monomer M3 binds to form the complex ETM_1–M_2M_3 from which the trimer product M_1–M_2–M_3 is released. As in the classical irreversible Michaelis–Menten mechanism, the elongation and product release steps have been combined into one step with rate constant k_5 (the effect of making both steps explicit will be discussed later). Binding steps are considered to be reversible, while the ligation and product release steps are considered to be irreversible (it is assumed that the monomers of template-directed condensation reactions are usually activated by the attachment of a good leaving

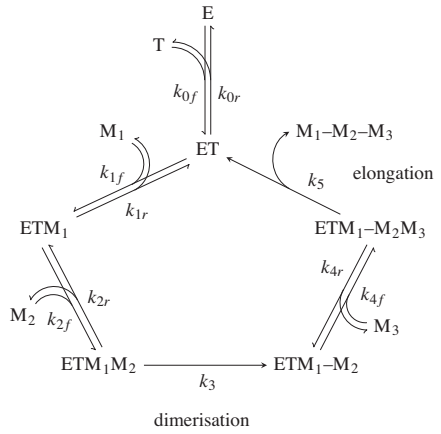

Fig. 3. Reaction scheme of a catalysed, template-directed polymerisation reaction. M_1, M_2, M_3 denote the monomers, E the free enzyme, T the free template, ET the enzyme–template complex, ETM_1 the ET–monomer complex, ETM_1M_2 the complex of ET with two unligated monomers, ETM_1–M_2 the ET–dimer complex, ETM_1–M_2M_3 the complex of ET–dimer with the next monomer, ETM_1–M_2–M_3 the ET–trimer complex, and M_1–M_2–M_3 the final trimer product. The half-headed arrows denote the reversible binding and dissociation steps, and the single-headed arrows denote irreversible catalytic steps.