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Mechanisms of telomerase-dimer catalysis

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Abstract

There is evidence that human telomerase acts as a dimer [Wenz, C., Enenkel, B., Amacker, M., Kelleher, C., Damm, K., Lingner, J., 2001. Human telomerase contains two cooperating telomerase RNA molecules. EMBO J. 20, 3526–3534]. Three possible mechanisms have been proposed. We translate those proposals into three detailed mechanistic models for telomerase action, also introducing optional isomerizations with equilibrium constants inversely related to the number of bound nucleotides. To distinguish between these models by in situ experiments we propose a microscopic system which uses two-photon excitation of fluorescence in a volume of about $0.5\,\mu m^3$. A variety of detection strategies and experimental designs are considered; we focus on those best suited to observation of a small volume under limitations imposed by diffusion to and from the reacting micro-volume, and consequently restrict ourselves to constant flow. Numerical simulation is used to help identify an optimal experimental design. The detection of mechanistic changes hinges on linking fluorescence reporters to selected reaction components, either directly (chemically) or indirectly (via an indicator reaction). We show that rapid mixing experiments are better than chemical relaxation experiments, as the statistics of single molecule kinetics affects the latter more than the former. However, some fast reaction steps can only be revealed by chemical relaxation coupled with mixing experiments. We explore connections between our methods and studies of HIV and other systems with RNA to DNA transcription.

Keywords: Telomerase; Cooperative behavior; Rapid mixing; Chemical relaxation; Two-photon absorption

1. Introduction

Telomerase, a reverse transcriptase, was discovered by Greider and Blackburn (1985). They obtained the enzyme from tetrahymena. Morin (1989) used the same method to confirm the presence of telomerase in certain human cells. In humans telomerase is only active in germ cells, stem cells and proliferating tumor cells (Blackburn, 1991). Ideas about the functioning of the enzyme were first described by Greider and Blackburn (1989). More complete mechanistic details were developed by Lingner et al. (1997). However, exactly how the initial telomere–telomerase complex is formed remains unclear (Baumann and Cech, 2001). There is some indication that the molecular weight of fully active human telomerase is very high (1000 kDa or more, Schnapp et al., 1998). A recent review of telomerase was given by Cech (2004).

Wenz et al. (2001) conducted experiments which point to human telomerase acting as dimers. They proposed two models (later expanded to three) which we investigate further. One of these models is an allosteric model. The concept of allosterism was introduced by Monod et al. (1965). Czerlinski (1968) studied the chemical relaxation of allosteric models, including the polymerizing case described originally by Klapper and Klotz (1968). We expand the original three models to six by adding an isomerization of the nucleotide complexes with isomerization constants which become smaller as the number of nucleotides bound increases. To determine how to distinguish experimentally between these six models we simulate their kinetic behavior numerically, in the context of an experimental design which allows observation of subcellular changes. We aim to identify distinguishing features and optimal conditions for their detection by rapid mixing (with one component spatially fixed) and chemical relaxation, avoiding complications described earlier (Czerlinski et al., 2003).

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The proposed experimental setup is shown in Fig. 1 (a vertical section through the surface of the horizontal object glass in the direction of flow above the surface, with one reactant attached to the surface) with Table 1 showing typical dimensions. The 'beam' refers to a fluorescence emission beam, with fluorescence excited by two-photon absorption (coaxial beams) in a volume of less than 1 μm^3 (Denk et al., 1991). The solution with the other reactant(s) flows from left to right at constant speed. The reactant attached to the glass surface covers the whole distance $b+d$. Distance e is the observation distance along the flow; in situ $b+d$ may be as small as e. Distance d, which may be reduced to e to measure small chemical relaxation times, is termed the flow transit distance.

Table 1 also lists characteristic diffusion times for stopped flow computed using the simplified diffusion equation (Moore, 1960) for each of two typical diffusion constants. While these diffusion times could possibly be modified by adjusting the design parameters of the flow cell, it is clear that diffusion may interfere with observation of the in stopped flow. Such concerns were previously discussed in detail (Czerlinski and Ypma, 2005, 2007). We thus focus on constant flow, where interference by diffusion is generally negligible.

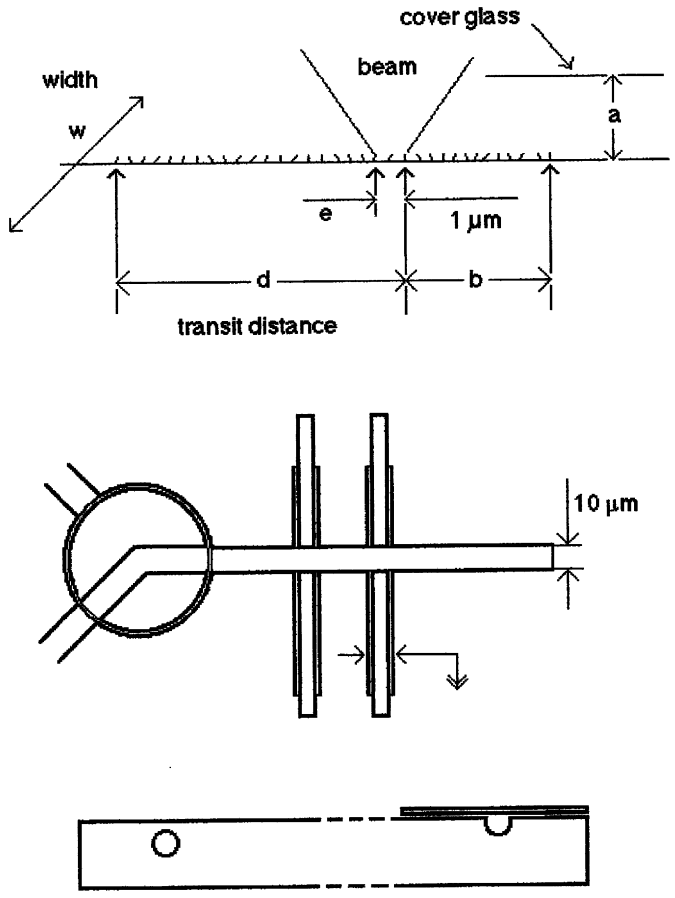

Fig. 1. Object glass design for small volumes. Upper part shows vertical cross-section in the horizontal plane of the central flow portion where observation takes place. To enhance the definition of the reaction volume, the valves are not shown. Middle portion shows valves for switching fluids on the left and a closing valve system for stopped flow (four plungers linked together). Lower part shows two object glass options for the opening for the flowing liquid: either a bore in the solid glass (left) or a groove with cover glass cemented on.

For constant flow, in which mobile components are held at fixed concentrations, interference from mixing is a concern if the flow transit time is not considerably smaller than the chemical relaxation time. The latter concept was defined by Eigen (1955), while the former may be defined as follows: with f denoting the linear flow velocity of the solution with the mobile reactant(s), the flow transit time t_0 is defined as $t_0 = d/f$. To avoid interference from mixing we thus need $t_0 \ll t_c$ with t_c—chemical relaxation time. If the two time constants are similar, one might instead spread out the chemical kinetics over the total length $b+d$ and scan points along this length, or observe the kinetic change only at selected points along the flow.

2. Methods

2.1. Allosteric model

The allosteric model shown in Fig. 2 was developed from proposals made by Wenz et al. (2001), but introduces isomerizations between components 11 and 18 as well as their compounds with the desoxyribonucleotides. Component X is assumed to be a promoter of the dissociation of the extended telomere chain from its complex with telomerase. As this component is only involved at a later stage, it does not appear in our model equations and is not used in our simulations as indicated by the dashed arrow in Fig. 2 (similarly in Figs. 3 and 4). The reaction components are detailed in Table 2, which also lists their initial concentrations. We refer to this mechanism as Model 1.

Allosteric models combine the interacting units into one molecule; see Monod et al. (1965) regarding thermodynamics and Czerlinski (1968) regarding kinetics. For the bi-telomerase system we combine two telomere ends into one molecule by distinguishing between unaligned pairs (component 1 to which telomerase binds poorly, resulting in a large dissociation constant K_1) and aligned pairs (component 7 to which telomerase binds strongly, resulting in a small K_3). Thus 'aligned' refers to 'oriented for optimal binding of telomerase', with dissociation constants K_3, $K_4 \ll K_1$, K_2. While there may in fact be a distribution of degrees of alignment, we omit such considerations here. Components 9 and 11 in Fig. 2 also have aligned pairs of telomere ends, consequently components 12–24 contain aligned pairs fixed in space as well.

Fig. 2 identifies the equilibrium constants and the rate constant k_{77} for Model 1. Definitions and values for the equilibrium constants are listed in Table 3 and values for the corresponding rate constants are given in Table 4. Forward rate constants have odd subscripts and move from the top left in Fig. 2 (component 1) to the bottom right (component 24), while reverse rate constants have even subscripts and move in the opposite direction. The values