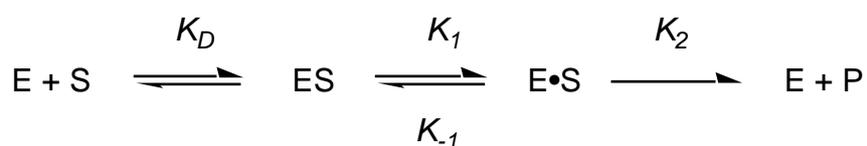


GENERAL INFORMATION

This spreadsheet has been built to simulate the reaction kinetics of enzymatic epoxide hydrolysis by / hydrolase fold epoxide hydrolases (please, note that there are bacterial EHs that belong to other structural families of enzymes and thus presumably work by different mechanisms). Users can change the *tunable variables* in order to explore the implication of these in the reaction kinetics. The general reaction scheme underlying the simulation is:



Definition of symbols:

E = free enzyme (epoxide hydrolase)

S = substrate (epoxide)

ES = Michaelis-Menten complex

E•S = enzyme-substrate ester intermediate

P = reaction product (diol)

K_D = dissociation constant

K_1 = rate constant for the formation of the ester intermediate

K_2 = rate constant for the ester hydrolysis

K_{-1} = rate constant for the epoxide formation from the ester intermediate
(we consider this an unlikely event)

Additional symbols used in the spreadsheet:

E_0 = concentration of the free enzyme before substrate addition

S_0 = concentration of the free substrate before first interaction with the enzyme

Δt = time increment for the stepwise calculation of the kinetics

ΔS = change in substrate concentration over time due to continuous substrate supply (for steady state kinetics)

K_m = Michaelis constant

ES_{ini} = initial equilibrium concentration of the Michaelis-Menten complex before the first chemical reaction

SPREADSHEET DESIGN

The spreadsheet has been built in Microsoft Excel and is compatible with the Microsoft Office '98 (Mac) or the Microsoft Office '97 (PC) version of this software package. The screen has been formatted for a monitor resolution of 832 x 624 pixel (or higher). It is composed of three separate sheets: (i) the Simulation Table (Fig. 3A), (ii) Fig. 3B, and (iii) Fig. 3C. The main sheet is the Simulation Table, sheets (ii) and (iii) are appended to give the data for the respective figures from our publication. These have been worked out using the Simulation Table and will not be further discussed in the following.

The spreadsheet opens with the Simulation Table being in front. On the left side of the screen, a graphical representation of the concentrations of the free substrate

(red line), the Michaelis-Menten complex (pink line), the ester intermediate (orange line) as well as of the product (green line) are shown over time. On the right side, the variables that determine the course of the concentration-time curves are listed. These variables, with the exception of the calculated variables K_m and ES_{ini} , are intended to be changed by the user to explore the influence of these changes on the time course. The present values are those for styrene oxide turnover by human microsomal epoxide hydrolase *in vivo*, as given in the paper. Thus, the graph presently resembles Fig. 3A of this reference.

The main calculations for the simulation are carried out in the cells between the coordinates A31 and J16384, allowing for the computation of 16350 separate incremental steps on the time scale. In order to speed up the time to build up the graphics screen, these data are reduced by a factor of 50 by placing a copy of a reduced set of cells in the area between coordinates M31 and S334. These are the reference cells for the graphical representation, which is thus built from 1200 separate data points (300 per separate curve).

CALCULATIONS

Here is how the computations are performed. The principle is that changes in the concentrations of single reactants are calculated over small discrete time increments. The first line of the calculation table fixes the equilibrium conditions previous to any chemical reaction. Values in this line are calculated as follows:

$$t [s] = \text{is set to zero}$$

$$E [\mu M] = E_0 - ES_{ini}$$

E_0 is the concentration of the free enzyme before substrate addition. It can be modified by the user as required.

ES_{ini} is the initial concentration of the Michaelis-Menten complex immediately after substrate addition. ES_{ini} and the other calculated variable K_m are calculated as follows:

$$ES_{ini} [\mu M] = (K_D + E_0 + S_0)/2 - \text{square root } (((K_D + E_0 + S_0)/2)^2 - E_0 \times S_0)$$

$$K_m [\mu M] = K_D \times K_2 / (K_1 + K_2)$$

$$S [\mu M] = S_0 - ES_{ini}$$

S_0 is the initial substrate concentration before any interaction with the enzyme. It can be modified by the user as required.

$$ES [\mu M] = ES_{ini}$$

$$E \cdot S [\mu M] = \text{is set to zero}$$

$$P [\mu M] = \text{is set to zero}$$

From the next line on, time increments by the step defined in the user-defined variable delta t and the concentrations of the reaction partners are calculated as follows (the subscript n designates the position of a given value on the time scale; thus, the subscript n-1 designates the respective value immediately preceding the former, ie these two are separated by just one incremental step on the time scale):

$$t_n [s] = t_{n-1} + \text{delta } t$$

$$E_n [\mu\text{M}] = E_0 - ES_n - E \bullet S_n$$

$$S_n [\mu\text{M}] = S_0 - ES_n - E \bullet S_n - P_n + t_n \times \text{delta } S$$

$t_n \times \text{delta } S$ represents the optional continuous substrate supply, which is required to calculate steady-state conditions.

$$ES_n [\mu\text{M}] = (K_D + E_{n-1} + S_{n-1})/2 - \text{square root } (((K_D + E_{n-1} + S_{n-1})/2)^2 - E_{n-1} \times S_{n-1})$$

$$E \bullet S_n [\mu\text{M}] = E \bullet S_{n-1} + \text{delta } E \bullet S_n - \text{delta } P_n - \text{delta } E \bullet S \text{ back}_n$$

The three "delta" variables are used to keep the calculations in this program as simple and transparent as possible. Delta $E \bullet S$ is the amount of $E \bullet S$ formed from ES in the present time increment, delta P is the amount of product formed from $E \bullet S$ in the present time increment, and delta $E \bullet S$ back is the amount of ES formed from $E \bullet S$ in the present time increment (the unlikely back reaction). These three values are calculated as follows:

$$\text{delta } E \bullet S_n = ES_{n-1} \times K_1 \times \text{delta } t$$

$$\text{delta } P_n = E \bullet S_{n-1} \times K_2 \times \text{delta } t$$

$$\text{delta } E \bullet S \text{ back}_n = E \bullet S_{n-1} \times K_{-1} \times \text{delta } t$$

$$P_n [\mu\text{M}] = P_{n-1} + \text{delta } P_n$$

LIMITATIONS

The incremental steps in the time scale (delta t) should not be significantly larger than the time frame of a single event, and thus have to match with the variables K_1 , K_2 , and K_{-1} . The higher the largest of these values is, the smaller delta t must be. We would recommend not to allow more than one event per incremental step, ie for the highest rate constant being 200 s^{-1} , delta t should not exceed 0.005 s .

IF YOU GET LOST

If you loose your way on exploring the different parts of the spreadsheet (it is huge, and roughly contains 300 000 cells) just get back to the origin (cell A1 in the upper left corner) which brings you back to the original setting. The graphic occupies roughly the area of cells A1 to J28, and the *tunable variables* are found in the cells K2 to L9. You should not hesitate to experiment with the spreadsheet. In order to avoid a loss of essential calculations in cells that are not supposed to be changed (they are not locked, however, to allow the ambitious user to customes the spreadsheet to the maximum extend), we would recommend that you keep a back-up copy of the spreadsheet that is not used for exploration. If any problems or question should arise, you may contact us under the following email address:

arand@toxi.uni-wuerzburg.de

Of course, other comments and suggestions for improvements are also highly appreciated.

APPENDIX

We have tried to keep the mathematics as simple as possible and believe that most of the equations used to calculate the different concentrations are more or less self-explaining. One exception may be the calculation of ES and ES_{ini}, which is therefore explained in more detail in the following:

Combining the three equations

- (1) $S = S_0 - ES$
- (2) $E = E_0 - ES$
- (3) $K_D = S \times E / ES$

leads to the following term, that, after transformation, can be used to calculate ES_{ini}

$$(4) \quad K_D = (S_0 - ES) \times (E_0 - ES) / ES = (S_0 \times E_0 - S_0 \times ES - E_0 \times ES + ES^2) / ES \quad \Rightarrow$$

$$K_D \times ES = - S_0 \times ES - E_0 \times ES + S_0 \times E_0 + ES^2 \quad \Rightarrow$$

$$(K_D + S_0 + E_0) \times ES = S_0 \times E_0 + ES^2 \quad \Rightarrow$$

$$ES^2 - (K_D + S_0 + E_0) \times ES = - S_0 \times E_0 \quad \Rightarrow$$

$$ES^2 - (K_D + S_0 + E_0) \times ES + ((K_D + S_0 + E_0)/2)^2 = ((K_D + S_0 + E_0)/2)^2 - S_0 \times E_0 \Rightarrow$$

$$(ES - (K_D + S_0 + E_0)/2)^2 = ((K_D + S_0 + E_0)/2)^2 - S_0 \times E_0 \quad \Rightarrow$$

$$ES = (K_D + S_0 + E_0)/2 - \text{square root } (((K_D + S_0 + E_0)/2)^2 - S_0 \times E_0)$$

In order to use this for the calculation of ES throughout the time course, ES_n is calculated by essentially the same equation, with the exception that S₀ is substituted by S_{n-1} and E₀ is substituted by E_{n-1}:

$$ES_n = (K_D + S_{n-1} + E_{n-1})/2 - \text{square root } (((K_D + S_{n-1} + E_{n-1})/2)^2 - S_{n-1} \times E_{n-1})$$