Thermodynamic consistency of complex enzymatic reactions with empty routes

Dmitry Yu. Murzin\textsuperscript{a,}\textsuperscript{*}, Alexander K. Avetisov\textsuperscript{b}

\textsuperscript{a}Åbo Akademi University, Biskopsgatan 8, FIN-20500 Turku/Åbo, Finland
\textsuperscript{b}Karpov Institute of Physical Chemistry, Vorontzovo pole 10, 103064 Moscow, Russia

Received 16 November 2006; received in revised form 10 June 2007; accepted 13 July 2007
Available online 20 July 2007

Abstract

Complex reaction schemes in heterogeneous, homogeneous or enzymatic catalysis often contain reaction routes, which do not result in chemical transformations. Existence of these empty routes imposes thermodynamic constraints on the values of kinetic parameters, e.g. rate coefficients in the steps, which constitute the empty routes. Thermodynamic analysis is presented in connection with reaction of 7,8-dihydrofolate and NADPH to form 5,6,6,8-tetrahydrofolate and NADP, catalyzed by dihydrofolate reductase.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Complex reactions; Empty routes

1. Steady-state kinetics of complex reactions

Kinetic modelling of complex reaction networks, consisting of a variety of participating species, has been extensively discussed in the literature for homogeneous catalytic, biochemical and heterogeneous catalytic reactions (Horiuti, 1973; Temkin, 1979; Murzin and Salmi, 2005; Murzin and Touroude, 1993).

Overall reaction equations are linear combinations of chemical equations of steps, and they are obtained by the addition of chemical equations of stages multiplied by certain numbers (positive, negative or zero). Following Horiuti (1973) these numbers (could be also fractional) are called stoichiometric and must be chosen in such a way that the overall equations contain no intermediates.

A set of stoichiometric numbers of the stages producing an overall reaction equation is called after Horiuti a “reaction route”. Routes must be essentially different and it is impossible to obtain one route from another through multiplication by a number, although their respective overall equations can be identical. In fact, any linear combination of routes $N^{(1)}$ and $N^{(2)}$ of a given reaction (infinite combinations) will also be a route of the reaction $N^{(3)} = C_1 N^{(1)} + C_2 N^{(2)}$, where $C_1$ and $C_2$ are arbitrary constant numbers. Although the basis of routes (e.g. a set of linear independent reaction routes, none of which can be represented as a linear combination of others) can be chosen in different ways, the number of basis routes for a given reaction mechanism is determined in a unique way. The number of basic routes is calculated according to Horiuti–Temkin rule (Horiuti, 1973; Temkin, 1979; Murzin and Salmi, 2005; Murzin and Touroude, 1993): $P = S + W - J$, where $P$ is the number of routes. $W$ reflects the way the intermediates are linked to each other and most often is the number of conservation (balance) equations, relating the surface coverage of adsorbed species and vacant sites in heterogeneous catalysis or concentrations of homogeneous catalyst(enzyme)–substrate complexes and free forms of homogeneous catalysts(enzymes). Additionally $W$ could account, for example, for overall neutrality of catalyst surface in case of ionic intermediates (Murzin and Touroude, 1993) or stoichiometry of intermediates generated and consumed in the same steps (Babkova et al., 1970). $J$ is the number of intermediates (including vacant sites or free forms of homogeneous catalysts), $S$ is the number of steps.

Linear independence of routes does not mean that corresponding overall equations are linearly independent. The route is an empty one, as it does not result in any chemical transformation.

In case of reactions with empty routes the main focus in the literature is in analyzing the kinetic equations for non-empty routes. However, presence of intermediates, which
are not directly involved in these non-empty routes, but appear in empty routes, through a complex relation influences the overall kinetics.

The analysis of linear mechanisms in case of multi-route reactions and derivation of the corresponding steady-state kinetic equations valid for enzymatic reactions as well were a subject of several contributions (King and Altman, 1956; Volkenstein and Golstein, 1966; Yablonskii et al., 1991).

2. Thermodynamic consistency of enzymatic reactions

Once the reaction network is established it is essential for kinetic modelling to specify kinetic and thermodynamic parameters. Since parameters in a mechanistic model have a physicochemical meaning, there are certain constraints. The rates of forward and reverse reactions are related through thermodynamics, e.g. the De Donder equation \( r_+/r_- = e^{\Delta G/RT} \), where \( A \) is the reaction affinity, expressed by \( A = RT \ln K \prod C_i^{n_i-2} \). These relationships provide possibility of determining the value of a rate constant for the reverse reaction, if the rate of the forward one and equilibrium constants are known. For an empty route with the overall changes in Gibbs energy should be equal to zero, the equilibrium constant (i.e., multiplication of equilibrium constants of reaction steps, which are defined as the ratio of forward and reverse constants) is equal to unity (Helfferich, 2001). In the present contribution, analysis of thermodynamic consistency of rate constants is performed in connection with a complex enzymatic reaction.

We would like to consider reaction of 7,8-dihydrofolate (A) and NADPH (B) to form 5,6,6,8-tetrahydrofolate (D) and NADP (C), catalyzed by dihydrofolate reductase (E). The chemistry of this reaction was reviewed recently by Benkovic and Hammes-Schiffer (2003). This example was addressed several times in the literature (Happel and Sellers, 1992, 1995; Poland, 1989; Chen and Chern, 2003) in connection to derivation of reaction rates in complex networks. The rate constants for the rather complicated dihydrofolate reaction network; referred as four-node pyramidal in the literature (Poland, 1989) gives \( K_{eq}^{N(5)} = K_{11} K_{10}/K_{11} \).

Calculation of this value using the rate constants reported in the literature (Poland, 1989) gives \( K_{eq}^{N(5)} = 0.544 \) or 0.68, if the value of \( k_{-6} \) is taken from Benkovic and Hammes-Schiffer (2003). This result is remarkably close to unity, especially taking into account the errors associated with the measurements of kinetic binding. For instance deviations reported for kinetic binding constants \( k_{-1} = 3.5 \pm 1.5 \) and \( k_{-11} = 85 \pm 20 \) (Fierke et al., 1987) could be the reason for these somewhat negligible deviations of the equilibrium constant from unity. Using the data from Benkovic and Hammes-Schiffer (2003) it is enough to assume that \( k_{-1} = 2.38 \) (from the interval 2 / 5) to get \( K_{eq}^{N(5)} = 1 \), which does not mean necessarily that this particular constant should be corrected, but is presented here just for illustration purposes.

In addition to the thermodynamic consistency of the empty route, discussed above, one should also verify such consistency for a combination of steps, which does not, however, necessarily constitutes a reaction route. For example there should be a relationship between the parameters for steps 1 and 2 and steps 3 and 4, respectively, in Table 1. It is clear that these two

---

Table 1

<table>
<thead>
<tr>
<th>Steps</th>
<th>( N^{(1)} )</th>
<th>( N^{(2)} )</th>
<th>( N^{(3)} )</th>
<th>( N^{(4)} )</th>
<th>( N^{(5)} )</th>
<th>( k_+ )</th>
<th>( k_- )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E + B = EB</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>EB + A = EA</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>E + A = EA</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>EA + B = EB</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>EAB = ECD</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>EC = E + C</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>7</td>
<td>ECD = D + EC</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>ECD = C + ED</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2.4</td>
</tr>
<tr>
<td>9</td>
<td>ED = D + E</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>300</td>
</tr>
<tr>
<td>10</td>
<td>EB + C = EBC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>11</td>
<td>EC + B = EBC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-1</td>
<td>1</td>
</tr>
</tbody>
</table>
combinations of steps give the same equation, e.g. E + A + B = EAB, which means, that the changes in the Gibbs energy for these two combinations should be the same, thus $K_1 K_2 = K_3 K_4$. Analogously it can be demonstrated that there is a relationship between thermodynamic parameters for the following sequences of steps 6, 7 and 8, 9, leading to $K_6 K_7 = K_8 K_9$. Analysis of the terms $K_1 K_2 / K_3 K_4$ and $K_6 K_7 / K_8 K_9$ utilizing the data from Table 1 demonstrates that they are equal to 1.03 and 1.01, once again highlighting remarkable thermodynamic consistency of the values of kinetic parameters reported in Poland (1989) and Chen and Chern (2003), although they were not checked in the original publications for the adherence to thermodynamics.

3. Conclusions

Complex reaction networks often contain empty routes, e.g. sequence of elementary steps that do not result in chemical transformations. Thermodynamic analysis of empty routes requires, that the overall changes of the Gibbs energy along these routes are zero, meaning also, that the equilibrium constant $K$ is equal to unity. As an example of enzymatic catalysis, reaction of 7,8-dihydrofolate and NADPH to form 5,6,6,8-tetrahydrofolate and NADP, catalyzed by dihydrofolate reductase was considered and thermodynamic consistency of experimentally determined values of kinetic parameters was evaluated.

References