RELAXATION SPECTRA OF TWO-STEP MECHANISMS AS MEASURED BY THE NADH-BINDING TO GLUTAMATE DEHYDROGENASE

Manfred Kempfle
Physikalisch-chemische Abteilung des Physiologisch-chemischen Instituts der Universität, Nussallee 11, 5300 Bonn, Germany

Temperature-jump relaxation experiments performed with glutamate dehydrogenase and the reduced coenzyme nicotinamide adenosine dinucleotide show two clearly separated relaxation times. Three of the simplest and most plausible mechanisms attributable to two relaxation times are treated here theoretically and the concentration dependence of the relaxation times is determined in each case with different experimental conditions. This makes it possible to distinguish between the mechanisms and also to determine the rate constants of the different reaction steps.

Relaxation kinetic experiments are not only a powerful tool in determining the single rate constants of chemical reaction steps but also in deciding between different possible reaction mechanisms.

This paper deals with the second case: it will be shown from the concentration dependence of the measured relaxation times we are able to conclude which reaction mechanism is present.

For those who are not familiar with relaxation kinetics a very brief introduction is given. For more detailed information they should refer to recent publications (Eigen and DeMaeyer, 1963; Czerlinsky, 1966; Hammes, 1968; Yapel and Lumry, 1971).

The concept of relaxation in a physical process relates to the time delay between a sudden change in external conditions and the readjustment of the system in equilibrium to the change. Almost any chemical equilibrium can be
perturbed rapidly by stepwise or periodical changes of external parameters according to the van t'Hoff equation

\[ \delta \ln K = \left( \frac{\partial \ln K}{\partial T} \right) \delta T_{\text{p},E} + \left( \frac{\partial \ln K}{\partial p} \right) \delta p_{\text{T},E} + \left( \frac{\partial \ln K}{\partial E} \right) \delta E_{\text{T},p} + \ldots, \]

where \( K \) represents the equilibrium constant of the reaction, \( T \) the temperature, \( p \) the pressure, and \( E \) the electric field strength.

The following internal re-equilibration (the "relaxation process") is now characterized by a time constant \( \tau \), the so-called relaxation time, and can be monitored by suitable techniques. If this chemical re-equilibration process includes several reactions, there is a spectrum of time constants (the relaxation spectrum) which can be expressed in terms of the rate constants by well-defined mathematical transformations (Eigen, 1957). The analysis of the relaxation phenomena becomes fairly simple if the perturbation is kept small to admit the linearization of the rate equations in terms of deviations of the concentrations from their reference values (deviations not larger than 10%).

The one-step equilibrium reaction between an enzyme \( E \) and a ligand \( L \) is a useful introduction to the general principle in the derivation of the expression for the relaxation times, because of its simplicity:

\[ E + L \xrightleftharpoons[{k_1}]{k_2} EL, \quad K = \frac{k_{21}}{k_{12}} = \frac{[E][L]}{[EL]} \]  

where \([E], [L], [EL]\) are the equilibrium concentrations and \( k_{12}, k_{21} \) are the rate constants for the forward (from left to right in (1)) and for the backward reaction step respectively.

As a consequence of a rapidly applied perturbation the system is suddenly produced in a nonequilibrium state. From it the system relaxes to a new equilibrium. The expression for the occurring single relaxation time of this mechanism is obtained by analyzing the time-dependence of system (1) as it shifts to achieve its new equilibrium conditions.

Let us assume that the perturbation has been carried out by a temperature jump and that the equilibrium constant \( K \) for the reaction (1) is dependent on temperature (due to the van t'Hoff equation \( \Delta H^0 \neq 0 \)). It is immaterial whether \( \Delta H^0 \) is positive or negative, since the mathematical expression for the relaxation time is independent of the direction in which the equilibrium shifts after the perturbation.

Using the law of mass action (the general principle of reaction kinetics) we can describe the rate equations for the equilibrium

\[ -\frac{dE}{dt} = -\frac{dL}{dt} = \frac{d[EL]}{dt} = k_{12}E \cdot L - k_{21}C, \quad C = EL. \]