# Dynamic Analysis of Enzyme Systems

## An Introduction

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With 318 Figures and 68 Tables

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# Preface

This book is concerned with a quantitative analysis of dynamic behavior of various enzymatic reaction systems by computer simulation. The authors and coworkers have been engaged in cooperative research since 1975, seeking to clarify the catalytic and regulatory characteristics of enzymatic reactions *in vivo* and control mechanisms suitable for enzyme technology. Rather than "enzyme kinetics" generally known in enzymology, this research has employed an approach called "enzyme dynamics" which concentrates on the exact schematic representation of an actual reaction mechanism, derivation of rate equation on the basis of the scheme, and computer simulation of its dynamic behavior (numerical solution of the rate equation and explanation of kinetic and regulatory properties of the enzymatic reaction).

A rate equation representing the behavior of enzymatic reactions is generally expressed by a set of nonlinear differential equations. The analytic solution of rate equations is therefore impossible in general, making it necessary to introduce some approximations in order to analyze the experimental data in enzyme kinetics. For example, under an assumption of excess substrate against enzyme in a closed system, we commonly use the linear approximation for the early period of reaction, the quasi-steady-state approximation based on putative maintenance of steady state in enzyme species, and the rapid-equilibrium approximation assuming instantaneous equilibration in complex formation and between complexes. The kinetic characteristics obtained by these approximations do not always reflect the dynamic behavior of actual enzymatic reactions. Furthermore, enzymatic reactions in *in vivo* systems and reactors in enzyme technology operate in open systems, often violating the underlying conditions for the approximations. This may also be true for some cases of multi-enzyme systems in a closed system.

A new approach to the analysis of dynamic behavior of actual enzyme systems is desired and has been developed as enzyme dynamics in conjunction with the progress in molecular description of biochemical pro-

cesses and simulation techniques using computers. Quantitative analysis of dynamic behavior of biochemical reactions in the cellular environments leads to an elucidation of the relationship between the structure and function of biological systems on the molecular basis, which is one of the most important goals in biology. Dynamic analysis by computer simulation will now furnish the biochemical research in this direction with a potent and quantitative means for understanding the *in vivo* behavior of enzymatic reaction and its regulation at the molecular level. The dynamic simulation can readily be extended to the systems exploited in biochemical and biomedical engineering.

Our cooperative research has resulted in the development of a computer program of numerical integration suitable for stiff nonlinear differential equations, dynamic analysis of basic enzymatic reactions and complex enzyme systems, and formulation of optimization methods applicable to estimation of reaction schemes and determination of reaction control processes. The research results were published by Japan Scientific Societies Press in 1981 in the form of a book in Japanese titled "Dynamics of Enzymatic Reactions" with the support of a grant-in-aid for publication of scientific research results from the Ministry of Education, Science and Culture of Japan. The English translation of that edition serves as the basis for the present volume with the results of our more recent research added to revise and expand the content. Our publication objective with this edition is to broadly disseminate the methods and applications of the approach of enzyme dynamics and also to encourage research with this approach which will lead to further understanding of the dynamic characteristics of *in vivo* systems of enzymatic reactions and detailed mechanisms of metabolic processes.

The many illustrations of time courses obtained from simulations of important enzymatic reactions in the book provide a comprehensive introduction to the methods and applications of enzyme dynamics for students and researchers in biochemistry, physiology and bioengineering. Accordingly, the book is written and arranged in the form of a textbook rather than as an edited compilation of chapters by individual contributors, although the contents of each chapter are based on the results of contributors' own research. We acknowledge the cooperation of Yoichi Aso and Satoru Kuhara of Kyushu University, Masahiro Okamoto of the University of Michigan, and Yukihiro Eguchi and Kiyokazu Nemoto of the Mitsui Knowledge Industry. In writing we attempt as clear a description as possible and use the same notations throughout the book. Explanations emphasize the analysis of dynamic behavior rather than the mathematical treatment of models and rate equations. As the material reflects the results

of research by the contributors, the corresponding results of other researchers are not necessarily reviewed or referred to exhaustively.

The introductory chapters deal with the derivation and approximation methods for rate equations of enzymatic reactions. The major portion is devoted to the dynamic simulation and analysis of some fundamental and important systems such as reactions of the Michaelis-Menten-type and allosteric enzymes, linear chain systems with feedback loops, branched reaction system, and complex systems with particular characteristics. The dynamic simulation is performed by numerical integration of the rate equations with the computer procedures and their principle and applicability are described in detail. Analysis emphasizes the dynamic aspects of the systems functioning under various conditions in closed and open systems as well as reaction-diffusion system. The simulation is further applied to the determination of reaction schemes and parameters for some enzyme systems.

Our cooperative research began with a research project of enzyme technology supported by the Office for Life Science Promotion of the Institute of Physical and Chemical Research. We wish to thank Prof. Akiyoshi Wada of the University of Tokyo, the project leader, and Dr. Shotaro Kohtsuki of the Mitsui Knowledge Industry for their encouragement and management of the research at that stage. Comments and remarks received from many scientists are appreciated regarding our research results and the Japanese edition.

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## Contents

# INTRODUCTION

# Role of Dynamic Analysis of Enzyme Systems in Biochemical Research

Intensive studies in biochemistry and molecular biology have sought an elucidation at the molecular level of the relationship between the structures and functions of living systems such as cell organelles, cells, tissues, organs and individual organisms. Progress in the field has revealed that the biological functions all stem from biochemical reactions within the systems. Furthermore, it is now established that such biochemical reactions are integrated into a biochemical network which circulates the flows of matter-energy and information in order to operate and regulate the biological processes responsible for certain functions of the system [1]. In fact, every network of biochemical reactions in a living system is decomposed into their constituent enzyme systems like metabolic pathways, and the sequence of individual reactions in each enzyme system is identified by the specific enzymes and metabolites involved. We have now accumulated a wealth of knowledge on the molecular mechanisms of enzymatic reactions as well as the molecular properties of enzymes and metabolites.

**Objectives of dynamic analysis of enzyme systems**

The next stage of the research is concerned with the molecular dynamics of enzyme systems which describes the mechanism for generation of dynamic behavior in terms of molecular structure. That is, we attempt to examine in detail how the molecules of enzymes and metabolites participating in enzymatic reactions are integrated to play their roles in the function and structure of an enzyme system. This investigation will eventually lead to understanding of the mechanisms by which the biochemical networks generate and regulate the biological processes.

This chapter was written by Naoto Sakamoto.

Thus, it is essential for determination of the relationship between structure and function in a biochemical network that we make a dynamic analysis of the enzyme systems working as network constituents. By dynamic analysis we mean analysis of the dynamic behavior of a system to explain input-output relations in that system. The dynamic behavior of enzyme systems can, in turn, be related to the functions of an entire network by integrating these systems into the network.

An enzyme system is basically formed of sequential enzymatic reactions and enzymes certainly play most important roles in it. The physico-chemical properties of enzyme molecules have been extensively studied, for example, for their molecular weight and subunit structure, sedimentation and diffusion coefficients, and extinction coefficient. The catalytic activity is characterized by the Michaelis constant and maximum velocity, and further determination of the rate constants themselves is steadily progressing. The catalytic mechanisms are now investigated even at the level of quantum chemistry.

The analysis of these data on structural and functional properties of enzymes provides fundamental knowledge for dynamic analysis in temporal and spatial dimensions of every individual enzyme in an enzyme system. Hence, we can say that we have reached the stage to start the dynamic analysis of enzyme systems. Furthermore, it is hoped that such analysis will lead to the molecular dynamics of enzyme systems.

**System model and computer simulation**

A powerful procedure for the dynamic analysis of enzyme systems is furnished by a system model and computer simulation. The model represents a postulated molecular mechanism for input-output relations of the enzyme system. Computer simulation of such a model can yield a quantitative description of the relationships between system's dynamic behavior and molecular structure.

The approach using modeling and simulation can be related to the understanding of structure and function of a biochemical network as shown in Fig.1. "Biochemical network" is a real system to be studied, which is comprised of enzyme systems as structural components of the system. Input-output relations for the functions of the system correspond to the flows of matter-energy and information through the biochemical network. Experiments and observations on a biochemical network are performed to obtain data regarding its structure and dynamic behavior. These data are analyzed to construct a system model for the network which describes the input-output relations of the system. "Modeling" thus is the process of constructing a system model based on an analysis of the data of a real system.

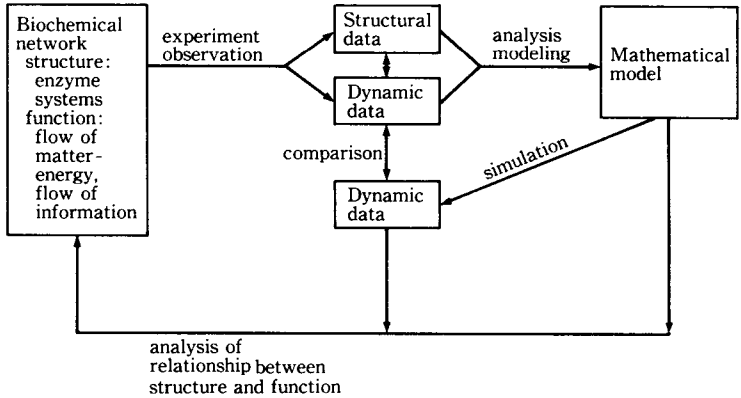


**Fig. 1** Dynamic analysis of biochemical network.

By a system model we mean a mathematical model in general, since it is basically a set of instructions for generating output (*i.e.*, behavioral data of the model) from input through "simulation." The simulation is a computational process which is now carried out commonly by a digital computer according to the model instructions suitably encoded as a program. A mathematical model representing the dynamic behavior of a biochemical network is often expressed by nonlinear differential equations. Its simulation is performed by numerical solution of these equations using computer procedures. Thus, computer simulation or, more specifically, numerical solution is often interchangeable with simulation in our terminology. With respect to the flow of data in Fig. 1, we can say essentially that a real system is a source of behavioral data, supplying "real system data" through experiment and observation, and that a system model is a source of generating other behavioral data, "model-generated data" through simulation [2].

In this approach the authenticity of the system model is of greatest concern, that is, how accurately a model represents the real system of interest. In order to understand how a real system works, we use models to embody hypotheses about the underlying and often inaccessible structure of reality. The criterion for accuracy or validity of a model is the degree of agreement between real system data and model-generated data. A model is "replicatively valid" if it matches data already acquired from the real system, and "predictively valid" if it can provide data before they are acquired from the real system. A model is "structurally valid" if it not only reproduces the observed behavior of the real system, but truly reflects the

way in which the real system operates to produce this behavior [2].

**Enzyme dynamics and enzyme kinetics**

In the dynamic analysis of enzyme systems, we aim at the complete construction of structurally valid models for biochemical networks. As indicated in Fig. 1, comparison of model-generated data with real system data can lead to a prediction of the unknown structure and function of a biochemical network and a suggestion of experiments and observations to be performed for their detection. The real system data newly obtained are analyzed to construct a more structurally valid model which can generate yet more refined data. With this procedure we can gradually explain the complex structures and functions and finally their relationships in the biochemical networks.

Hence, emphasizing its objectives and procedures, we call the dynamic analysis of enzyme systems "enzyme dynamics." In contrast, so-called "enzyme kinetics" is mainly concerned with the estimation of kinetic parameters and reaction schemes for enzymatic reactions. These static data from the kinetics can be related to the dynamic behavior of systems through the dynamics. An effective accomplishment with the dynamics is naturally dependent on productive experiments and good models.

**Open and closed systems**

Most kinetic experiments with individual enzymatic reactions have been performed in a closed system (*i.e.*, in a test tube, or a common laboratory system), and much data are available on the kinetic properties of many enzymes. The biochemical networks of living systems, on the other hand, operate in open system in almost all situations. Hence, the relevant data of dynamic behavior in open system are essential for the dynamic analysis of enzyme systems.

Originally, open and closed systems arise from the concept defined in thermodynamics. The thermodynamic system is defined as a geometrical space in the universe, which is the objective of our interest for experiment or observation. The system is classified in three types with respect to the property of its boundary which exists between the system and its surroundings:

isolated (adiabatic) system : Neither energy nor matter can penetrate the boundary.

closed system : Energy can go through the boundary, but matter cannot.

open system : Both energy and matter are exchangeable through the boundary.

In the isolated and closed systems, equilibrium is the most stable state in

which the entropy does not change according to the laws of equilibrium thermodynamics.

An open system, however, has to be treated with nonequilibrium thermodynamics, which describes the state of a system using time and boundary conditions in addition to state variables. In an open system a nonequilibrium state, called a steady state, can be generated when no change in entropy takes place because of the balance between its production in processes of the system and the influx from the exterior through the exchange of energy and matter. At a steady state in an open system neither entropy nor concentrations of chemical species change temporally. Hence, the steady state is also called "dynamic equilibrium." Although the concentrations are constant through time, a molecular exchange of chemical species continuously occurs between the system and the surroundings, because the steady state cannot be maintained without the flow of energy and matter through the boundary.

As mentioned above, the elucidation of relationships between the structures and functions of biochemical networks in living systems requires the accumulation and analysis of data concerning the dynamic behavior of enzyme systems in open system. Such experiments and observations, however, have not been seriously performed, not only because they are not fully recognized as essential, but also because an appropriate design of experimental systems is difficult. At present, dynamic analysis through modeling and simulation would be a rather efficient means of accumulating the behavioral data of enzyme systems in open system. Moreover, the analysis could suggest the experimental systems which produce useful real system data of the behavior in open system.

**Methods for dynamic analysis**

Dynamic analysis is performed for various types of enzyme systems from a single enzymatic reaction to an entire cellular metabolism consisting of thousands of reactions in linear, branched or cyclic pathways. Enzyme systems also display diversified functions such as autocatalysis, feedback and feedforward loops, a coupled reaction system subject to interactive inhibition and activation, and a two-factor system with an output of threshold characteristics. In studying the dynamic characteristics of enzyme systems, their environments such as closed or open, and homogeneous or distributed systems have to be taken into consideration in addition to their variety of structure and function. It is naturally expected that a system behaves differently in various environments and plays different roles.

Finding of a method and/or principle for treating this diversity in a unified manner would lead to the discovery of a fundamental law relating

the functions to the structures in living systems. For the time being, however, the diversified enzyme systems are classified into similar groups with respect to structure or function so that each group is analyzed by its most effective method specifically devised. This would currently be the best approach to dynamic analysis, although an ultimate unification should continue to be sought. Indeed, many groups of enzyme systems can be efficiently analyzed employing the method of enzyme dynamics, which is the primary subject of this book.

As mentioned, the construction of mathematical models for enzyme systems plays an essential role in enzyme dynamics. For the process of modeling, it is of basic importance to clearly specify the conditions under which dynamic behavior is to be characterized by the model. First, we have a problem as to whether the reaction process in an enzyme system is to be described as a deterministic or stochastic process in the model. In many cases the deterministic description has been widely applied to the time course of concentration change of chemical species in a system based on the mass-action law.

On the other hand, from the microscopic viewpoint of molecular dynamics, chemical reaction evolves from an efficient collision of reactant molecules, which is a stochastic process at the molecular level. The stochastic approach, however, introduces considerably more variables and parameters in the model than the deterministic approach, resulting in practical difficulty in computation even for the behavior in steady states. If an averaging operation with respect to the number of molecules is done to reduce the computational load, the results can only prove the findings obtained from the deterministic model. Neither the steady-state assumption nor averaging operation are desirable for enzyme dynamics. It is necessary, in any event, that we first choose either the deterministic or stochastic approach to describe the individual enzymatic reactions in the model.

Secondly, the standpoint in modeling is dependent on whether the system is autonomous or nonautonomous. In enzyme systems the activity is regulated to meet one of two types of requirements. One type keeps a constant production of necessary metabolites in order to maintain the homeostasis in living systems. The other leads the time course of the system in a definite direction for the homeoresis such as development, differentiation and morphogenesis. The enzyme systems for the former type are autonomous since the structure and kinetic parameters are constant temporally and the regulatory mechanisms in the system are closed. The enzyme systems for the latter type are nonautonomous; the time term appears explicitly in the model and the structure and kinetic

parameters of the system vary with respect to time. The regulatory mechanism governing the nonautonomous system, however, remains unknown as to the manner of change of the structure and kinetic parameters. Kinetic study of these systems is possible solely by imbedding the system in a more extended and complex system or by introducing a macroscopic approximation in the model.

Consequently, this book concentrates on the analytical procedure employing the deterministic and autonomous treatments, which is most commonly applicable to enzyme systems. These treatments have the following characteristics and shortcomings. The first is a problem of nonlinearity. Enzymatic reaction is intrinsically nonlinear owing to the formation mechanism of the enzyme-substrate complex and application of the mass-action law in the deterministic approach. Allosteric enzymes in particular acquire important functions in metabolic regulation from the higher-order nonlinearity due to polymeric structure and cooperativity. Hence, the enzyme system as a whole becomes very highly nonlinear and complex.

The nonlinear differential equations of rate equations represent the time course of the system, and in general have no analytic solutions. Some approximations should be employed to examine the dynamic behavior of the system. Methods for this purpose have been developed over many years and are classified into two kinds. One includes the quasi-steady-state, rapid-equilibrium and linear approximations, which essentially remove the nonlinearity from the rate equation and transform it into an appropriate form for analytical treatment. The other type of approximation is performed by the method for numerical solution of a rate equation in using digital computers.

Since the pioneering research of Michaelis and Menten on enzyme kinetics, approximation by assuming the rapid equilibrium or steady state among enzyme species has been commonly used to transform a rate equation (a system of ordinary differential equations) into a system of algebraic equations, from which the relationship between reaction rate and substrate concentration ($S$-$v$ relationship) is derived. In fact, the Michaelis constant ubiquitously employed for characterizing the kinetic property of enzyme is determined from treatment of the experimental data with a relationship based on the quasi-steady-state approximation. Enzyme kinetics thus aims mainly at an estimation of kinetic parameters and reaction scheme for every enzymatic reaction from the experimental data applying the $S$-$v$ relationship in the form of rational function of substrate concentration.

In reaction kinetics further evaluation is done for rate constants which