METABOLIC INHIBITORS

volume IV

Metabolic Inhibitors

A Comprehensive Treatise

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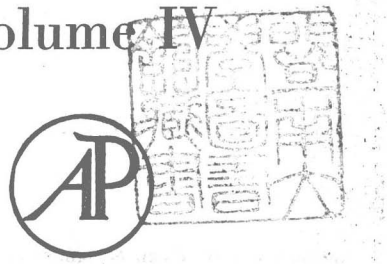
Volume IV



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Preface

Volume IV of this treatise continues the coverage begun in Volume III of recent developments in studies of inhibition of metabolic and enzymic processes. Articles on inhibition of photosynthesis, blood clotting, protein synthesis, fatty acid metabolism, and phospholipid metabolism are included as well as on inhibition of specific enzyme reactions such as amino acid activation, amino acid hydroxylation, and cyclic AMP formation. This volume also contains a contribution dealing specifically with allosteric inhibition and allosteric inhibitors.

It is inevitable that some overlapping of subject matter will be found in Volume IV, redundancy is difficult to avoid or rectify in a treatise such as this. However, the reader will probably benefit from any inadvertent repetition since coverage of similar topics from different points of view is bound to make for a deeper understanding of the subject.

Much of the burden of preliminary organization of the articles was carried by our coeditor Rolf Hochster; we dedicate this volume to his memory.

Our sincere thanks are due the contributors for their efforts in preparing their articles and for their patience in the face of the unavoidable delays encountered. We are grateful to the many authors and publishers who gave permission to use previously published material and to the staff of Academic Press for their fine spirit of cooperation during production of this volume. Mr. Paul Deroo again prepared the Subject Index for which we express our sincere appreciation.

Morris Kates
J. H. Quastel

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CHAPTER 1

Allosteric Inhibitors

R. W. Miller

I. INTRODUCTION[1]

Feedback inhibition has become a well-established concept in the search for metabolic control mechanisms that regulate the rate of metabolite synthesis. The discovery that end products of biosynthetic pathways may inhibit directly the initial reactions of the pathway provides an attractive explanation for the precise metabolic homeostasis that is essential in all organisms. However, direct *in vivo* observations of enzymic feedback phenomena generally are not possible due to problems of transport of metabolites across membranes and the presence of other regulatory processes such as enzyme repression. Therefore, the study of the effects of inhibitors on the quaternary structure, tertiary structure, catalytic activity, and affinity for substrates of purified enzymes has produced a great deal of information as to how inhibitors may modulate the rates of catalyzed processes.

A particularly significant type of enzyme inhibition has been examined intensively in recent years and has emerged as a plausible mechanism for the general hypothesis of feedback regulation of metabolic transformations. Enzymes that display anomalous order (higher than one) with respect to a subtrate or coenzyme produce sigmoidal plots of reaction velocity versus ligand concentrations. Inhibitors are often observed to increase the order of the reaction with respect to substrate or to give inhibition patterns that lead to a change in the V_{max}, K_m (substrate concentration which gives half-maximal reaction velocity, referred to as $S_{0.5}$), or other parameters that describe binding and rates of catalysis by enzymes. Inhibitors that bind at a site remote from the catalytic site and produce effects of this type are referred to as allosteric inhibitors or negative effectors as opposed to compounds that modify, sterically block, bind at, or otherwise interfere directly with the catalytic site. Depending on the type of chemical transformation inhibited (i.e., single- or multiple-substrate reactions), characteristic and diagnostic kinetic data are obtained which may lead to detailed physical and chemical studies. These combined methods have greatly increased knowledge of regulatory phenomena. In this area it is somewhat artificial to distinguish between allosteric inhibitors and allosteric activators since similar mechanisms have been proposed (*1*) for both. However, since

[1] Standard abbreviations are used for purine and pyrimidine nucleotides. NAD and NADP are used for nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, respectively.

allosteric inhibitors account for many cases of feedback regulation this chapter will emphasize the former. Table I lists allosteric inhibitors and the enzymes they may control. It is apparent that by far the largest number of compounds represented are purine and pyrimidine nucleotides. Indeed, Atkinson (*2*, *3*) has proposed that many enzymes respond to the relative concentrations of adenine nucleotides (AMP, ADP, ATP, cyclic 3′,5′-AMP) rather than to the concentration of a single inhibitor or activator.

Many enzymes have more than one negative effector. While there are many well-known examples of feedback inhibition listed in Table I, there is no apparent limitation of allosteric inhibition to end products of metabolic pathways. Inorganic anions and cations, antibiotics, complex carbohydrates, and other seemingly unrelated compounds can bind at remote receptor sites and cause allosteric effects which, while they may represent control mechanisms in the general sense, are not associated with feedback control of metabolism. However, metabolic regulation is clearly an extremely complex and subtle process, and relationships between biosynthetic reactions and their allosteric inhibitors are not necessarily always as obvious as end-product inhibition.

II. LIMITING MODELS FOR ALLOSTERIC INHIBITION

Several limiting mechanistic models have been proposed to explain the behavior of allosteric enzymes. The models attempt to account for the sigmoidal shape of plots of reaction velocity (or fractional binding-site saturation) against substrate or effector concentration. Data of this type have generally suggested cooperative interactions between subunits of an oligomeric protein where the binding of effector molecules at a regulatory site may change the binding constant for substrate (*K*-type system) or may influence the activity of the catalytic site (*V*-type system) or both. Curve 2 of Fig. 1 (*4*) shows cooperative behavior for a well-documented allosteric enzyme, aspartate transcarbamylase. This enzyme catalyzes the first step in pyrimidine biosynthesis and is inhibited by the end product of that pathway, cytidine triphosphate. Cytidine triphosphate is a true allosteric inhibitor; that is, it decreases the reaction rate at low substrate concentrations by increasing the dependence of the rate on the concentration of substrate molecules.

Control curve 2 of Fig. 1 shows a dependence on substrate concentration that is anomalous since ordinary substrate dependence would

give a typical hyperbolic curve. An effector molecule could modify this cooperative effect so as to produce more or less cooperativity. The negative effector CTP increases the order of the dependence of activity on substrate concentration in the case of aspartate transcarbamylase (curve 3). The cooperative interactions associated with molecules of the same ligand are designated homotropic, while interactions involving the inhibitor (CTP) or the activator (AMP) and the substrate are heterotropic. The allosteric activator AMP changes the curve to a rectangular hyperbola which is the response expected in the absence of substrate-induced cooperative interactions. Heating the enzyme also eliminates cooperativity (Curve 1, Fig. 1). With the native enzyme the rate, in the absence of ligands other than substrate, is slower than expected from a normal hyperbolic response. This effect suggests that the substrate must

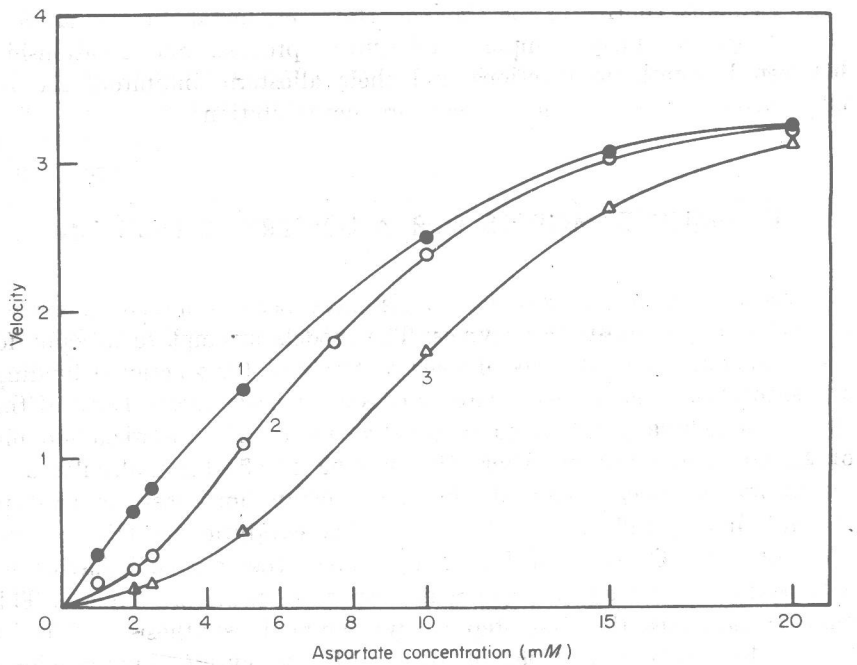

FIG. 1. Dependence of reaction rate on aspartate concentration at pH 7.0. Velocity expressed as units of activity per milligram of protein $\times 10^{-3}$. Legend: ○, native enzyme; ●, heated enzyme; and △, native enzyme in the presence of 0.1 m*M* CTP. Assay mixtures contained 0.05 *M* tris at pH 7.0, 3.3 m*M* carbamyl phosphate, asparatate as indicated, and 0.75 μg enzyme protein/ml. [Reprinted from Weitzman and Wilson (*4*) by permission of the copyright owner. Copyright (1966) by the American Society of Biological Chemists.]

convert the enzyme to a more active form for maximum reaction velocity to occur and is designated positive homotropic cooperativity. If in the presence of an activator the rate of reaction at low substrate concentration is greater than that expected from a hyperbolic relationship, the implication is that increased substrate concentrations convert the enzyme to a less active form. This type of interaction is labeled negative homotropism.

Since both types of behavior are reported for some enzymes it is important to diagnose the type of cooperativity present in a given system. Also, some models allow only positive homotropic cooperativity and kinetic analysis enables the investigator to distinguish between models. Allosteric inhibitors lead to more positive homotropic cooperativity. In this chapter inhibitors falling in the category of effectors that change cooperativity between substrate molecules by binding at a site remote from the catalytic center will be considered mainly. Inhibitors such as reaction products that bind or interfere directly at the catalytic site, some of which also may be described as feedback inhibitors, will not be considered. Also, noncompetitive inhibition (i.e., change of V_{max}), which is often observed with multisubstrate enzymes, will not be discussed unless evidence for interaction of the inhibitor at a separate allosteric site or for an effect on the cooperative behavior of one of the substrates is available. Noncompetitive inhibition can result from direct interaction with the active site in multisubstrate cases. Just as some allosteric activators may produce negative homotropic interactions for substrate molecules, allosteric inhibitor molecules may exhibit negative or positive homotropic interactions depending on whether increasing the concentration of the inhibitor produces more or less drop in activity than expected from a normal hyperbolic inhibitor response. Again, diagnosis of the type of cooperativity for the inhibitor being observed is important in ascribing kinetic phenomena to an appropriate mechanistic model.

Allosteric inhibition may be competitive, in the sense that it is completely overcome at high substrate concentrations (pure K-type system), or partially noncompetitive. Lineweaver–Burk plots obtained with allosteric enzymes may be straight lines but are generally curved [see Fig. 2 (*5*)]. Thus, in the presence or absence of heterotropic ligands a hyperbolic response of reaction velocity to substrate concentration yields a straight Lineweaver–Burk plot, while positive homotropic interactions give an upward curvature and negative homotropism causes a downward curvature. Mahler and Cordes (*5a*) and Farago and Denes (*5b*) give further graphical information that is useful in identifying the different types of cooperative interactions.