Laboratory Guide to Biochemistry, Enzymology, and Protein Physical Chemistry

A Study of Aspartate Transcarbamylase

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Foreword

The study of a single well-chosen substance, here aspartate transcarbamylase, can provide an excellent basis for a laboratory course. The student is introduced to a variety of scientific ideas and to many experimental and interpretive techniques. This enzyme is readily available, is relatively stable, has an extensive literature, and its behavior has many facets: substrate inhibition, a large change in structure upon homotropic activation by substrates, allosteric stimulation by ATP, allosteric inhibition by CTP synergistic with UTP, positive cooperativity for substrates, negative cooperativity for CTP binding, and dissociation and reassembly of subunits C_3 and R_2 from the holoenzyme C_6R_6. In addition to the known biochemical aspects of these properties, the results obtained here can be interpreted in the light of the high-resolution X-ray diffraction structures of the *T* and *R* forms, the low-angle X-ray scattering results, and the large number of mutants now available by recombinant DNA methods. Future development of this course could also involve part of these methods, as well as the carefully chosen experiments described here.

This approach resembles research more than the approaches one usually finds in biochemical laboratory courses. A consistent development of ideas about a single enzyme, which shows so many facets in its behavior, is sure to hold the interest of the student. Moreover, one explores a depth, and reasons to move forward, that are an essential part of research.

Some years ago, I conceived and put into practice a somewhat similar plan for the laboratory part of a first semester of general chemistry. The student prepared a transition metal complex, made a chemical analysis, analyzed spectra (UV, visible, infrared, NMR), and then meas-

ured kinetics of a subsequent transformation of the complex. This program was very successful in that it incorporated preparative, analytical, and physical–chemical methods as essential parts of a larger purpose. I expect similar success with the plan so well described in this guide.

William N. Lipscomb

Harvard University

Preface

This book presents an integrated set of laboratory experiments devised for the teaching of biochemistry, enzymology, and protein physical chemistry. The program relies entirely on the use of a unique enzyme, aspartate transcarbamylase, which exhibits all of the catalytic and regulatory properties characteristic of allosteric enzymes. It can be easily and reversibly dissociated into subunits that have only the catalytic properties and thus behave as a simple Michaelian enzyme. A comprehensive study of this system leads to the use of numerous biochemical techniques.

Biochemistry and molecular biology are young sciences, under rapid development. It is desirable that students of these subjects have the benefit of concrete training directly connected to today's practices as developed in research laboratories. Aspartate transcarbamylase is presently the object of high-level fundamental research, and the following teaching program benefits directly from the results obtained. An up-to-date list of references guides the reader to more detailed information.

The book begins with a general presentation of aspartate transcarbamylase and of the concepts of cooperativity and allostery. A study of the expression of the gene coding for the enzyme is then described, followed by a chapter dealing with the purification of the enzyme and its dissociation into subunits. The next chapter describes a wide experimental program concerning the structure and physical chemistry of the enzyme and its subunits. Finally, an extensive study of the catalytic and regulatory properties of these proteins is proposed. Each chapter begins with a presentation of the underlying theory. Appendixes provide useful information, including constants and units. Within each experimental section several questions are posed, and answers are given at the end of the book.

Initially devised for the course Biochemistry and Cellular Biology and Physiology at the Pierre et Marie Curie University of Paris (Paris VI), this successful program is now used in other universities both in France and abroad. It is addressed primarily to undergraduate biochemistry students as well as to those following courses that involve biochemistry (medical schools; schools of pharmacy and agronomy). The book is sufficiently detailed to be of interest also to graduate students. It is a flexible program that can be adapted in terms of the number and nature of experiments performed in order to organize 50 to 200 hours of laboratory classes.

Teachers will find at the end of the book a nonexhaustive list of complementary pedagogic possibilities that are offered by the system studied but are not presented in detail in this edition. The authors are at your disposal should you wish to examine closely any of these possibilities, as well as those emerging from current research. Criticisms and suggestions from our teaching colleagues or students are welcome.

We wish to thank all of our friends who have already helped us with their advice and encouragement, in particular Dr. Anthony Else, who efficiently improved the English version of this book. We especially thank Ms. Jocelyne Mauger (Laboratoire d'Enzymologie of CNRS) for her skillful collaboration in its framing.

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Contents

Chapter 6

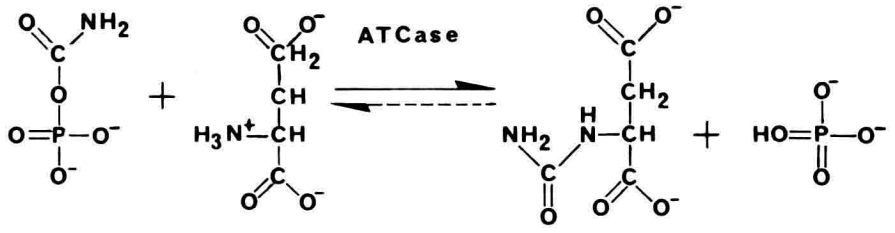
COMPLEMENTARY EXPERIMENTS

APPENDIX

Chapter 1

Aspartate Transcarbamylase

Aspartate transcarbamylase (ATCase) catalyzes the carbamylation of the amino group of aspartate by carbamylphosphate according to the following reaction:



CARBAMYL PHOSPHATE **ASPARTATE** **CARBAMYL ASPARTATE** **PHOSPHATE**

This reaction is the first step of the pyrimidine pathway. This important enzyme is present in all organisms, and it is the ATCase from *Escherichia coli* that will be considered here. In this microorganism, the catalytic activity of ATCase is feedback inhibited by the end product CTP, which in this way limits its own production. On the other hand, its activity is stimulated by a purine, ATP. This antagonism is one of the phenomena which, in the cell, tend to balance the production of purines and pyrimidines for the biosynthesis of nucleic acids (Fig. 1).

As in most cases of feedback inhibition or activation, the effectors CTP and ATP have molecular structures that are very different from those of the substrates, and they bind to the enzyme on specific sites that

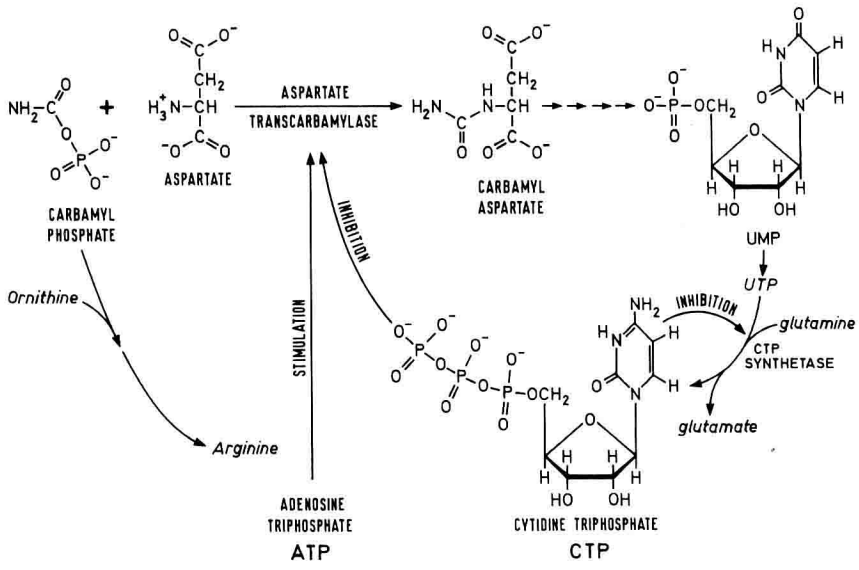


Figure 1. Metabolic regulation of ATCase activity in *E. coli*.

are distinct from the catalytic site. For this reason, they are called allosteric effectors.

The term "allosteric enzyme" applies to enzymes that exhibit feedback inhibition or activation and was extended to enzymes or proteins that show cooperative effects for substrate binding to the catalytic sites. This is the case with ATCase, to which the substrate aspartate binds cooperatively at the catalytic sites. The binding of the first molecule of this substrate facilitates the binding of subsequent molecules. The effectors CTP and ATP bind competitively to the regulatory sites.

1. STRUCTURE OF ATCase

ATCase is a globular protein of molecular mass 305,646 Da and diameter about 100 Å. It is made up of two *catalytic subunits* (relative molecular mass M_r = 101,913) which are trimers of identical *catalytic chains* (M_r = 33,971) and of three *regulatory subunits* (M_r = 33,940) which are dimers of identical *regulatory chains* (M_r = 16,970). Thus, the native enzyme contains six chains of each type (Fig. 2).

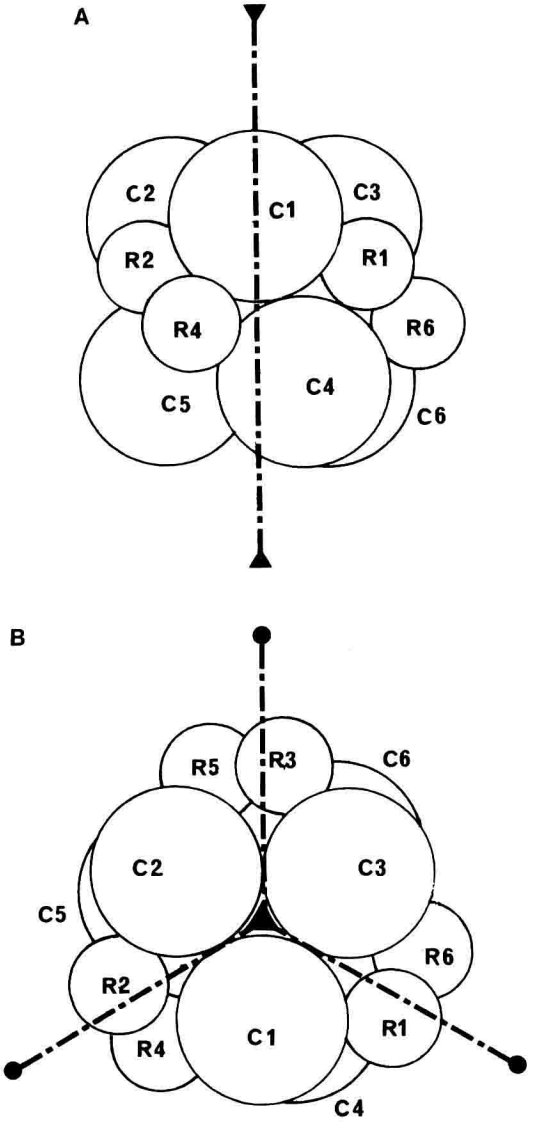


Figure 2. Schematic representation of the quaternary structure of ATCase. The catalytic, C, and regulatory, R, chains are numbered from 1 to 6. (A) View of ATCase perpendicular to the threefold axis. (B) View of ATCase along the threefold axis; the twofold axes of symmetry are indicated. (C) Expanded view of A allowing visualization of the organization of the molecule in trimers of catalytic chains (Cat) and dimers of regulatory chains (Reg). (Adapted from Honzatko *et al.*, 1982, with permission from W. Lipscomb.)

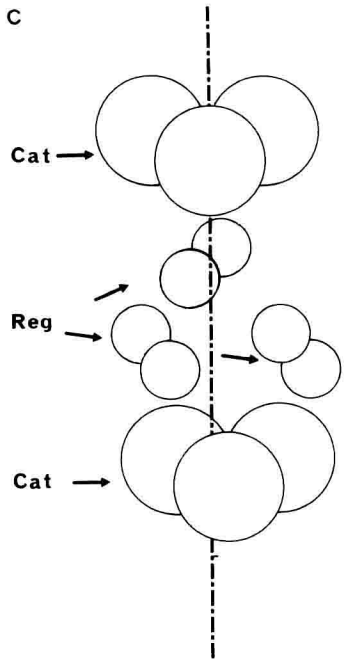


Figure 2. (*continued*)

The catalytic sites are located at the interface between two catalytic chains within the same trimer and involve side chains of amino acids from both chains (Fig. 3).

The two catalytic subunits (C_1-C_2-C_3 and C_4-C_5-C_6) are maintained in contact by the three regulatory dimers (R_1–R_6, R_2–R_4, R_3–R_5). Figure 4 shows how a regulatory dimer (for example, R_1–R_6) links two catalytic chains (C_1 and C_6) that do not belong to the same trimer.

The primary structure (sequence of amino acids) of the two types of chains is known (Fig. 5). Among the noticeable features are the following: the regulatory chain does not contain tryptophan, but contains four cysteine residues, clustered in the C-terminal region of this chain, which play an important structural role, as discussed later. The catalytic chain contains two tryptophan residues whose presence can be used for fluorescence studies (Royer *et al.*, 1987).

The three-dimensional structure of ATCase was determined by X-ray crystallography in W. Lipscomb's laboratory (Harvard University)

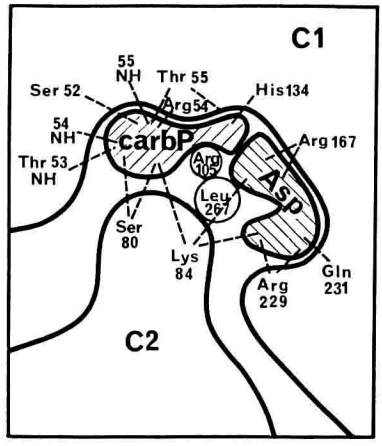


Figure 3. Schematic representation of the active site indicating the amino acids that are involved in substrate binding. CarbP and Asp refer to the approximate positions of the two substrates. The dashed lines indicate the ionic and hydrogen bonds and other polar contacts between groups that are separated by less than 3.5 Å. Numbers associated with the designations for amino acids indicate position in the primary structure. The same is true for some α-amino groups (NH). (Adapted from Volz *et al.*, 1986, with permission from W. Lipscomb.)

and is presently known to a resolution of 2.5 Å. The structure of a catalytic chain-regulatory chain pair is schematically represented in Fig. 6, which also shows the position of the zinc atom that is associated to the regulatory chain R_1. Each regulatory chain contains such a zinc atom, which is linked to the sulfur atoms of the four cysteine residues. The presence of the zinc ensures the correct structural organization of this domain of the regulatory chains, allowing their interaction with the

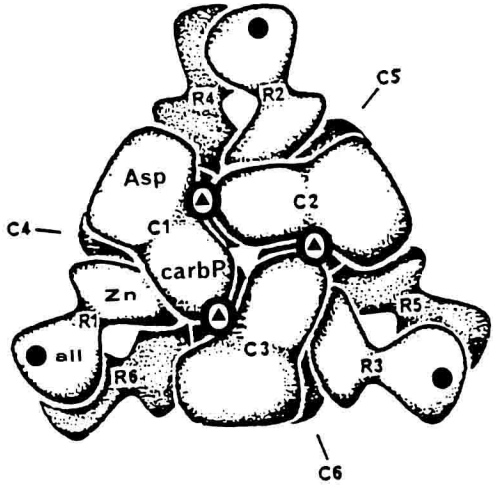


Figure 4. Representation of the quaternary structure of ATCase. ▲, Region of the catalytic site; ●, region of the regulatory site. Asp, Aspartate binding domain of the catalytic chain; carbP, carbamylphosphate binding domain of the catalytic chain; Zn, zinc domain of the regulatory chain; all, allosteric domain of the regulatory chain where the effector binding sites are localized. (Adapted from Krause *et al.*, 1987, with permission from W. Lipscomb.)