Enzymes in Industry

Production and Applications

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The *cover illustration* shows the Stuart-Briegleb model of lysozyme. It was produced by E. E. POLYMEROPOULOS of ASTA Pharma AG, Frankfurt, Federal Republic of Germany. The data were taken from the Cambridge Crystallographic Data System (F. H. Allen, O. Kennard and R. Taylor, *Acc. Chem. Res.* **16**, 146–153 (1983)). The picture was generated on a Silicon Graphics workstation using the molecular modelling program MOLCAD of the Darmstadt Institute of Technology, Darmstadt, Federal Republic of Germany.

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Foreword

"Enzymes" was the last major manuscript I edited for "*Ullmann's Encyclopedia of Industrial Chemistry*" – and it was the most rewarding article in my ten-year period as executive editor of Ullmann's.

Its careful planning was headed by Professor Günter Schmidt-Kastner at Bayer AG in Wuppertal, Federal Republic of Germany, who invited two other German enzyme specialists to a small symposium with the sole purpose of determining the contents of the article and proposing potential authors. The two other specialists were Professor Maria-Regina Kula of Düsseldorf University and Dr. Georg-Burkhard Kreße of Boehringer Mannheim, Tutzing. The help and advice given by these three experts are gratefully acknowledged.

The response of the suggested authors was very enthusiastic. The list of authors (page XIII) reads like an international "Who is Who" of industrial enzymology.

I accepted the publisher's invitation to make a monograph from the Ullmann article with a great deal of pleasure and cooperation with the authors has once again been perfect. All chapters have been updated, and the literature is covered until late 1989. Even the latest nomenclature of DNA modification methyltransferases has been included.

The book is recommended to all those non-enzymologists who want to know what is really happening as regards enzymes in industry. The term "industry" is used in its broadest sense: it includes the production of enzymes as well as their use in the production of bulk products, such as detergents, glucose, or fructose; in fine chemicals synthesis; in food processing and food analysis; in clinical diagnosis and therapy; and – last but obviously not least – in genetic engineering.

I hope that the reader will derive as much pleasure from reading this book as I did from producing it.

The Editor

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Abbreviations

A	adenosine
ACA	acetamidocinnamic acid
ACL	α-amino-ε-aprolactam
ADH	alcohol dehydrogenase
ADI	acceptable daily intake
ADP	adenosine 5′-diphosphate
Ala	alanine
Arg	Arginine
AMP	adenosine 5′-monophosphate
ATC	D,L-2-amino-Δ^2-thiazoline-4-carboxylic acid
ATP	adenosine 5′-triphosphate
C	cytidine
cDNA	copy DNA
CL	citrate lyase
CMP	cytidine 5′-monophosphate
CoA	coenzyme A
CS	citrate synthetase
CTP	cytidine 5′-triphosphate
d	deoxy
dam	gene locus for *E. coli* DNA adenine methylase (N^6-methyladenine)
*dcm*I	gene locus for *E. coli* DNA cytosine methylase (5-methylcytosine)
dd	dideoxy
ddNTP	dideoxynucleoside 5′-triphosphate
DEAE	diethylaminoethyl
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxynucleoside 5′-triphosphate
DOPA	3-(3,4-dihydroxyphenylalanine)[3-hydroxy-L-tyrosine]
dpm	decays per minute
ds	double-stranded
E.C.	Enzyme Commission
F6P	fructose 6-phosphate
fMet	*N*-formylmethionine
FMN	flavin mononucleotide
$FMNH_2$	flavin mononucleotide, reduced
G	quanosine
GDP	guanosine 5′-diphosphate

Glu	glutamic acid
Gly	glycine
GMP	guanosine 5′-monophosphate
GOD	glucose oxidase
GOT	glutamate – oxaloacetate transaminase
G6P	glucose 6-phosphate
GPT	glutamate – pyruvate transaminase
GTP	guanosine 5′-triphosphate
3-HBDH	3-hydroxybutyrate dehydrogenase
HFCS	high-fructose corn syrup
*hsd*M	*E. coli* gene locus for methylation
*hsd*R	*E. coli* gene locus for restriction
*hsd*S	*E. coli* gene locus for sequence specificity
IDP	inosine 5′-diphosphate
Ile	isoleucine
INT	iodonitrotetrazolium chloride
ITP	inosine 5′-triphosphate
LDH	lactate dehydrogenase
Lys	lysine
^{m}(superscript)	methylated
MDH	malate dehydrogenase
Met	methionine
M6P	mannose 6-phosphate
mRNA	messenger RNA
MTT	3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide
N	any nucleotide
NAD	nicotinamide – adenine dinucleotide
NADH	nicotinamide – adenine dinucleotide, reduced
NADP	nicotinamide – adenine dinucleotide phosphate
NADPH	nicotinamide – adenine dinucleotide phosphate, reduced
NMN	nicotinamide mononucleotide
NTP	nucleoside 5′-triphosphate
p	phosphate groups
^{32}P	phosphate groups containing ^{32}P phosphorus atoms
p_i	inorganic phosphate
PEP	phosphoenolpyruvate
6-PGDH	6-phosphogluconate dehydrogenase
Phe	phenylalanine
PMS	5-methylphenazinium methyl sulfate
poly(dA)	poly(deoxyadenosine 5′-monophosphate)
pp_i	inorganic pyrophosphate
Pro	proline
PRPP	phosphoribosyl pyrophosphate
Pu	purine
Py	pyrimidine

r	ribo
RNA	ribonucleic acid
RNase	ribonuclease
SAM	S-adenosylmethionine
SMHT	serine hydroxymethyltransferase
ss	single-stranded
T	thymidine
TMP	thymidine 5′-monophosphate
tRNA	transfer RNA
TTP	thymidine 5′-triphosphate
U	uridine
UMP	uridine 5′-monophosphate
UTP	uridine 5′-triphosphate
Val	valine

Bacteriophages:

fd
ghl
M13
N4
PBS1
PBS2
SPO1
SP6
SP15
T3
T4
T5
T7
XP12
λ
λgt11
ΦSM11
ΦX174

Plasmids:

pBR322
pBR328
pSM1
pSP64
pSP65
pSPT18, pSPT19
pT7-1, pT7-2
pUC 18, pUC 19
pUR222

Eukaryotic viruses:

Ad2
SV40

Contents

1. Introduction

Enzymes are the catalysts of biological processes. Like any other catalyst, an enzyme brings the reaction catalyzed to its equilibrium position more quickly than would occur otherwise; an enzyme cannot bring about a reaction with an unfavorable change in free energy unless that reaction can be coupled to one whose free energy change is more favorable. This situation is not uncommon in biological systems, but the true role of the enzymes involved should not be mistaken.

The activities of enzymes have been recognized for thousands of years; the fermentation of sugar to alcohol by yeast is among the earliest examples of a biotechnological process. However, only recently have the properties of enzymes been understood properly. Indeed, research on enzymes has now entered a new phase with the fusion of ideas from protein chemistry, molecular biophysics, and molecular biology. Full accounts of the chemistry of enzymes, their structure, kinetics, and technological potential can be found in many books and series devoted to these topics [1.1]–[1.5]. This chapter reviews some aspects of the history of enzymes, their nomenclature, their structure, and their relationship to recent developments in molecular biology.

1.1. History

Detailed histories of the study of enzymes can be found in the literature [1.6], [1.7].

Early Concepts of Enzymes. The term "enzyme" (literally "in yeast") was coined by KÜHNE in 1876. Yeast, because of the acknowledged importance of fermentation, was a favorite subject of research. A major controversy at that time, associated most memorably with LIEBIG and PASTEUR, was whether or not the process of fermentation was separable from the living cell. No belief in the necessity of vital forces, however, survived the demonstration by BUCHNER (1897) that alcoholic fermentation could by carried out by a cell-free yeast extract. The existence of extracellular enzymes had, for reasons of experimental accessibility, already been recognized. For example, as early as 1783, SPALLANZANI had demonstrated that gastric juice could digest meat in vitro, and SCHWANN (1836) called the active substance pepsin. KÜHNE himself appears to have given trypsin its present name, although its existence in the intestine had been suspected since the early 19th century.

Enzymes as Proteins. By the beginning of the 20th century, the protein nature of enzymes had been recognized. Knowledge of the chemistry of proteins drew heavily on the improving techniques and concepts of organic chemistry in the second half of the 19th century; it culminated in the peptide theory of protein structure, usually credited to FISCHER und HOFMEISTER. However, methods that had permitted the separation and synthesis of small peptides were unequal to the task of purifying enzymes. Indeed, there was no consensus that enzymes were proteins. Then, in 1926, SUMNER crystallized urease from jack bean meal and announced it to be a simple protein. Against this, WILLSTÄTTER argued that enzymes were not proteins but "colloidal carriers" with "active prosthetic groups." However, with the conclusive work by NORTHROP and his colleagues who isolated a series of crystalline proteolytic enzymes, beginning with pepsin in 1930, the protein nature of enzymes was established.

The isolation and characterization of intracellular enzymes was naturally more complicated and, once again, significant improvements were necessary in the separation techniques applicable to proteins before, in the late 1940s, any such enzyme became available in reasonable quantities. Because of the large amounts of accessible starting material and the historical importance of fermentation experiments, most of the first pure intracellular enzymes came from yeast and skeletal muscle. However, as purification methods were improved, the number of enzymes obtained in pure form increased tremendously and still continues to grow. Methods of protein purification are so sophisticated today that, with sufficient effort, any desired enzyme can probably be purified completely, even though very small amounts will be obtained if the source is poor.

Primary Structure. After the protein nature of enzymes had been accepted, the way was clear for more precise analysis of their composition and structure. Most amino acids had been identified by the early 20th century. The methods of amino acid analysis then available, such as gravimetric analysis or microbiological assay, were quite accurate but very slow and required large amounts of material. The breakthrough came with the work of MOORE and STEIN on ion-exchange chromatography of amino acids, which culminated in 1958 in the introduction of the first automated amino acid analyzer [1.8]. Modern machines have lowered the time required for an analysis to less than 1 h and the amount of protein required to < 1 μg [1.9].

The more complex question – the arrangement of the constituent amino acids in a given protein, generally referred to as its primary structure – was solved in the late 1940s. The determination in 1951 of the amino acid sequence of the β-chain of insulin by SANGER and TUPPY [1.10] demonstrated for the first time that a given protein does indeed have a unique primary structure. The genetic implications of this were enormous. The introduction by EDMAN of the phenyl isothiocyanate degradation of proteins stepwise from the N-terminus, in manual form in 1950 and subsequently automated in 1967 [1.11], provided the principal chemical method for determining the amino acid sequences of proteins. The primary structures of pancreatic ribonuclease [1.12] and egg-white lysozyme [1.13] were published in 1963. Both of