Methods of Enzymatic Analysis

edited by H. U. Bergmeyer

METHODS OF ENZYMATIC ANALYSIS

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Hans-Ulrich Bergmeyer

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Foreword

An analytical method is of value when its specificity, reproducibility and sensitivity are high and when the expenditure of labour, time and material are low. Even at the time when enzymes were understood and defined solely by their action many enzymatic methods were suggested to fulfil these conditions. However, on the whole these methods were not adopted, mainly because of the unreliability of the enzyme preparations and the elaborate nature of the assays.

During the last decade *Otto Warburg*'s methods of enzymatic analysis (crystalline enzymes used in single and combined "optical tests") provided a new basis for work in this field. The majority of the methods used at present are based on measurements of the 340 mμ band of DPNH and TPNH and were developed during this period. The renewed interest in enzymatic analysis began mainly with the determination of alcohol (ADH method) in forensic medicine. At the same time other enzymatic methods of analysis were thoroughly revised and new types worked out.

In addition to the general progress in enzymology and intense activity in the special field of enzymatic analysis, a new factor has appeared: the commercial production of biochemical reagents for analysis. We are indebted to the pioneer spirit of a few firms, but more especially to the research workers who have continued their work in this field in spite of all difficulties.

By and large, the transition from the possible use of enzymatic analysis to its varied applications in pure and applied biochemistry has been made. This then is the situation at the time of publication of this collection of methods and is therefore an argument in favour of the considerable effort required. Many of the workers who have been engaged in recent developments report on their experience here. It is hoped that this book will increase the exchange of ideas between various groups and thus will attract new recruits to the field of enzymatic analysis.

This book and the methods described are intended to be of practical service. I hope that it will succeed.

Marburg/Lahn (Germany), March 1963 *Theodor Bücher*

Preface

Today enzymes are much more widely used as analytical tools than in the past. New methods have been worked out for the use of those enzymes which are now available in a high state of purity, and existing techniques have been improved.

This laboratory manual contains the working directions for carefully tested procedures. The analytical methods have been contributed by authors who have had many years of experience in their particular field of study. Consequently, the reader is certain to have reliable experimental directions which represent the latest advances in this branch of science.

Any type of laboratory can make good use of this book, since it is designed on strictly practical lines. The individual chapters are arranged according to the substances to be determined (not according to the enzymes used). Grouping by substrates is employed since today the reagents are commercially available (with the exception of a few special enzymes). For these exceptions a short résumé of isolation techniques is included. The possibility of attempting the preparation of these enzymes is then easily judged by the reader, bearing in mind the facilities available to him.

The book is divided into four sections. The first section outlines the basis of enzymatic analysis and gives general experimental instructions for the techniques of measurement and for the disintegration of cells and tissues. The two main sections which follow give detailed directions for the determination of substrates and assay of enzyme activities. The commercially available enzymes, coenzymes, substrates and some less common reagents are described in the fourth section "Biochemical Reagents". Once again the practical aspects are emphasized, and information necessary for the application of these reagents to enzymatic analysis, such as sources, stability and purity required, is given.

The publisher and the editor agreed not to use the International Nomenclature for enzymes and coenzymes proposed by the International Union of Biochemistry at present, apart from referring to it in the Section on "Biochemical Reagents".

I am especially grateful to Prof. Th. Bücher, Marburg, for useful advice, both in the planning of the book and with regard to particular details. My thanks are also due to all the authors for their sympathetic co-operation, without which the book could not have been written, and for the willingness with which they adapted their contributions to the proposed form of the text.

I wish to thank Dr. H. Grünewald of Verlag Chemie for his valuable help in dealing with the large amount of material. I was also greatly helped by him in the editing of the manuscripts. To the publishers go my best thanks for their fruitful co-operation.

Tutzing/Oberbayern (Germany), March 1963 *Hans-Ulrich Bergmeyer*

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Abbreviations

Enzymes

ADA	Adenosine deaminase Adenosine aminohydrolase
ADH	Alcohol dehydrogenase Aldehyde reductase Alcohol:NAD oxidoreductase
ALD	Aldolase Fructose-1,6-diphosphate aldolase Ketose-1-phosphate aldehyde-lyase
AP	Phosphatase, alkaline Phosphomonoesterase I
ARS	Arylsulphatase Steroid sulphatase Sulphatase Sterol sulphate sulphohydrolase
CAH	Carbonic anhydrase
CAT	Catalase H_2O_2:H_2O_2 oxidoreductase
CCE	Citrate cleavage enzyme
CE	Citrate condensing enzyme
ChE	Cholesterin esterase
CHTR	Chymotrypsin
CPK	Creatine phosphokinase Creatine kinase ATP-creatine transphosphorylase ATP:creatine phosphotransferase
DNase	Deoxyribonuclease
EN	Enolase Phosphopyruvate hydratase D-2-Phosphoglycerate hydrolase
ETF	Electron transferring flavoprotein
FDPase	Fructose-1,6-diphosphatase
FUM	Fumarase
GAD	General acyl-CoA dehydrogenase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase D-Glyceraldehyde-3-phosphate: NAD oxidoreductase (phosphorylating)
GDH	Glycerol-1-phosphate dehydrogenase α-Glycerophosphate dehydrogenase L-Glycerol-3-phosphate:NAD oxidoreductase
GK	Glycerokinase ATP:glycerol phosphotransferase
Gl-I	Glyoxalase I S-Lactoyl-glutathione methyl-glyoxal-lyase (isomerizing)
GlDH	L-Glutamic dehydrogenase L-Glutamate:NAD oxidoreductase (deaminating)
Gly-R	Glyoxylic acid reductase Glycollate:NAD oxidoreductase
GOD	Glucose oxidase β-D-Glucose:O_2 oxidoreductase
GOT	Glutamate-oxaloacetate transaminase L-Aspartate:2-oxoglutarate aminotransferase
G6Pase	Glucose-6-phosphatase
G6P-DH	Glucose-6-phosphate dehydrogenase Zwischenferment D-Glucose-6-phosphate:NADP oxidoreductase
GPT	Glutamate-pyruvate transaminase L-Alanine:2-oxoglutarate aminotransferase
GR	Glutathione reductase NAD(P)H_2:glutathione oxidoreductase
GRD	β-Glucuronidase β-D-Glucuronide glucuronohydrolase
HK	Hexokinase ATP:D-hexose-6-phosphotransferase

HOADH	β-Hydroxyacyl-CoA dehydrogenase L-(+)-β-Oxyacyl dehydrogenase β-Hydroxybutyryl-CoA dehydrogenase L-3-Hydroxyacyl-CoA:NAD oxidoreductase
ICDH	Isocitric dehydrogenase L$_S$-Isocitrate:NADP oxidoreductase
IDH	Isocitric dehydrogenase L$_S$-Isocitrate:NADP oxidoreductase
LAP	Leucine aminopeptidase
LDH	Lactic dehydrogenase L-Lactate:NAD oxidoreductase
MDH	Malic dehydrogenase L-Malate:NAD oxidoreductase
MK	Myokinase, adenylate kinase ATP:AMP phosphotransferase
OCT	Ornithine carbamyl transferase
PFA	1-Phosphofructoaldolase Fructose-1-phosphate aldolase
6-PG-DH	6-Phosphogluconic dehydrogenase Gluconic-6-phosphate dehydrogenase 6-Phospho-D-gluconate:NADP oxidoreductase (decarboxylating)
PGI	Phosphoglucose isomerase Glucose phosphate isomerase Phosphohexose isomerase D-Glucose-6-phosphate ketol-isomerase
PGK	Phosphoglycerate kinase ATP:D-3-phosphoglycerate 1-phosphotransferase
PGluM	Phosphoglucomutase D-Glucose-1,6-diphosphate:D-glucose-1-phosphate phosphotransferase
PGM	3-Phosphoglycerate mutase D-2,3-Diphosphoglycerate:D-2-phosphoglycerate phosphotransferase
PK	Pyruvic kinase Pyruvate kinase ATP:pyruvate phosphotransferase
PL-D	Phospholipase D Lecithinase D
POD	Peroxidase Donor:H_2O_2 oxidoreductase
PTA	Phosphotransacetylase Acetyl-CoA:orthophosphate acetyltransferase
RDH	Ribitol dehydrogenase
RNase	Ribonuclease Polyribonucleotide:2-oligonucleotidotransferase (cyclizing)
R5P-I	Ribose-5-phosphate isomerase
SDH	Sorbitol dehydrogenase Polyol dehydrogenase
SDPase	Sedoheptulose-1,7-diphosphatase
SP	Phosphatase, acid
TIM	Triosephosphate isomerase D-Glyceraldehyde-3-phosphate ketol-isomerase
TK	Thiokinase
TR	Trypsin
UDPG-DH	Uridine diphosphoglucose dehydrogenase UDPglucose:NAD oxidoreductase
XOD	Xanthine oxidase
ZF	Zwischenferment (see G6P-DH)

Coenzymes and Substrates

ADP	Adenosine-5′-diphosphoric acid Adenosine-5′-pyrophosphoric acid
A-2′,5′-DP	Adenosine-2′,5′-diphosphate
A-3′,5′-DP	Adenosine-3′,5′-diphosphate
AMP	Adenosine-5′-monophosphoric acid Muscle adenylic acid
A-2′-MP	Adenosine-2′-monophosphate

A-3′-MP	Adenosme-3′-monophosphate
AP-DPN	Acetylpyridine analogue of diphosphopyridine nucleotide
ATP	Adenosine-5′-triphosphoric acid
Bz-CoA	Benzoyl-CoA
C-2′,3′-MP	Cytidine-2′,3′-monophosphate, cyclic
CoA	Coenzyme A
CoA-SH	Coenzyme A
CoA-S-S-CoA	Coenzyme A, oxidized
CP	*Creatine phosphate* Phosphocreatine
DAP	Dihydroxyacetone phosphate
DPN	Diphosphopyridine nucleotide Nicotinamide-adenine dinucleotide (NAD)
DPNH	Diphosphopyridine nucleotide, reduced Nicotinamide-adenine dinucleotide, reduced (NADH)
E-4-P	D-Erythrose-4-phosphate
FAD	Flavine adenine dinucleotide
FDP	D-Fructose-1,6-diphosphate *Harden-Young* ester
FMN	Flavine mononucleotide Lactoflavine phosphate
F-1,6-P_2	D-Fructose-1,6-diphosphate
F-1-P	D-Fructose-1-phosphate
F-6-P	D-Fructose-6-phosphate *Neuberg* ester
GAP	D-Glyceraldehyde-3-phosphate
Ga-1-P	D-Galactose-1-phosphate
α-GP	L-Glycerol-1-phosphate α-Glycerophosphate
G-1-P	D-Glucose-1-phosphate
G-6-P	D-Glucose-6-phosphate *Robison* ester
GSH	Glutathione
GSSG	Glutathione, oxidized Glutathione disulphide
HMG-CoA	β-Hydroxy-β-methylglutaryl-CoA
HXN	Hypoxanthine
ISN	Inosine
KG	α-Ketoglutaric acid α-Oxoglutaric acid
OAA	Oxaloacetate
PEP	Phosphoenolpyruvate
2-PG	D-2-Phosphoglycerate
3-PG	D-3-Phosphoglycerate *Nilsson-Lohmann* ester
6-PG	D-6-Phosphogluconic acid
1,3-PG-P	1,3-Diphosphoglycerate
1,3-diPG	1,3-Diphosphoglycerate
2,3-PG-P	2,3-Diphosphoglycerate
2,3-diPG	2,3-Diphosphoglycerate
Py	Pyruvate
RNA	Ribonucleic acid
R-5-P	D-Ribose-5-phosphate
Ru-1,5-P_2	D-Ribulose-1,5-diphosphate
Ru-5-P	D-Ribulose-5-phosphate
SDP	D-Sedoheptulose-1,7-diphosphate
S-1,7-P_2	D-Sedoheptulose-1,7-diphosphate
S-7-P	D-Sedoheptulose-7-phosphate
TPN	Triphosphopyridine nucleotide Nicotinamide-adenine dinucleotide phosphate (NADP)
TPNH	Triphosphopyridine nucleotide, reduced Nicotinamide-adenine dinucleotide phosphate, reduced (NADPH)
TPP	Thiamine pyrophosphate Cocarboxylase Aneurin pyrophosphate
UDPG	Uridine diphosphoglucose Cowaldenase
UDPGal	Uridine diphosphogalactose
UDPGA	Uridine diphosphoglucuronic acid
U-2′,3′-MP	Uridine-2′,3′-monophosphate, cyclic
UTP	Uridine-5′-triphosphate

Contents

*) For determination with aldolase/TIM/GDH, see p. 246.