

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 m μ 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.

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Abbreviations

Enzymes

ADA	Adenosine deaminase	GDH	Glycerol-1-phosphate dehydrogenase
	Adenosine aminohydrolase		
ADH	Alcohol dehydrogenase		α -Glycerophosphate dehydrogenase
	Aldehyde reductase		
	Alcohol:NAD oxidoreductase		L-Glycerol-3-phosphate:NAD oxidoreductase
ALD	Aldolase		
	Fructose-1,6-diphosphate aldolase	GK	Glycerokinase
	Ketose-1-phosphate aldehyde-lyase	Gl-I	ATP:glycerol phosphotransferase
AP	Phosphatase, alkaline		Glyoxalase I
	Phosphomonoesterase I		S-Lactoyl-glutathione methylglyoxal-lyase (isomerizing)
ARS	Arylsulphatase	GIDH	L-Glutamic dehydrogenase
	Steroid sulphatase		L-Glutamate:NAD oxidoreductase (deaminating)
	Sulphatase		
	Sterol sulphate sulphohydrolase	Gly-R	Glyoxylic acid reductase
CAH	Carbonic anhydrase		Glycollate:NAD oxidoreductase
CAT	Catalase	GOD	Glucose oxidase
	H ₂ O ₂ :H ₂ O ₂ oxidoreductase		β -D-Glucose:O ₂ oxidoreductase
CCE	Citrate cleavage enzyme	GOT	Glutamate-oxaloacetate transaminase
CE	Citrate condensing enzyme		
ChE	Cholesterin esterase		L-Aspartate:2-oxoglutarate aminotransferase
CHTR	Chymotrypsin		
CPK	Creatine phosphokinase	G6Pase	Glucose-6-phosphatase
	Creatine kinase	G6P-DH	Glucose-6-phosphate dehydrogenase
	ATP-creatine transphosphorylase		Zwischenferment
	ATP:creatine phosphotransferase		D-Glucose-6-phosphate:NADP oxidoreductase
DNase	Deoxyribonuclease		
EN	Enolase	GPT	Glutamate-pyruvate transaminase
	Phosphopyruvate hydratase		L-Alanine:2-oxoglutarate aminotransferase
	D-2-Phosphoglycerate hydrolase		
ETF	Electron transferring flavoprotein	GR	Glutathione reductase
FDPase	Fructose-1,6-diphosphatase		NAD(P)H ₂ :glutathione oxidoreductase
FUM	Fumarase		
		GRD	β -Glucuronidase
GAD	General acyl-CoA dehydrogenase		β -D-Glucuronide glucuronohydrolase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase		
	D-Glyceraldehyde-3-phosphate:NAD oxidoreductase (phosphorylating)	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	PGluM PGM	Phosphoglucomutase D-Glucose-1,6-diphosphate: D-glucose-1-phosphate phosphotransferase 3-Phosphoglycerate mutase D-2,3-Diphosphoglycerate:D-2-phosphoglycerate phosphotransferase
ICDH	Isocitric dehydrogenase L ₅ -Isocitrate:NADP oxidoreductase	PK	Pyruvic kinase Pyruvate kinase ATP:pyruvate phosphotransferase
IDH	Isocitric dehydrogenase L ₅ -Isocitrate:NADP oxidoreductase	PL-D	Phospholipase D Lecithinase D
LAP	Leucine aminopeptidase	POD	Peroxidase Donor:H ₂ O ₂ oxidoreductase
LDH	Lactic dehydrogenase L-Lactate:NAD oxidoreductase	PTA	Phosphotransacetylase Acetyl-CoA:orthophosphate acetyltransferase
MDH	Malic dehydrogenase L-Malate:NAD oxidoreductase	RDH	Ribitol dehydrogenase
MK	Myokinase, adenylate kinase ATP:AMP phosphotransferase	RNase	Ribonuclease Polyribonucleotide:2-oligonucleotidotransferase (cyclizing)
OCT	Ornithine carbamyl transferase	R5P-I	Ribose-5-phosphate isomerase
PFA	1-Phosphofructoaldolase Fructose-1-phosphate aldolase	SDH	Sorbitol dehydrogenase Polyol dehydrogenase
6-PG-DH	6-Phosphogluconic dehydrogenase Gluconic-6-phosphate dehydrogenase 6-Phospho-D-gluconate:NADP oxidoreductase (decarboxylating)	SDPase SP	Sedoheptulose-1,7-diphosphatase Phosphatase, acid
PGI	Phosphoglucose isomerase Glucose phosphate isomerase Phosphohexose isomerase D-Glucose-6-phosphate ketol-isomerase	TIM TK TR	Triosephosphate isomerase D-Glyceraldehyde-3-phosphate ketol-isomerase Thiokinase Trypsin
PGK	Phosphoglycerate kinase ATP:D-3-phosphoglycerate 1-phosphotransferase	UDPG-DH XOD ZF	Uridine diphosphoglucose dehydrogenase UDPgucose:NAD oxidoreductase Xanthine oxidase Zwischenferment (see G6P-DH)

Coenzymes and Substrates

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

A-3'-MP	Adenosme-3'-monophosphate	ISN	Inosine
AP-DPN	Acetylpyridine analogue of diphosphopyridine nucleotide	KG	α -Ketoglutaric acid α -Oxoglutaric acid
ATP	Adenosine-5'-triphosphoric acid	OAA	Oxaloacetate
Bz-CoA	Benzoyl-CoA	PEP	Phosphoenolpyruvate
C-2',3'-MP	Cytidine-2',3'-monophosphate, cyclic	2-PG	D-2-Phosphoglycerate
CoA	Coenzyme A	3-PG	D-3-Phosphoglycerate
CoA-SH	Coenzyme A		<i>Nilsson-Lohmann ester</i>
CoA-S-S-CoA	Coenzyme A, oxidized	6-PG	D-6-Phosphogluconic acid
CP	Creatine phosphate Phosphocreatine	1,3-PG-P	1,3-Diphosphoglycerate
DAP	Dihydroxyacetone phosphate	1,3-diPG	1,3-Diphosphoglycerate
DPN	Diphosphopyridine nucleotide	2,3-PG-P	2,3-Diphosphoglycerate
	Nicotinamide-adenine dinucleotide (NAD)	2,3-diPG	2,3-Diphosphoglycerate
DPNH	Diphosphopyridine nucleotide, reduced	Py	Pyruvate
	Nicotinamide-adenine dinucleotide, reduced (NADH)	RNA	Ribonucleic acid
E-4-P	D-Erythrose-4-phosphate	R-5-P	D-Ribose-5-phosphate
FAD	Flavine adenine dinucleotide	Ru-1,5-P₂	D-Ribulose-1,5-diphosphate
FDP	D-Fructose-1,6-diphosphate	Ru-5-P	D-Ribulose-5-phosphate
	<i>Harden-Young ester</i>	SDP	D-Sedoheptulose-1,7-diphosphate
FMN	Flavine mononucleotide	S-1,7-P₂	D-Sedoheptulose-1,7-diphosphate
	Lactoflavine phosphate	S-7-P	D-Sedoheptulose-7-phosphate
F-1,6-P₂	D-Fructose-1,6-diphosphate	TPN	Triphosphopyridine nucleotide
F-1-P	D-Fructose-1-phosphate		Nicotinamide-adenine dinucleotide phosphate (NADP)
F-6-P	D-Fructose-6-phosphate	TPNH	Triphosphopyridine nucleotide, reduced
	<i>Neuberg ester</i>		Nicotinamide-adenine dinucleotide phosphate, reduced (NADPH)
GAP	D-Glyceraldehyde-3-phosphate	TPP	Thiamine pyrophosphate
Ga-1-P	D-Galactose-1-phosphate		Cocarcboxylase
α-GP	L-Glycerol-1-phosphate		Aneurin pyrophosphate
	α -Glycerophosphate	UDPG	Uridine diphosphoglucose
G-1-P	D-Glucose-1-phosphate		Cowaldenase
G-6-P	D-Glucose-6-phosphate	UDPGal	Uridine diphosphogalactose
	<i>Robison ester</i>	UDPGA	Uridine diphosphoglucuronic acid
GSH	Glutathione	U-2',3'-MP	Uridine-2',3'-monophosphate, cyclic
GSSG	Glutathione, oxidized	UTP	Uridine-5'-triphosphate
	Glutathione disulphide		
HMG-CoA	β -Hydroxy- β -methylglutaryl-CoA		
HXN	Hypoxanthine		

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