Enzyme Assays

A Practical Approach

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Preface

Virtually all chemical reactions in living systems are catalysed by enzymes, and the assay of enzyme activity is probably one of the most frequently encountered procedures in biochemistry. Most enzyme assays are carried out for the purpose of estimating the amount of active enzyme present in a cell or tissue, or as an essential part of an investigation involving the purification of an enzyme (see also *Protein Purification: A Practical Approach*). They are also a manifestly integral component of the determination of kinetic parameters, or the investigation of catalytic mechanism.

All too frequently, however, the investigator may choose an assay that is inappropriate to the purpose. It is hoped that this book will help the experimentalist select, and if necessary modify, existing assays, and interpret the data obtained correctly and to the maximum advantage. There is no ideal assay for any enzyme and, in general, the appropriateness of an assay will depend on the nature of the enzyme, its purity, and the purpose of the assay. For following the progress of a purification, convenience and speed may be the prime considerations for which a sacrifice in accuracy or precision may be tolerated. For kinetic and mechanistic work, accuracy and reproducibility are obviously essential. This book will also aid in the design of new assay methods that may be more suitable to the purpose of the investigation than those appearing in the literature, or in the improvement of existing assays.

The assay of enzyme activity is essentially a kinetic measurement and as such there are many pitfalls for the unwary. The first chapter of the book deals with the general principles of enzyme assay and is a comprehensive account of how to avoid these pitfalls, whilst also alerting the worker at the bench to intrinsic properties of the enzyme that may manifest themselves through kinetic assays.

The range of techniques used to measure the rate of an enzyme-catalysed reaction is vast and will depend on the nature of the chemical change and the ingenuity of the investigator. Within these limits a wide scope of methodology is available, and the six subsequent chapters discuss the instrumental techniques most frequently used. The techniques described in Chapters 2–7 are, admittedly, discussed in detail in many excellent texts, review articles, and monographs, and reference to these is made in the individual chapters. However, although theory and applications are discussed in those articles, they do not in general address the unique problems arising from the use of these techniques in enzyme assays.

As several thousand enzyme-catalysed reactions are known, it would have been impossible in a book of this size to deal with all the possible applications of the techniques described here to every known enzyme. However, the

techniques chapters in this book contain experimental protocols that have been carefully chosen to represent the various types of enzyme-catalysed reactions amenable to assay using that particular technique. These then can be adapted to assay enzymes other than those specifically described. The theory underlying each method is introduced together with a description of the instrumentation, sensitivity, and sources of error. The methods discussed cover those most used for enzyme assay and include photometric, electrochemical, radiochemical, and HPLC techniques. The assay of enzymes after gel electrophoresis is an important application, and a separate chapter is devoted to special methods for detecting enzyme activity under these conditions.

Most enzymes are intracellular, and their measured activities may well depend on the method used to disrupt the cells. An associated problem is maintaining enzyme activity in cell extracts or in purified, or partially purified, fractions. The catalytic activity of an expiring enzyme is of little use. Accordingly, a chapter is included on the techniques involved in enzyme extraction, and in stabilizing enzyme activity.

Determination of kinetic parameters is usually undertaken to characterize an enzyme, to provide a quantitative evaluation of substrate specificity, and to study kinetic mechanisms. The increasing availability of desk-top computers and associated software for analysing kinetic data has sometimes led, in our experience, to uncritical application of statistical methods. Such an approach may well mask features of the data that might reveal interesting properties of an enzyme. The penultimate chapter describes how one should apply statistical methods in a rational manner to the analysis of kinetic data, an important topic missing from many enzymology texts.

The final chapter is a critical discussion of buffers and methods of protein estimation and will provide a realistic basis for choosing a system appropriate to the enzyme under investigation.

In summary, this book is a guide to the principles and practice of enzyme assays. It is intended for all those in the life sciences who are concerned with practical enzymology.

Bath R.E.
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Abbreviations

A_{340}	Absorbance at 340 nm
Ace	2-[(2-Amino-2-oxoethyl)-amino]ethanesulphonic acid
ACV	δ-(L-α-Aminoadipyl)-L-cystinyl-D-valine
Ada	*N*-(2-Acetamido)-2-aminodiacetic acid
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate, Adenylate
amp	Ampere
APAD	Acetylpyridine adenine dinucleotide (oxidized)
APADH	Acetylpyridine adenine dinucleotide (reduced)
ATEE	Acetyltyrosine ethyl ester
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
BAEE	Benzoylarginine ethyl ester
Bes	*N*,*N*-Bis(2-Hydroxyethyl)-2-aminoethanesulphonic acid
Bicine	*N*,*N*-Bis (2-hydroxyethyl)glycine
Bis-Tris	Bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane
BSA	Bovine serum albumin
BV_{ox}, BV_{red}	Benzylviologen (oxidized, reduced)
Caps	3-(Cyclohexylamino)-2-hydroxy-1-propanesulphonic acid
CDP	Cytidine diphosphate
Ches	2-(*N*-Cyclohexylamino)ethanesulphonic acid
CI	covalently immobilized
Ci	Curie (2.2×10^6 decompositions per second)
CM	Carboxymethyl
CoA,CoASH	Coenzyme A
DAD	Diode array detector
DAP	Diaminopimelic acid
dATP	Deoxyadenosine triphosphate
DBM	Diazabenzyloxymethyl
DCI	3,4-Dichloroisocoumarin
dCTP	Deoxycytidine triphosphate
DEAE	Diethylaminoethyl
DEHPA	Bis(diethylhexyl)phosphoric acid
dGTP	Deoxyguanosine triphosphate
DHF	Dihydrofolate
DHFR	Dihydrofolate reductase
DHQ	Dihydroquinozolinium
Dipso	3-[*N*,*N*-Bis(2-hydroxyethyl)amino]-2-hydroxypropanesulphonic acid

DME	Dropping mercury electrode
DMSO	Dimethylsulphoxide
DNase	Deoxyribonuclease
DOPA	Dihydroxyphenylalanine
d.p.m.	Disintegrations per minute
dTDP	Deoxythymidine diphosphate
DTNB	5,5′-Dithiobis(2-nitrobenzoate)
dTTP	Deoxythymidine triphosphate
E	Absorbance (extinction) coefficient
$E_{1/2}$	Half-wave potential
EC	Enzyme commission
EDTA	Ethylenediamine tetra-acetic acid
EGTA	Ethyleneglyco-bis(β-aminoethyl ether)N,N,N′,N′-tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
f	Activity coefficient
FCCP	Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone
FMN	Flavin mononucleotide (oxidized)
$FMNH_2$	Flavin mononucleotide (reduced)
g	Relative centrifugal force
GDP	Guanosine diphosphate
GOT	Glutamate oxalacetate transaminase
GTP	Guanosine triphosphate
Hepes	*N*-(2-Hydroxyethyl)piperazine-*N*′-(2-ethanesulphoric acid)
Hepps	*N*-(2-Hydroxyethyl)piperazine-*N*′-(2-propanesulphonic acid)
Heppso	*N*-(2-Hydroxyethyl)piperazine-*N*′-(2-hydroxypropanesulphonic acid)
Hip	Hippuric acid
HPLC	High performance liquid chromatography
I	Intensity of light
I	Ionic strength
Kat	Katal
k_{cat}	Catalytic rate constant
K_i	Inhibition constant
K_m	Michaelis constant
LLD	Lower limit of detection
Mes	2-(*N*-Morpholino)ethanesulphonic acid
Mops	3-(*N*-Morpholino)propanesulphonic acid
Mopso	3-(*N*-Morpholino)-2-hydroxypropanesulphonic acid
MPDP	1-Methyl-4-phenyl-2,3-dihydropyridine
M_r	Relative molecular mass (molecular weight)
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide

MV_{ox},MV_{red}	Methylviologen (oxidized, reduced)
NAD	Nicotinamide adenine dinucleotide (oxidized)
NADH	Nicotinamide adenine dinucleotide (reduced)
NADP	Nicotinamide adenine dinucleotide phosphate (oxidized)
NAT	*N*-acetyl transferase
NBT	Nitroblue tetrazolium
σ	Standard error or standard deviation
OAB	*O*-Aminobenzaldehyde
OAT	Ornithine aminotransferase
OPA	*O*-Phthaldehyde
PABA	*p*-Aminobenzoate
PAGE	Polyacrylamide gel electrophoresis
PEP	Phosphenolypyruvate
PFK	Phosphofructokinase
P_i	Inorganic orthophosphate
Pipes	Piperazine-*N*,*N*'-bis(2-ethanesulphonic acid)
PK	Pyruvate kinase
PMS	Phenazine methosulphate
PMSF	Phenylmethanesulfonylfluoride
Popso	Piperazine-*N*,*N*'-bis(2-hydroxypropanesulphonic acid)
PP_i	Inorganic pyrophosphate
RI	Refractive index
RIA	Radioimmuno assay
RNase	Ribonuclease
RPC	Reverse phase chromatography
SCE	Standard calomel electrode
SDS	Sodium dodecyl sulphate (Sodium lauryl sulphate)
SEC	Size exclusion chromatography
SPA	Scintillation proximity assays
Taps	*N*-Tris(hydroxymethyl)methyl-3-aminopropane sulphonic acid
Tapso	3-[*N*-Tris(hydroxymethyl)methylamino]-2-hydroxypropane-sulphonic acid
TBA	tert-Butylammoniuim hydroxide
TCA	Trichloroacetic acid
TCC	2,3,5-Triphenyltetrazoluim chloride
TEMED	*N*,*N*,*N*'*N*'-Tetramethylethylene diamine
Tes	2-(Tris[hydroxymethyl]methylamino)ethanesulphonic acid
THF	Tetrahydrofolate
THF	Tetrahydrofuran (Chapter 3 only)
TLC	Thin layer chromatography
Torr	mmHg
Tricine	*N*-Tris(hydroxymethyl) methylglycine
Tris	Tris (hydroxymethyl) aminomethane

UDP	Uridine diphosphate
UV	Ultraviolet
v	Velocity
V	Volts
v_o	Initial velocity
V_{max}	Maximum velocity
ε	Absorption (extinction) coefficient
λ	Wavelength
λ_{max}	Wavelength of maximum light absorption

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