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M Weller

Protein Phosphorylation

The nature, function, and metabolism of proteins which contain covalently bound phosphorus

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Preface

It has been known for over a century that certain proteins contain covalently bound phosphorus, and it is equally well-established that, if animals are injected with ^{32}P-labelled inorganic phosphorus, radioactivity is rapidly incorporated into many tissue proteins, showing that protein-bound phosphorus has a rapid turnover. It is, however, only in the last ten years or so that studies on protein phosphorylation have really gained momentum. The reason for the current interest in the topic is that cyclic AMP has been shown to stimulate the phosphorylation of certain proteins, resulting in important changes in their properties. These observations have naturally led many workers to consider the possibility that protein phosphorylation may have as important and widespread a function in regulation as does cyclic AMP itself. Many protein phosphorylation reactions are, however, unaffected by cyclic AMP, and much interesting work on these reactions is in danger of being overshadowed by the current interest in cyclic-AMP-stimulated protein kinase activities. Indeed, the properties of certain phosphorylated proteins have been much more thoroughly investigated than others and there are many gaps in our knowledge.

The literature on protein phosphorylation, and on proteins which contain covalently bound phosphorus, is now very large and continues to increase rapidly. Consequently it seems to me to be a very suitable time to present a book which attempts to summarise, review, and critically discuss the present state of our knowledge on these subjects and to bring together previously unrelated observations, put forward possible new hypotheses, and indicate where gaps occur. I should feel especially rewarded if this book, by indicating some of these gaps, in any way helped to identify and stimulate suitable fields for future research.

At the risk of occasionally being repetitive I have divided the text into a series of chapters each of which is complete in itself and may be read independently, so that the reader who is interested in only one type of phosphoprotein, or one aspect of protein phosphorylation, will not have to read the whole book in order to gain the relevant information about his topic. The individual chapters are further divided into sections for easy reference.

I trust that the book will prove to be of value to anyone carrying out research on protein phosphorylation, or on proteins which contain covalently bound phosphorus, and that it will provide instructive reading to advanced students interested in these subjects.

In conclusion, it is a great pleasure to thank Miss Wilma Laing and Miss Jeanne Rousseau as well as my wife, Jennifer, for their help in the preparation of the manuscript for this book. I am also indebted to those of my colleagues who have provided information about their current work.

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List of abbreviations

The abbreviations used in the text are those accepted by the Biochemical Journal [*Biochem. J.* (1966) **101** 1–7]. In addition the following abbreviations are used:

ACTH adrenocorticotropic hormone,
DFP diisopropylphosphofluoridate,
EGTA ethyleneglycol-*bis*(β-aminoethyl ether)*N*,*N*′-tetraacetic acid,
MSH melanocyte stimulating hormone,
NEM *N*-ethylmaleimide,
PCMB *p*-chloromercuribenzoate,
SDS sodium dodecyl sulphate.

In peptide sequences the symbols are joined by hyphens where the sequence is known, or separated by commas if the sequence is not known. Phosphorylated amino acids are represented by the following abbreviations:

aspP phosphoasparate,
gluP phosphoglutamate,
serP phosphoserine,
thrP phosphothreonine.

Contents

1

General aspects and functions of proteins which contain covalently bound phosphorus

This chapter is written with the intention of introducing the reader to some general considerations of phosphoproteins and phosphorylated amino acids.

1.1 Classification and nomenclature of proteins which contain covalently bound phosphorus—the phosphoproteins

The first proteins to be discovered which contained covalently bound phosphorus were phosvitin (from egg yolk) and casein (from milk). These proteins, not unnaturally, were called 'phosphoproteins'. It was many years after these initial discoveries that it was found that other proteins could contain covalently bound phosphorus, though often in quite small amounts. This has led to some confusion in nomenclature. The term 'phosphoprotein' has been used for such a long time only to describe casein and phosvitin that people tend to be hesitant about using it to describe a protein which is newly discovered to contain covalently bound phosphorus, particularly if the concentration is low. Few people, for example, would think of describing a histone as a phosphoprotein even though it contains phosphorus, and the same applies to enzymes such as phosphorylase *a* which also contain phosphorus.

The term phosphoprotein, however, means, quite simply, a protein which contains covalently bound phosphorus and I shall use it in this sense even if the protein in question contains only a very low level of phosphorus.

Because of the great diversity of proteins which contain covalently bound phosphorus, and because of our frequent lack of knowledge about them, a proper classification is impossible. The phosphoproteins which have been at least partially purified and about which we know most may be divided into two groups: those which have enzymic activity and those which do not. The former group will be described in chapter 6 and the latter in chapter 5.

The properties and functions of proteins which contain covalently bound phosphorus will also be discussed in relation to their subcellular location in the appropriate chapters.

1.2 Phosphorylated amino acids which occur in proteins, and their chemical properties

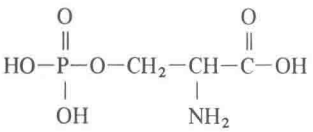
In all the phosphoproteins so far investigated phosphorus has been found to be covalently bound only to serine, threonine, histidine, lysine, aspartic acid, or glutamic acid residues. The structures of these phosphorylated amino acids are shown in figure 1.1.

There was some early confusion but protein-bound phosphorylated amino acids are now known to occur almost entirely as phosphomonoesters,

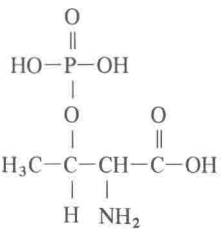
although the presence of serine phosphodiesters has been reported in certain peptides derived from nervous tissue[1,2].

Phosphoserine is by far the most commonly found protein-bound phosphorylated amino acid, though phosphothreonine is also frequently detected. These are the only phosphorylated amino acids which occur in casein and phosvitin and their presence has been recorded in many animal tissues[3] as well as in certain bacteria[4], viruses (chapter 13, page 143), and plant tissues. Many enzymes also contain phosphoserine (chapter 6).

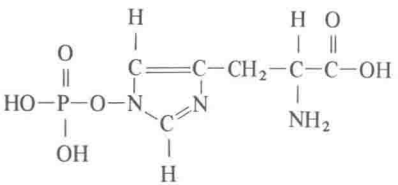
It has been suggested that in certain circumstances the phosphoserine of phosvitin might occur in an enol form which would be of high energy[5].



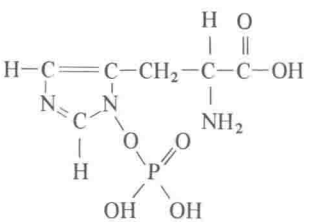
phosphoserine



phosphothreonine



3-phosphohistidine



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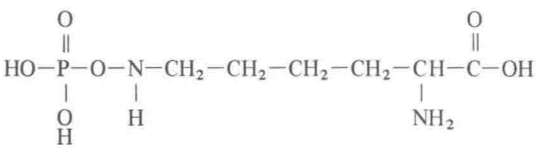
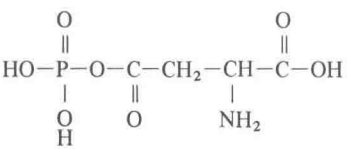
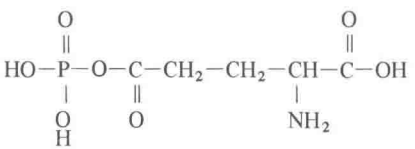


N^{ϵ}-phospholysine



phosphoaspartic acid



phosphoglutamic acid

Figure 1.1. The structures of the phosphorylated amino acids.