liquid electrode interfaces, semiconductor–liquid electrodes, liquid–liquid interfaces, proteins, etc.

Marcus' work has greatly stimulated experimental work in chemistry and it makes predictions concerning such widely differing phenomena as the fixation of light energy by green plants, photochemical production of fuel, chemiluminescence, the conduction of electrically conducting polymers, corrosion, the methodology of electrochemical synthesis and analysis, etc.

Editor

Regulatory role of protein phosphorylation recognized

This year's Nobel prize for physiology or medicine was awarded to Prof. Edwin G. Krebs of the Department of Pharmacology, University of Washington, Seattle, USA, and to Prof. Edmond H. Fischer of the Department of Biochemistry of the same university for their pioneering work on 'reversible protein phosphorylation as a biological regulatory mechanism'. The first protein whose function (in this case enzyme activity) was shown to be regulated by reversible phosphorylation was glycogen phosphorylase[1,2] (also known simply as phosphorylase), an enzyme involved in the breakdown of glycogen in muscle and liver. They not only discovered this covalent modification reaction but also identified and purified the enzymes involved in this regulatory process, namely phosphorylase kinase and phosphorylase phosphatase. Their fundamental findings initiated an area of research which is one of the most active and wide-ranging.

Regulation of phosphorylase by reversible phosphorylation was discovered in mid-fifties. Till late sixties it was believed that protein phosphorylation was not widespread. The only enzymes shown to be regulated by reversible protein phosphorylation, till then, were those involved in glycogen metabolism namely phosphorylase, phsophorylase kinase[3] and glycogen synthase[4]. However, the situation changed rapidly after Krebs and his colleagues[5] in 1968 showed that cyclic AMP activates a protein kinase, now known as cyclic AMP-dependent protein kinase. Since then the function of many enzymes and non-enzymatic proteins has been shown to be regulated by reversible protein phosphorylation and this process has emerged as one of the major devices by which eukaryotic cells control their response to extracellular stimuli such as hormones, neurotransmitters, growth factors, etc.

Discovery of the first regulatory protein phosphorylation

When Krebs and Fischer started their work on the regulation of enzyme activity of phosphorylase it was known from the work of Cori[6] in the forties that phosphorylase in muscle exists in two forms. One of the forms called phosphorylase *a* was active in the absence of AMP whereas the other form, phosphorylase *b*, required AMP for its enzyme activity. The work of Krebs and Fischer in the mid-fifties showed that phosphorylase *b* can be converted to phosphorylase *a* in presence of ATP and a divalent metal ion[1]. The requirement for ATP provided the first clue for this conversion from *b* to *a* form to be a phosphorylation reaction. They also isolated the enzyme (phosphorylase kinase) which catalysed the conversion of phosphorylase *b* to phosphorylase *a* Using ^{32}P-labelled ATP they were able to show[2] that during this conversion, the radioactive label was tightly bound to phosphorylase *a* and that this labelled phosphorylase *a* could be converted to the *b* form which did not have the bound ^{32}P. This conversion to the *b* form was catalysed by what was known as PR enzyme[7] (now known as phosphorylase phosphatase). The term PR enzyme (prosthetic group removing enzyme) was originally given because it was thought to remove a prosthetic group from phosphorylase *a*. Interestingly, the presence of phosphate in phosphorylase *a* was shown as early as 1943 (ref. 7).

The regulation of the enzyme activity of phosphorylase is, however, much more complex. The *b* form of this enzyme is active in presence of AMP which is an allosteric regulator. ATP acts as an inhibitor by competing with AMP. Glucose 6-phosphate is also an inhibitor of phosphorylase *b*. Phosphorylase *b* is generally inactive under physiological conditions due to inhibitory effects of ATP and glucose 6-phosphate. Phosphorylase *a* is fully active and is not affected by ATP, AMP or glucose 6-phosphate. The relative rates of phosphorylation and dephosphorylation determine the proportion of the active enzyme, the *a* form. Almost all of the enzyme is in inactive *b* form in resting muscle which gets converted to the active *a* form when the levels of hormone, epinephrine, increase in the blood.

The enzyme phosphorylase kinase was shown to be regulated by phosphorylation[3]. Like phosphorylase it is also activated by phosphorylation; dephosphorylation converts it into the inactive (or less active) form. The phosphorylation

Edmond H Fischer (left) and Edwin G Krebs

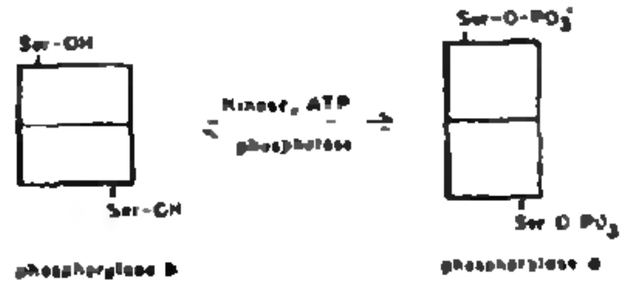

Figure 1. A schematic diagram showing regulation of enzyme activity of phosphorylase by phosphorylation and dephosphorylation at a single serine residue per subunit Phosphorylation of Ser by phosphorylase kinase results in conformational change in the enzyme, converting it to active form

of phosphorylase kinase is brought about by cyclic AMP-dependent protein kinase. These protein kinases are quite specific, the cyclic AMP-dependent protein kinase does not phosphorylate phosphorylase *b*.

Regulation of enzyme activity by reversible phosphorylation has several advantages to the cell as compared to *de novo* synthesis of an enzyme. It is energy efficient, only 2 moles of ATP are required to activate 1 mole of phosphorylase. The response is fast and the signal gets amplified; one molecule of phosphorylase kinase can phosphorylate several molecules of phosphorylase.

Protein phosphorylation regulates many cellular processes

The pioneering work of Fischer and Krebs led to an increase in the interest in the area of protein phosphorylation particularly after the discovery of cyclic AMP-dependent protein kinase. In the seventies and eighties many protein kinases were discovered and many enzymes and non-enzymatic proteins were shown to be regulated by reversible phosphorylation[8-10]. Cell growth, cell division and transformation by viruses and other agents, cell differentiation, transcription, translation and many other cellular processes are now known to be affected directly by phosphorylation of proteins.

Phosphorylation of proteins occurs on many residues but mainly Ser, Thr and Tyr are phosphorylated. Of these, Tyr phosphorylation represents less than 1% of protein phosphorylation whereas Ser (90–95%) and Thr (5–10%) account for bulk of the phosphorylation. It is estimated that a eukaryotic cell has about 1000 genes coding for different protein kinases[9] and perhaps as many for protein phosphatases[10].

The examples of phosphorylase and phosphorylase kinase may give an impression that phosphorylation always results in increase in enzyme activity. However, that is not the case. The activity of some enzymes like glycogen synthase is decreased upon phosphorylation[4]. Positive and negative regulatory phosphorylation sites are sometimes present in the same enzyme. In such cases at least two kinases and two phosphatases are required for regulating the enzyme activity (for a recent review see ref 11).

The recent excitement

Work in many laboratories during late seventies and the early eighties showed that transforming genes of many viruses code for proteins with tyrosine-specific protein kinase activity and this activity was necessary for cell transformation. At about the same time many growth factor receptors were also shown to be tyrosine-specific protein kinases. Unregulated tyrosine phosphorylation was thought to be the main culprit for the uncontrolled growth of many tumour cells. Tyrosine phosphatases help in maintaining the phosphorylated state of a protein at appropriate level.

The interest in this area of research was levelling off when, in the late eighties, an unexpected discovery was made. Protein phosphatases were thought to be of little significance since they were believed to function constitutively, regulation being carried out at the protein kinase level. Purification of first protein tyrosine phosphatase in the laboratory of Fischer and sequence analysis of the purified human placental enzyme showed that it had significant homology with CD45, the leukocyte common antigen which occurs on the surface of white blood cells[12]. Soon after they showed that CD45 which is a transmembrane receptor-like protein, had intrinsic tyrosine phosphatase activity[13]. Later work in many laboratories established that transmembrane tyrosine phosphatases constitute a novel class of receptors that make up an independent signalling pathway in the cell[14,15]. Tyrosine phosphatases have been identified in viruses, bacteria, yeast, insects and mammals. The biological significance of only a few tyrosine phosphatases is known. The CD45 is required for T-cell activation. Another tyrosine phosphatase whose biological significance is known is the one isolated from a bacterium *Yersinia* which is the causative agent of plague. The gene coding for tyrosine phosphatase carried by this bacterium is essential for virulence in this pathogen[10].

The area of protein phosphorylation research has been full of surprising and exciting discoveries. After the original discovery of the regulatory role of phosphorylation in the control of phosphorylase, each decade had towards its end major surprises. In 1968 it was cyclic AMP-dependent protein kinase, the late seventies showed the tyrosine protein kinases (coded by receptors and viral oncogenes) and the late eighties came up with receptor-type tyrosine phosphatases. Of course, there have been many other important discoveries that one could easily argue that the ones not listed above were equally important such as regulation of cell cycle by cdc kinase and phosphatase[16] and regulation of transcription factor function by phosphorylation[17]. It would be hazardous to guess what is in store for the nineties. Considering the importance of the regulatory role played by protein phosphorylation in eukaryotic cells, its recognition by Nobel foundation has come rather late.

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