PHOSPHORYLATION OF A PRECURSOR OF SUBSTANCE P (α-PROTACHYKININ) BY A CATALYTIC SUBUNIT OF PROTEIN KINASE A

ELŻBIETA HRABEC, MAŁGORZATA STREK and ZBIGNIEW HRABEC
Department of Medical Biochemistry, Medical University of Łódź, Lindleya 6, 90-131 Łódź, Poland

The neuropeptide substance P (SP) is a member of the tachykinin family of peptides that are involved in the regulation of many different biological processes. SP-containing neurons are widely distributed throughout the central and peripheral nervous systems. For example, SP neurons originate in the striatum and project to the midbrain, where SP functions as a modulator of the activity of dopaminergic neurons.

It is generally accepted that a small biologically active peptide, such as substance P, is initially synthesized as a larger precursor – α-protachykinin. Precursors of regulatory peptides, such as α-protachykinin, undergo a maturation process, which includes proteolytic processing. In the cells, peptides are produced from precursors through cleavage at pairs of basic amino acids (Lys-Lys or Arg-Arg). Although enzyme activities specific for cleavage at adjacent basic amino acids within mammalian peptide precursors have been reported on, up till now, little or nothing has been learned about the regulation of this process. Reversible modifications of α-PT, e.g. phosphorylation, might be one of many factors which are of significance in the regulation of SP release from α-PT.

The reaction mixture for phosphorylation of α-PT contained: 50 mM Tris-HCl buffer, pH 7.5, 10 mM MgCl₂, 1 mM EDTA, 1 mM dithiothreitol, 5 μM [γ-32P]ATP, 40 ng of the catalytic subunit of protein kinase A, and 600 ng of α-PT. The incubation was carried out at 37°C for 1 h. Then the reaction was stopped and samples were applied on 18% polyacrylamide SDS gels. Proteins were visualized by silver staining, the gels were dried and the labeled α-PT was detected by autoradiography.

The recombinant precursor of substance P (α-PT) prepared from E.coli was readily phosphorylated by the catalytic subunit of protein kinase A. To our knowledge, this is the first report on the in vitro phosphorylation of α-PT.

It was found that, for its action, protein kinase A requires basic residues surrounding the target serine or threonine. The presence of positively charged amino acids on the C-terminal tail of α-PT suggests that two or three different seryl residues might serve as phosphorylation sites. However, precise identification of the phosphorylation sites requires further investigation.