Cyclic AMP-dependent protein phosphorylation in primate kidney

NATALIE A. LAMINISKI,* KATHRYN D. HAMMOND* and ANTHONY M. MEYERS†
Departments of *Medical Biochemistry and †Medicine, University of the Witwatersrand Medical School, Johannesburg 2193, South Africa

The functions of cell types within different tissues are coordinaté by a variety of hormones. These increase the concentration of second messengers, such as cyclic nucleotides, leading to activation of protein kinases and phosphorylation of specific proteins [1]. The kidney is the target organ for a number of hormones which activate adenylate cyclase and hence cyclic AMP-dependent kinases [2]. The purpose of this study was to gain further understanding of the regulation of kidney metabolism by measuring protein phosphorylation resulting from the action of cyclic AMP-dependent protein kinases. Specific proteins phosphorylated were identified using electrophoresis and autoradiography. Studies were performed on cytosol and membrane preparations from primate kidney.

Kidneys were obtained from recipient baboons undergoing transplant, normal human kidneys were donor kidneys which had not been used for transplant. All procedures were carried out at 4°C. Kidneys were decapsulated and outer cortex homogenized in sucrose medium. Membrane vesicles, including basolateral and brush-border membranes, were prepared using the method of Fitzpatrick et al. [3]. Cytosol was obtained by ultracentrifugation (100 000 × g, 1 h) of the initial low-spin supernatant from the membrane preparation. Purity of fractions was assessed using the marker enzymes adenosine triphosphatase and γ-glutamyltransferase.

After incubating tissue fractions with 0.1% (v/v) Triton X-100 at 4°C for 10 min, phosphorylation was measured using a modification of the assay of Szoka & Ettinger [4]. Fractions (100–200 μg of protein) were incubated with 40 mM-Tris/HCl, pH 7.2, 10 mM-MgCl₂ and 0.3 mM-EGTA in the presence or absence of 25 μM-cyclic AMP for 30 min at 4°C. After 1 min at 30°C the reaction was initiated by the addition of 10 μM-[γ-³²P]ATP (0.16 × 10⁶ d.p.m./pmol). The reaction was terminated after 5 min by the addition of ice-cold 25% (w/v) trichloroacetic acid. Protein was precipitated by centrifuging for 15 min at 800 g. The resulting pellet was washed three times in 25% (w/v) trichloroacetic acid, resuspended in 1 M-NaOH overnight and then counted in scintillant.

For the identification of phosphorylated proteins by means of electrophoresis and autoradiography, tissue fractions were treated as above except that the reaction was initiated by addition of 10 μM-[γ-³²P]ATP (0.5 × 10⁶ d.p.m./pmol) and incubation continued for 1 min at 30°C. The reaction was terminated by addition of 0.125 mM-Tris/HCl, pH 6.8, 4% (w/v) SDS, 20% (v/v) glycerol and 1% (v/v) mercaptoethanol followed by boiling for 1 min. SDS/polyacrylamide-gel electrophoresis [5] employing a 4% stacking gel (6 cm) and a 7.5% resolving gel (6 cm) was carried out at 15 mA/gel for 1.5 h. The gels were then dried and autoradiographed. Rainbow markers (Amersham, Bucks, U.K.) were used to estimate relative molecular mass.

Phosphorylation occurred very rapidly (< 1 min) and then remained constant for at least 20 min. Incorporation increased with increasing ATP concentrations from 10 to 250 μM and then remained constant at 500 μM-ATP. Phosphate incorporation after a 5 min incubation period was determined and expressed as pmol/mg of protein. In the absence of cyclic AMP, phosphate incorporation in baboon membrane was 30.7 ± 13.1 (n = 5) and in cytosol was 18.5 ± 6.1 (n = 6) pmol/mg of protein; in the presence of cyclic AMP, the respective values were 34.2 ± 18.7 and 18.8 ± 4.2 pmol/mg of protein.

Fig. 1 shows an autoradiograph for baboon cytosol demonstrating that eight major proteins, of molecular mass in the range 31–135 kDa, were phosphorylated in the absence of cyclic AMP; three bands of mass 45, 55 and 66 kDa were intensified in the presence of cyclic AMP. Similar results were found for normal human kidney cytosol as

Vol. 17

Fig. 1. Autoradiograph showing phosphorylated proteins in cytosol of baboon and human kidney

Lanes 1–5, baboon cytosol; lanes 6–10, human cytosol. Preparations without cyclic AMP were electrophoresed on lanes 1, 2, 6, 7 and 8. Preparations with cyclic AMP were electrophoresed on lanes 3, 4, 5, 9 and 10. The bands intensified in the presence of cyclic AMP are indicated. The direction of migration was from top to bottom.
Lipid–peptide interactions: \(^{31}\)P-nuclear magnetic resonance and differential scanning calorimetric study of staphylococcal \(\delta\)-lysin on liposomes

MANMOHAN BHAKOO,* RUTHVEN N. A. H. LEWIS† and RONALD N. McELHANEY‡

*Department of Biological Sciences, University of Keele, Staffordshire ST5 5BG, U.K., and †Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

Staphylococcal \(\delta\)-lysin (26 amino acid residues) has a high percentage of hydrophobic and non-ionizable side-chained amino acids. \(\delta\)-Lysin exhibits lateral amphipathicity and surface activity, being soluble in aqueous and organic solvents (42% \(\alpha\)-helical in water). This peptide interacts with artificial lipid membranes [1] and lyses a variety of cells including leucocytes, erythrocytes, lysosomes and mitochondria.

The lytic activity of \(\delta\)-lysin is inhibited by phospholipids [2], indicating the possibility of preferential interactions with phospholipids. These interactions were studied by Bhakoo et al. [3, 4] using numerous phospholipid monolayers and bilayers. The ability of \(\delta\)-lysin to penetrate into both hydrophobic and hydrophilic environments and its high affinity for lipid membranes could be useful for studying lipid–peptide interactions.

We have investigated the effect of \(\delta\)-lysin on thermotropic properties of liposomes composed of dimyrystoylphosphatidylcholine (DMPC) using high-sensitivity differential scanning calorimetry (DSC). Possible changes in the structure of phospholipid assemblies formed in the presence of the peptide were studied by \(^{31}\)P-n.m.r. spectroscopy. Briefly, lipid–peptide samples for DSC and \(^{31}\)P-n.m.r. were obtained by addition of \(\delta\)-lysin to a film of DMPC in chloroform dried by evaporation to vacuo. The mixture was vortexed and incubated above \(T_m\) (the phase transition temperature) of DMPC. The lipid concentrations for DSC and \(^{31}\)P-n.m.r. were 1–4 mg/ml and 10 mg/ml, respectively. Samples (1.23 ml) were equilibrated at 17°C (between the pretransition temperature, \(T_p\), and \(T_m\) of DMPC) and DSC heating thermograms were recorded at a rate of 11.6°C/h and repeated after equilibration. A series of DSC thermograms (Fig. 1) of various mixtures of DMPC/\(\delta\)-lysin (molar ratios 15:500:1) and DMPC alone were produced. The DMPC thermogram shows two usual endotherms, pretransition and gel/liquid–crystalline transition.

Abbreviations used: DMPC, dimyrystoylphosphatidylcholine; DSC, differential scanning calorimetry.

Received 13 June 1989

Fig. 1. High sensitivity DSC heating endotherms of various ratios of DMPC/\(\delta\)-lysin mixtures

The thermograms shown were recorded at 11.6°C/h after equilibration of samples at 0–4°C. The two normal endotherms (pretransition and gel/liquid–crystalline phase transition) are observed above 62.5:1 for DMPC/\(\delta\)-lysin. \(\Delta H\) (area under the peak) decreased with an increase in \(\delta\)-lysin concentration. The arrows indicate the onset and offset of the gel/liquid–crystalline transition.