STRUCTURE AND FUNCTION OF 14-3-3 ISOFORMS

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INTRODUCTION

There are seven major mammalian brain isoforms of 14-3-3 (named due to their migration position on DEAE cellulose chromatography and starch gel electrophoresis). Epithelial cells contain a specific isoform called HME1 or stratifin (Leffers *et al.*, 1993) and a distinct isoform has been identified in T cells. The 14-3-3 family is highly conserved (homologues are found in plants, insects, amphibians, yeast, and the nematode worm, *C. elegans*. Many functions have been suggested for this widely distributed family of dimeric, eukaryotic proteins (reviewed in *Aitken et al.*, 1992).

The studies of our own group have focused on 14-3-3 as a protein kinase regulator (Toker *et al.*, 1992; Robinson *et al.*, 1994) and analysis of subcellular localisation and function of brain 14-3-3 (Martin *et al.*, 1994). Recent developments in our own research and in the studies of group of Frank McCormick (personal communication) have strongly implicated the involvement of 14-3-3 proteins in the MAP kinase cascade.

METHODS

We have expressed a number of these isoforms as recombinant proteins and made a variety of mutants and truncated proteins. We have raised antisera specific for acetylated synthetic peptides, based on the N-terminal sequence of mammalian 14-3-3 isoforms (Martin *et al.*, 1993). Electrospray mass spectrometry (ESMS) on a Fisons VG Platform instrument has been used to identify post-translational modifications in specific isoforms and the site of dimerisation. On-line trapping was used to purify/desalt proteins before introduction to the ESMS source (Kay and Mallet, 1993). This comprised a Polymer Labs. (UK) poly (styrene/divinyl-benzene) PLRP-S, 8 mm particle, 300A pore size, 0.75 mm microbore column (slurry-packed in-house).

The sample was loaded on this trapping column in a low concentration of organic modifier, washed free of interfering salts, etc, with acetonitrile/water/acetic acid 15:84:1 (v/v/v) at a flow rate of 2-500 μL min^{-1}. Proteins were eluted with acetonitrile/water/acetic acid 50:49:1 (v/v/v) at a flow rate of 10 μL min^{-1} by switching a Rheodyne valve to put this column on-line with the source.

RESULTS AND DISCUSSION

ESMS of 14-3-3 proteins gave results which are in very close agreement to the theoretical values and verified the presence of N-acetylation. Two sets of sheep and chicken brain isoforms differ in mass by 80 Da. This suggests the presence of phosphorylation in specific isoforms which is conserved across a wide range of species.

In addition, species of sheep α and β with masses c.a. 100 Da higher were detected, suggesting that they are additionally expressed (as proposed by Leffers *et al.*, 1993) using an alternative initator methionine codon six nucleotides upstream of the major initation site. With Thr (residue mass 101 Da) as the second amino acid, this initiator Met is predicted to be removed (Aitken, 1990) resulting in alternative species commencing with the amino terminal sequence, N-Ac.Thr-Met-Lys-Ser-, instead of N-Ac.Met-Lys-Ser-. The presence of the former has been verified by our new anti-peptide antiserum. We are currently identifying this site of phosphorylation which is present specifically on isoforms that have been shown to interact with the oncogene-related protein, c- Raf.

Two dimensional gel electrophoretic analysis of truncated 14-3-3 recombinant proteins has enabled us to identify the site of dimerisation at the amino terminus. Crystals of recombinant 14-3-3 and another protein kinase inhibitor (a Zn^2 + -binding protein) have been obtained for X-ray structure analysis. Initial diffraction data has been obtained on the latter by the group of G. Dodson, NIMR.

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PROGRESOS EN ESTUDIOS PRECLINICOS DEL FACTOR DE CRECIMIENTO EPIDERMICO HUMANO RECOMBINANTE EN CUBA

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INTRODUCCION

En el CIGB se han desarrollado las siguientes formas farmacéuticas con el Factor de Crecimiento Epidérmico humano recombinante (EGF-hr): crema de EGF-hr con sulfadiacina de plata (SDP) al 1%, crema de EGF-hr solo, solución viscosa de EGF-hr y colirio de EGF-hr. En esta revisión se presentan resultados obtenidos recientemente en los estudios de eficacia y seguridad del EGF-hr en animales de experimentación.

FARMACOLOGIA

En un estudio de **farmacodinamia** utilizando un modelo de heridas por ponchamiento en ratas Sprague Dawley, se aplicó crema de EGF-hr a diferentes concentraciones: 0,5; 5 y 10 μg/g para cada grupo y se evidenció re-epitelización acelerada en los grupos de 5 y 10 (curva dosis-efecto), así como maduración del tejido de granulación y deposición de colágeno más marcada. Además, se realizó **farmacodinamia** del efecto protector del EGF oral y subcutáneo (tabla 1) sobre la mucosa gástrica de ratas,

Tabla 1

Vía	Dosis	N
oral	10 μg/Kg	10
	30 μg/Kg	10
	100 μg/Kg	10
sub-cutánea	25 μg/Kg	10
	75 μg/Kg	10

evidenciándose efecto dosis-respuesta, las dosis más altas de ambas vías protegieron la mucosa de manera similar al Q-ulcer (control positivo).

En estudios de **farmacocinética** en ratas Sprague Dawley y perros Beagle (tabla 2), ocurrió rápido aclaramiento plasmático por la vía ev (a los 6 minutos niveles del 50%); biodistribución fundamentalmente en riñón, hígado, piel y estómago; y eliminación >75% por orina.

En la aplicación cutánea los niveles de radioactividad no fueron diferentes del fondo radiactivo siendo ligeramente superiores en piel lesionada.

Tabla 2

Modelo	Vía	Dosis	N
ratas	ev	EGF-hr 250 μg/Kg (10^6 cpm)	82
perros	ev	EGF-hr marcado 180 μg/Kg (2 x 10^7 cpm)	6
ratas	cutánea	crema SDP + EGF-hr 10 μg/g (10^6 cpm)	43
perros	cutánea	crema SDP + EGF-hr 10 μg/g (10^6 cpm)	3
perros	cutánea	crema SDP + EGF-hr 10 μg/g (10^6 cpm)	3