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A Highly Sensitive Immunoenzymometric Assay for the Determination of Angiogenin

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Summary: A polyclonal antibody to human recombinant angiogenin was prepared in rabbits using a $Pam_3CysSerGly$ conjugate. The antibody was then used to develop the first highly sensitive enzyme-labelled immunometric assay for this vascularisation inducing and tumour associated protein. The assay was suitable for quantification of angiogenin in body fluids between 2.5 and 0.05 µg/l. The mean intra-assay imprecision was 6.0% and the inter-assay imprecision 7.9%. Angiogenin in human plasma was found to lie in the range of 0.38 to 0.11 mg/l with a mean of 0.25 ± 0.07 mg/l.

Introduction

Angiogenin is a single chain M_r 14100 protein, first isolated and characterised from a HT-29 human adenocarcinoma cell line (1). It has been shown to be an inducer of vascular growth. The protein has 35% identity with pancreatic ribonuclease (2) and has been shown to inhibit protein synthesis in vitro (3). *Saxena* et al. described angiogenin as a cytotoxic, t-RNA-specific ribonuclease in the RNase A superfamily (4). Angiogenic and ribonucleolytic activities are blocked by a tight-binding placental ribonuclease inhibitor (5). Recently, it was shown that angiogenin supports endothelial and fibroblast cell adhesion (6). *Shapiro* et al. isolated angiogenin from human plasma (7). Its mRNA was detected predominantly in the adult liver, but was also detectable at a low level in other tissues (8) and cells (9). Detailed information on the presence of the protein in body fluids and tissues is not available due to the lack of a sensitive and easily performed assay.

Here we report the production of a polyclonal antibody, which was then used to develop the first sensitive immunometric assay for the determination of this protein. The method will facilitate an understanding of the role of angiogenin in physiological and pathophysiological processes.

Materials and Methods

Materials

Nunc Immuno plates II were obtained from Nunc (Roskilde, Denmark). Horseradish peroxidase[1]) was obtained from Boehringer Mannheim (Mannheim, Germany) and 2,2′-azino-bis(3-ethyl-benzthiazoline-6-sulphonic acid (ABTS) from Sigma (Deisenhofen, Germany). Human recombinant angiogenin was kindly donated by Behringwerke, Marburg. Blood samples were supplied by Prof. *Kleesiek*, Herzzentrum Bad Oeynhausen (Bad Oeynhausen, Germany). *S*-[2,3-bis(palmitoyloxy)propyl]-N-palmitoyl-cysteinyl-serinyl-glycine-succinimidyl-ester (Pam_3CysSerGlyOSu) and S-[(2*RS*)-2,3-bis(palmitoyloxy)-propyl]-N-palmitoyl-cysteinyl-lysyl-lysyl-lysyl-lysine (Pam_3Cys-Ser-Lys_4) were kindly donated by Prof. *Jung* (Tübingen, Germany).

Methods

Preparation of the S-*[2,3,bis(palmitoyloxy)propyl]-N-palmitoyl-cysteinyl-serinyl-glycine/angiogenin (Pam₃CysSerGly/angiogenin) conjugate*

Recombinant angiogenin (0.5 mg) was mixed with 5 mg $Pam_3CysSerGlyOSu$ in 500 µl freshly distilled dimethylformamide and incubated under continuous stirring for 15 h at room temperature. After removal of the dimethylformamide, the product was dissolved in *t*-butanol/water (3 + 1, by vol.) and lyophilised (10).

[1]) Enzyme
Horseradish peroxidase:
donor: hydrogen-peroxide oxidoreductase (EC 1.11.1.7)