Identification of an NAD-dependent 3-acetamidobenzamide-sensitive system in oncospheres from the tapeworm Hymenolepis diminuta

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Comprehensively little is known of the biochemistry of oncospheres of Hymenolepis diminuta, although a detailed histochemical study of the mechanism of egg hatching has been reported. Six layers surround the oncosphere and the hatching sequence follows from basic stages [1]. Activation of the oncosphere will only take place if both the shell and cytoplasmic layer are physically disrupted. It has been suggested that the activation stimulus may be related to a change in salt/water balance rather than chemical changes [2]. Oncospheres consume oxygen \((3.18 \times 10^{-5} \text{ ml of } \text{O}_2/\text{h})\); however, the larvae does not operate a cytochrome oxidase system [3]. It has been reported that some flavoprotein enzymes do exist and oxygen is reduced to hydrogen peroxide. α-Amino acid

References


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Fig. 1. Identification of cyclization sites

(a) Autoradiograph of RNA species which arise in a transcription reaction. Nomenclature, fragment sizes and experimental conditions are as in [4]. RNA circles are arrowed with their tentative sizes in nucleotides. Precursor (p), ribozyme (IVS), ligated exons (5E3E) and precursor in which the downstream exon has been hydrolysed (5EI) are depicted. (b) Autoradiograph of radiolabelled amplified DNA of the material used for electrophoresis in (a). Three doublets arising from DNA fragments of 108, 112 and 127 bp are observed. The left panel shows the four tracks of an M13 DNA sequence used as a size marker: the cross denotes nucleotide position 127 from the 5’ end of the universal primer.
Transfection of cos cells by normal and mutant $\alpha_{1}$-antitrypsin cDNA constructs: biochemical and immunocytochemical findings

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The Z mutation of $\alpha_{1}$-antitrypsin (AAT) or proteinase-inhibitor (PI) is associated with predisposition to developing liver disease in childhood and pulmonary emphysema in later life [1]. A single base substitution alters codon 342 (Glu→Lys). This mutation results in abnormal folding and newly synthesized protein accumulates in the endoplasmic reticulum (ER).

We have transfected cos cells with Simian virus 40 cDNA constructs for the normal PIM and mutant PIZ AAT [2] (a gift of Dr. A. McCracken, University of Nevada) and examined AAT production biochemically and by immunocytochemistry (ICC).

**Methods**

**Transfections.** Cells were grown to a density of 2 and 5×10⁶ cells/60 mm diameter plastic Petri dish. cDNA constructs (0, 2, 5, 10, 15 and 20 μg) were incubated with or without 20 μg of calf thymus DNA as carrier. The incubation of the cDNA with the cells was carried out in the presence of CaCl₂ (final concentration 125 mM) and the incubation times varied from 4 to 8 h.

Biochemical and immunological studies. Expression of AAT was examined by the production of specific mRNA, the measurement of protein in the culture medium and by ICC.

Total RNA was prepared by the method of Chomczynski & Sacchi [3]. A portion (5 μg) was applied to nylon membrane (Zeta probe) and AAT mRNA was quantified by hybridization with a full-length AAT cDNA probe and the signal was compared with $\gamma$-actin as an internal standard.

AAT protein was measured at various time points after transfection using an enzyme-linked immunoassay based on a monospecific antibody to human AAT (Dakopatts Ltd). Monolayers of untransfected and transfected cells were grown on polylysine-coated glass and plastic coverslips and fixed in formaldehyde-saline (4% formaldehyde in 150 mM NaCl). The cells were stained immunocytochemically using the antibody described above in conjunction with an indirect immunoperoxidase coupled to silver amplification (Amer sham Silver Enhancement Kit). For electron microscopy the immunostained cells on plastic coverslips were embedded in London White resin as previously described [4].

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