tobacco (*Nicotiana tabacum* L. cv. Petit Havana SR1) rootstock as described by Synková et al. (J. Plant Physiol. 155: 173-182, 1999) or as rooted plants (kanamycin resistant progeny of the transgenic grafts). Samples for TEM were taken from the central part of the young fully developed leaf or isolated chloroplast suspension and after overnight fixation in 3 % glutaraldehyde were embedded in Spurr's resin. Ultrathin sections were stained by uranyl acetate and Reynold's lead citrate and examined in JEM 1010 (Jeol, Japan). Analysis of serial sections by program IMOD 2.42 enabled three dimensional (3D) reconstructions of chloroplasts and pseudo-crystals. The size of basic structural unit was calculated using MRC Cambridge Image Processing System (1994).

3D reconstruction showed that pseudo-crystalline structures occupy up to 20 % of chloroplast volume (at least in that part of chloroplast which was studied).

The average size of basic cell unit was calculated as: a = 11 nm, b = 12 nm, = 100 .This size parameters support our hypothesis, although dehydration preceding embedding in epoxide resin cause a shrinkage of natural structures. Therefore further experiments are needed to prove it.

Acknowledgements

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THE ROLE OF STRUCTURAL DIFFERENCES OF FLAVANOLIGNAN SILYBIN STEREOIZOMERS IN BINDING TO HEPATOCYTES

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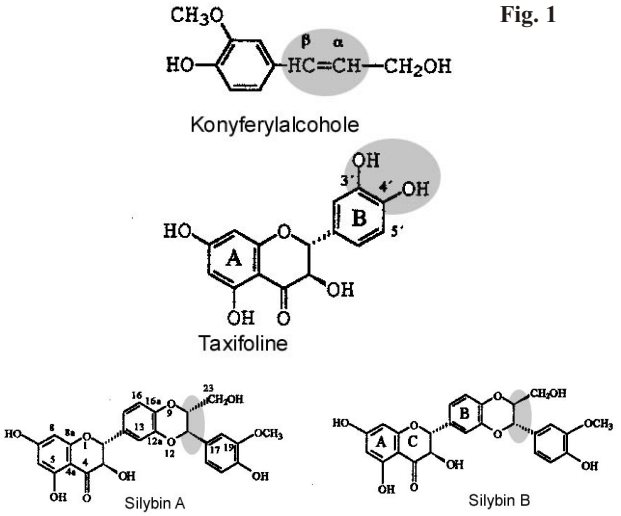
Hepatoprotective effects of Milk Thistle (*Silybum marianum*) have been known since ancient Greece and Roma very well. Flavanolignans (called silymarine) extracted from Milk Thistle seeds were shown to help against hepatotoxic effects of many natural toxins (i.e. alga toxin microcystine, mushroom toxins amanitin and phaloidin, fungal toxins cyclosporines, etc.). The main active substance of silymarin is silybin.

Recent studies revealed that many transport and metabolic processes in the cell are stereospecific. Silybin occurs in two stereoisomers (A and B) that differ in the bound between konyferyl and taxifolin (Fig. 1). We developed a new method for preparation and purification of these silybin stereoisomers and for their specific labelling by radioactivity (3H, 125I) at positions 6 and 8. Transport of four stereoisomers was studied. The best affinity of transport systems were found for 6-[125I]silybinA, which is taken 100 times better than the other silybin stereoisomers.

Fig. 1



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OVEREXPRESSION AND PURIFICATION OF RECOMBINANT MEMBRANE PROTEIN PSBH IN ESCHERICHIA COLI

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In this work we featured an expression system that enables the production of sufficient quantities of membrane PsbH protein (~mg´s quantities) for solid-state NMR as well as other biophysical studies. PsbH is a small membrane protein associated with the photosystem II complex in higher plants, algae and cyanobacteria. Although the exact role of PsbH is not clear, it seems to be important for the structure and function of photosystem II.

In this approach a synthetic psbH gene from cyanobacterium *Synechocystis sp.* PCC 6803 was cloned into a plasmid expression vector, which allowed a direct synthesis of the PsbH protein as a glutathione-S transferase (GST)

fusion protein in *E. coli* BL21(DE3) cells. A relatively large GST anchor overcome foreseeable problems with the low solubility of membrane proteins and the toxicity caused by protein incorporation into the membrane of the host organism. As a result, the majority of fusion protein was obtained in a soluble state and could be purified from crude bacterial lysate by affinity chromatography on immobilised glutathione under non-denaturating conditions. The PsbH protein was cleaved from the carrier protein with Factor Xa protease and purified on DEAE- cellulose column with yields of up to 2.1 μg protein/ml of bacterial culture. Details of sample optimization for small membrane proteins as well as the impact constitutive cell protection mechanism against host membrane proteins are discussed.

KINETIC AND STRUCTURAL CHARACTERIZATION OF TWO ACTIVE FORMS OF ASPARTIC PROTEINASE FROM MURINE INTRACISTERNAL A-TYPE PARTICLES

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Murine intracisternal type A particles (intracisternal A-particles, IAPs) are endogenous retroviruses encoded by many proviral elements within the mouse genome. They share sequence homology with the B-type mouse mammary tumor virus (MMTV), the D-type simian retroviruses (SRV) and C-type avian sarcoma virus. IAPs genetic elements have been shown to transpose within the genome of retrovirus-producing cells.

The IAP particles assemble and bud at the membranes of the endoplasmic reticulum (ER) where they accumulate as immature particles consisting exclusively of uncleaved polyproteins. They do not leave cell and horizontal transmission via free particles has not been achieved. Recent evidence has indicated that the lack of proteolytic processing is not due to a defective viral proteinase but rather is caused by the site of particle formation and can be rescued by an artificial redirection of the polyprotein to the plasmatic membrane.

The recombinant proteinase of murine intracisternal A-type particle 14 (MIA14 PR) undergoes N- and C- terminal autoprocessing at defined sites and is sequentially and functionally related to the B- and D-type retrovirus proteinases. An unusual feature of these proteinases as opposed to the C-type retrovirus proteinases is a 50 amino acid C-terminal extension of unknown function. In this study, we aim to analyse the possible role of the C-terminal extension of the proteinase in regulation of polyprotein processing.

We have cloned, expressed in *E. coli* and purified to homogeneity both the full-length MIA14 PR and its C-terminally truncated form. Both enzymes are active and have been used for *in vitro* kinetic studies using peptide substrates and inhibitors. The C-terminal extension of MIA14 PR has been cloned, expressed in *E. coli*, purified and its effect on catalytic activity of both MIA14 PR constructs evaluated.

THE VITAMIN B12 BIOSYNTHETIC PATHWAY: STRUCTURE ANALYSIS OF UROPORPHYRINOGEN-III C-METHYL-TRANSFERASE

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The biosynthesis of vitamin B_{12}, "the anti-pernicious anaemia factor", requires about 30 enzymes, and is further complicated by the appearance in nature of two separate pathways, representing aerobic and anaerobic routes, where the major difference seem to be concerned with the process of cobalamin ring contraction and cobalt chelatation. *Pseudomonas aeruginosa* is able to synthesise the vitamin in the absence of oxygen. However, the bacterium can also make B_{12} when grown aerobically. Thus, there must exist a pathway that can operate both in the presence and absence of molecular oxygen.

Uroporphyrinogen (uro'gen) III methyltransferase, a key enzyme in the biosynthetic pathways of vitamin B12 and siroheme, catalyzes the S-adenosyl-L-methionine (SAM)- dependent bismethylation of its substrate, uro'gen III, resulting in the formation of dihydrosirohydrochlorin (precorrin-2). The enzyme exists in at least two forms. One form, encoded by the *cob*A gene, is required for vitamin B_{12} synthesis in *Pseudomonas denitrificans*. The second form, encoded by the *cys*G gene, is required for siroheme in *E. coli*. Both forms of the enzyme perform the *in vivo* synthesis of precorrin-2, but in addition, CysG has NAD+-dependent precorrin-2 oxidase and ferrochelatase activities. The CysG enzyme mass is ~52 kDa, whereas the smaller CobA protein mass is of ~30 kDa and is homologous only to the C-terminal region of CysG.

CobA is a key regulatory enzyme in the branched tetrapyrrole biosynthetic pathway, and is sensitive to both substrate and product inhibition. To gain some molecular insight into how this enzyme exerts its control, we have crystallised the CobA protein and collected data on the SRS synchrotron in Daresbury. The molecular replacement method (AMoRe) has been used for the phase problem solution with the C-terminal domain of CysG as a search model. Structure refinement is currently under way.

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