13 01

Isolation of a putative protein methyltransferase

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Quantitative and qualitative changes in protein methylation have been observed during the treatment of alfalfa cell cultures with fungal elicitor. Suspension cultures were treated with elicitor and fed with [^{3}H-methyl]-methionine in the presence of cycloheximide to inhibit protein synthesis. Several proteins were selectively methylated in response to elicitation and by 48hrs protein methylation had increased five-fold. Similar results were obtained following the *in vitro* labelling, with S-adenosyl-[^{3}H-methyl]-methionine, of an elicited time course of protein extracts. A methylase involved in such protein methylation has been partially characterised from plants and cell cultures. It was hypothesised that increased protein methylation in the elicited tissue could be due, at least in part, to the action of the stress damage repair enzyme isoaspartyl methyltransferase. Using an *E. coli* mutant deficient for isoaspartyl methyltransferase (Li and Clarke PNAS 1992) we have cloned a putative protein methyltransferase from a maize expression library by complementation. Although there is little homology between the heterologous sequence and other isoaspartyl methyltransferases, the complemented mutant labelled proteins post-translationaly *in vivo* when fed with [^{3}H-methyl]-methionine.

13 02

Influence of light quality on adenylate kinase activity from the C_3-plant barley (*Hordeum vulgare* L.)

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Adenylate kinases (ATP : AMP phosphotransferase, E.C. 2.7.4.3.) are able to maitain equilibrium between the three adenin nucleotides: Mg*ATP + AMP⇔ Mg*ADP + ADP
For the C_4-plant maize, it was shown that this activity undergoes a rapid increase during transition from dark to light, while activity remained at a constant low level in continuous dark. In addition, it was concluded that adenylate kinase activity in C_3-plants would not undergo such a light-dependent change in activity. In contrast to this assumption, we were able to show that adenylate kinase activity from seedlings of barley increased during dark-light transition and remained at a lower constant level in continuous dark. In order to test the light dependance of this increase, dark-grown seedlings were transferred to different light qualities 5 d after sowing. In all cases adenylate kinase activity increased similarly, but red and far-red light were not as effective as white and blue light. Thus, no single light quality could be related to the observed phenomenon. Nevertheless, adenylate kinase activity from barley (C_3-plant) undergoes similar changes as described for maize (C_4-plant).

13 03

RUBISCO-degrading proteolytic activity induced by detergents and free fatty acids

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Purification of RUBISCO from the soluble fraction of isolated chloroplast of barley revealed associated proteolytic activity. Products of LSU degradation were observed (M_r 43, 37, 35 and 31 kD) even from chloroplasts which were previously treated with thermolysin. The degradation is triggered by ionic and non-ionic detergents and free fatty acids. The activity can be inhibited to 100 % with PMSF. It is concluded that a chloroplastic protease, probably a serin protease, is co-purified together with RUBISCO and that a hydrophobic environment is necessary for its activation.

13 04

Phosphinothricin reverts the ammonia-dependent enhancement of phosphoenolpyruvate carboxylase activity

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The effect of ammonium assimilation on phosphoenolpyruvate carboxylase (PEPCase) activity was investigated in detached leaves from N-limited barley plants. The time-course induction of PEPCase was dependent on the rate of ammonia assimilation and not on ammonia uptake or accumulation. Treatment of N-deprived leaves with phosphinothricin (an inhibitor of glutamine synthetase activity) caused inhibition of ammonia assimilation, resulting in the reversion of ammonia-dependent enhancement of PEPCase. The results indicate that glutamine level controls phosphoenolpyruvate carboxylase activation, consequently whether glutamine synthesis is inhibited PEPCase activity is not enhanced. Determination of malate content showed that as PEPCase activity increased in response to increasing ammonia assimilation, there was a linear decline in the level of that metabolite. We have also analyzed the *in vivo* effect of malate on ammonium-dependent activation of PEPCase. High malate uptake partially abolished PEPCase activation, but the induced PEPCase activity seems to be less sensitive to malate than the control one.
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13 05

Diversity of soluble inorganic pyrophosphatases in potato

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Inorganic pyrophosphatases (EC 3.6.1.1, 'PPases') catalyze the hydrolysis of pyrophosphate (PP_i) into orthophosphates and are sometimes considered as 'housekeeping' enzymes, making a range of anabolic processes thermodynamically possible. In addition, PP_i has a special status in the plant cell cytosol where it functions as a phosphoryl and energy vehicle in parallel to ATP. Its concentration in this compartment seems to be finely regulated but the control mechanisms are only poorly understood. In order to provide new insights into the importance of PPases and PP_i in plant metabolism, we have addressed the diversity of the enzymes at the protein and gene levels in potato. Protein purification work, immunological approaches, gene cloning and mapping have pointed to the existence of at least three isoforms encoded by separate genes. Molecular approaches will define their regulation and significance in terms of carbon use strategy during plant development.

13 06

Sucrose metabolism in soybean nodules: contributions of sucrose synthase and alkaline invertase

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A detached nodule system was developed in order to study carbohydrate metabolism in relation to N_2 fixation. After detachment apparent nitrogenase activity was maintained at reasonable rates for at least 2 hours. During this time exogenously supplied carbohydrate was readily taken up and metabolised into a variety of products (respired CO_2, anions, starch etc.). Fluorosucrose (FS, 1-deoxy-1-fluoro sucrose) and sucrose (both labelled with ^{14}C (U) in the glucosyl moiety) were used in this system to estimate the contributions of sucrose synthase (SS) and alkaline invertase (Alk I) to sucrose metabolism. *In vitro*, SS can metabolise sucrose 3.6 x faster than FS while Alk I metabolises sucrose 26 x faster than FS. Thus the ratio of ^{14}C derived from suc compared to the ^{14}C from FS in any product gives a measure of the relative contributions of the two enzymes. The results will be discussed in relation to the amount of extractable activity and other potential controlling factors in nodules at various stages of plant development.

13 07

The physiological role of storage proteins in Romanian wheat germplasm

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The proteins represent the most important components of the cell. The storage proteins are involved in different processes. The glutenins were investigated in individual kernels belonging to 15 genotypes by polyacrylamide gel electrophoresis. There were established eight proteinogram types. Our results evidenced the role of glutenin subunits with high molecular mass in a good bread making quality. These components also allow the use of their polymorphism in characterization and identification of varieties, being known that these proteins are coded by 3 genes and that their allelles are electrophoretically distinct.

13 08

Molecular characterization of sucrose-cleaving enzymes from carrot

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Mature tap roots of carrot (*Daucus carota*) accumulate high concentrations of sucrose, glucose and fructose in vacuoles of storage parenchyma cells. In carrot, sucrose is also the major transport form of assimilated carbon. Transport of the disaccharide from leaves into the storage organ seems to be controlled among other factors by the rate of sucrose utilization in the tap root and its transport through membranes.

Sucrose utilization is initiated by sucrose cleavage, which is catalyzed by two different enzymes with several isoforms differing in their subcellular locations: ß-fructosidase (invertase) and sucrose synthase. Carrot contains an extracellular acid ß-fructosidase ionically bound to the cell wall, two soluble acid ß-fructosidases located in the vacuole, and a soluble alkaline ß-fructosidase with a cytoplasmic location. Sucrose synthase appears to be encoded by only one gene and is also located in the cytoplasm.

As a step towards understanding the role of sucrose-cleaving enzymes in sucrose partitioning in carrot, we characterized them at the molecular level. The characterization of cDNA clones for sucrose synthase and the major forms of acid ß-fructosidase and their expression in developing plants will be presented. We will also describe the structures of some of the genes and their characterization.

13 09

Allantoinase and allantoate amidohydrolase activities in soybean cell cultures

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Allantoinase (ALNase) and allantoate amidohydrolase (ALAH) are enzymes involved the degradation of the ureides allantoin (ALN) and allantoic acid (ALC), major N compounds in N_2-fixing tropical legumes. The activity of ALNase and ALAH was determined in soybean cells during growth on media containing inorganic N (B5) or ALN. When cells were grown on B5, a steady increase of ALNase activity with culture age was observed, while ALAH activity peaked and further decreased. The cellular level of ALC increased with time while ALN level remained constant. In ALN-grown cells, ALNase activity reached a plateau, whereas ALAH activity slightly increased with time. ALNase was apparently inhibited or repressed at high ALC concentration in the cells. ALAH activity, limitant step of ureide assimilation, seemed to be affected by ALC levels as well as by other factors. Possible regulating mechanisms of ALAH activity are being studied at present.

13 10

Modeling phloem loading in peach leaves

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Sorbitol and sucrose export out of mature peach (*Prunus persica* L. Batsch) leaves was studied by simulating carbon fluxes. Photosynthesis and ^{14}C partitioning into sorbitol and sucrose were measured at the beginning of the photoperiod and the export rate of sorbitol and sucrose was modeled using saturable functions of the exportable carbohydrate content. The C fluxes were modeled using PSPICE (Simulation Program with Integrated Circuit Emphasis, University of Berkeley) simulator. The sorbitol and sucrose contents calculated by the simulator were compared with experimental carbohydrate contents. This allowed one to estimate the apparent K_m and V_{max} for sorbitol and sucrose phloem loading, and to quantify the effect of a theoretical small change in K_m or V_{max} on export rate. The estimated V_{max} values for both sorbitol and sucrose phloem loading were similar to the photosynthetic C flux into each exportable carbohydrate measured under the leaf growth conditions. However, the fate of sorbitol differed from that of sucrose. The leaf appeared to have a higher storage capacity for sorbitol than for sucrose. The content of sucrose seemed tightly regulated.

13 11

Significance of glutamine synthetase in *Solanum tuberosum* L.tubers

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Potato tubers represent an important reservoir of nitrogen both in the form of proteins and of a free aminoacid pool which consists mainly of glutamine and asparagine. Recent work in this laboratory supported the assertion that a cytosolic isoform of glutamine synthetase (GS) may be involved in the synthesis of glutamine for export. An approach was made to the characterization of GS isoforms in potato tubers, particularly at the stages of sprouting and of new tuber formation. On immunoblots of tuber extracts, a GS polypeptide of about 42 kDa, corresponding to the cytosolic form could be detected. The immunostaining of this polypeptide was more intense in extracts of tubers in the onset of sprouting and less intense a few weeks after sprouting. By Western tissue printing it was possible to localize GS in tuber tissues. In spite of the low resolution of this technique, it allows the visualization of large areas of tissue sections and it is possible to ascertain the expression confined to certain tissues, irrespective of the level of GS in the whole organ. Labelling appeared scattered through the parenchyma but it was preferentially localized in the internal phloem in a tissue specific distribution. In sprouting tubers, labelling could be seen in rows, possibly vascular traces, directed to the sprouts. Immunostaining was also very intense in the basal portion of the sprouts. The internal phloem was also heavily labeled in the sprouts.These data provide additional clues to the interpretation of the functional role of the phloem-located GS in the mobilization of nitrogen in the plant.

13 12

Macluralisin - a serine proteinase from *Maclura pomifera* Schneid fruits

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From *Maclura pomifera* fruits a serine proteinase was isolated by affinity chromatography on bacitracin-containing sorbents and gel-filtration. The enzyme, named macluralisin, is a glycoprotein with a molecular weight of 65000, its protein moiety corresponds to molecular weight of 48-50000. Its substrate specificity towards synthetic peptides and insulin B chain is similar to that of cucumisin - a subtilisin-like proteinase from melon fruit. Its amino acid composition resembles that of serine proteinase isolated from *Cucurbitaceae*, the N-terminal sequence reveals 33% of identical residues, when compared with the sequence of fungal subtilisin-like proteinase K. Hence, *Maclura pomifera* serine proteinase belongs to subtilisin family, which seems to be broadly distributed in plant kingdom.

13 13

Pyrroline-5-carboxylate dehydrogenase in *Nicotiana plumbaginifolia* cultured cells

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Proline synthesis represents in many species a mechanism by which plant cells contrast dehydration stress caused by various environmental factors: water deficiency, high salinity, freezing. Understanding the basis of such response would be of great help in research for obtaining crop varieties adapted to non permissive environments. In spite of the interest and efforts of many research groups, the metabolic routes through which proline is synthesized and catabolized in plants are still poorly understood.
Here we report the purification of an NAD-dependent pyrroline-5-carboxylate dehydrogenase [EC 1.5.1.12] from *Nicotiana plumbaginifolia* suspension cultured cells. The enzyme was characterized with respect to structural, kinetic and biochemical properties, and its modulation investigated in response to water stress conditions.

13 14

Characterization and partial purification of endoamylase from leaves of spinach

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Endoamylase from leaves of spinach (*Spinacia oleracea* L.) can be resolved into multiple forms by non-denaturing electrophoresis in polyacrylamide gels containing starch substrates. Enzyme-specific staining upon electrophoresis of leaf extracts in the presence of amylopectin revealed five major discretely migrating activity forms. The entire spectrum of these isoforms was found in chloroplasts isolated from the leaves. On the basis of leaf chlorophyll, however, chloroplasts appeared to contain only a small proportion of the total endoamylolytic potential of the entire leaf.

The endoamylase of crude leaf extracts was concentrated by ammonium sulfate precipitation, enriched by affinity chromatography on amylose and separated into the individual forms by anion exchange chromatography on Q-Sepharose and a Mono-Q column. Two of the forms were enriched 500- and 700-fold, respectively, in comparison with the crude extract. Data on the properties of the purified endoamylase forms will be presented.

13 15

Studies on the rape seed phospholipase D

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Phospholipase D (PLD, E.C.3.1.4.4), an enzyme hydrolysing the phosphodiester bond in phosphatidylcholine and related phospholipids was isolated from mature dry winter rape seed (*Brassica napus* L, cv Arabella) and its biochemical properties were characterised The rape seed PLD is mainly soluble, a minor quantity being identified in the microsomal fraction. Purification of the enzyme was achieved by ammonium sulphate precipitation and ion-exchange chromatography followed by gel filtration. The effect of sodium dodecylsulphate (SDS), Triton X-100 and Ca^{2+} on the enhancement of rape seed PLD activity was shown. The optimum PLD activity was assessed to pH 6 and at 45 °C. Its isoelectric point was at pH 4.8. PLD partly purified by isoelectric focusing was used to prepare antibodies against the rape seed PLD. These antibodies provide means for further studies on the enzyme, i.e. during storage concerning its molecular specific activity, as well as, for examining changes in the PLD enzymes present during the germinating stages of the rape seeds.

13 16

Carbohydrate metabolism studied by *in vivo* ^{13}C-NMR: embryogenic cell cultures of *Daucus carota* used as a model system

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^{13}C-NMR has been used to study primary carbon metabolism of plant cell suspensions using embryogenic cell cultures of carrot (*Daucus carota*) as a model system. $^{13}C_1$-labelled glucose was added to the medium and taken up within 6 h. Inside the cell, part $^{13}C_1$-labelled glucose was converted into $^{13}C_1$-labelled fructose, probably by hexose isomerase; both labelled hexoses were found in newly synthesized sucrose. Glucose and fructose were also present in the $^{13}C_6$-labelled form. This C_1-C_6-exchange will occur at the level of triose phosphates in the glycolysis, probably as a result of gluconeogenetic activity, in which pyrophosphate dependent PFP is supposed to play a key role. The rate of C_1-C_6-exchange depended on the level of O_2. The labelling of sugars in the cells was transient: all the NMR-visible label had disappeared after 8 - 10 h, probably by a combination of sugar degradation (yielding $^{13}CO_2$) and synthesis of (NMR-invisible) macromolecules. The total level of sugar did not show comparable changes, suggesting the presence of multiple sugar pools in the suspension. The implications of these results for the regulation of sugar metabolism and the possibilities of using ^{13}C-NMR in the study of primary metabolism will be discussed.

13 17

Molecular characterization of a leucine zipper transcription factor from *Hordeum vulgare*

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The expression pattern of several members of the barley multigene family of trypsin/α-amylase inhibitors (1) has been analyzed. The steady state levels of mRNA corresponding to trypsin-inhibitor BTI-CMe and to the three components of the tetrameric α-amylase inhibitor BTAI-CMa, BTAI-CMb and BTAI-CMd are greatly diminished in the developing endosperm of some high lysine mutants with respect to the wild type lines from which they were derived. Since the corresponding structural genes or their copy members have not been modified in the mutants, a missing or altered transcription factor(s) could be the cause of such low synthesis. This possibility has been fully demonstrated for BTI-CMe in cv Bomi and its high-lysine (low hordeins) mutant Riso 1508. In maize, the study of high lysine opaque-2 mutants has unveiled that the regulation of zein and of b32 protein synthesis is mediated by a transcriptional factor of the leucine zipper type. We have cloned from barley PCR fragments of the bzip domain of leucine zipper transcription factors, and have used these as probes for isolating the corresponding genomic clones. Data will be presented regarding the molecular characterization of one leucine zipper gene, its chromosomal mapping and its expression in several plant tissues from normal and high-lysine barley cvs. (1) P. Carbonero et al. (1993). In: Innovations in Proteases and their Inhibitors (F.X.Avilés ed). Walter de Gruyter Berlin/NY. pp. 333-348.

13 18

The effect of sugar supply under *in vitro* conditions on the efficiency of transfer of plants from *in vitro* to *ex vitro* conditions

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Plants grown *in vitro* are usually supported by exogenous sugars. It is possible to find optimum concentration for the best growth for particular plant species under *in vitro* conditions. However, it is not clear which *in vitro* conditions are favourable for the most successful transfer of plants to *ex vitro* conditions. The best growth of wheat plants derived from excised wheat embryos is correlated with the highest efficiency of the transfer to *ex vitro* in spite of the fact that the accumulation of non-structural carbohydrates is highest at the highest concentration of exogenous sugar used. We can conclude that there is no direct relationship between the amount of non-structural carbohydrates or degree of autotrophy and the efficiency of transfer to *ex vitro* conditions.

13 19

Problems and limitations in studying plant primary metabolism

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There are many powerful tools available for studying plant metabolism, and measurement of gene expression, protein content and enzyme activity, and metabolite steady-state levels, can be readily achieved. However, all of these measurements only look at part of the story, and it is not often easy to integrate such separate approaches into a consistent view of active metabolism in a given tissue or organ. Transgenic plants, with sense or antisense expression of defined genes, are often considered "the answer" to metabolic problems - but in practice they can uncover more puzzles than they answer. Simple measurements of enzyme activity and metabolite levels do not reveal the flux through a pathway, which is what we need to understand, particularly with regard to control and metabolic regulation. The advantages and disadvantages of available methods will be reviewed, using examples from nitrogen and amino acid metabolism. The often overlooked importance of trans-membrane fluxes will be discussed.

13 20

Effect of some enzymes on starch grains of chloroplasts

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The effect of different enzymes on starch grains of chloroplasts of mature leaves in *Loiseleuria procumbens* and *Aesculus hippocastanum* with electron microscope have been investigated. 1 % solutions of amylase, lipase, pronase in distilled water and β-mannosidase and alkaline proteinase in phosphate buffer were used. The sections exposed to distilled water and phosphate buffer were used as controls. These enzymes affected a starch grains differently. Starch grains were dissolved due to amylase and lipase. Pronase dissolved starch grains of *A. hippocastanum* and alkaline proteinase - that one of *L. procumbens*. β-mannosidase dissolved starch grains of *A. hippocastanum* and did not that one of *L. procumbens*. The solutions with various pH acted on starch grains differently. Starch grains of both species were not dissolved in distilled water with various pH. Hydrolyzation of starch grains in mature leaves occurring in two ways probably: enzymatic and not-enzymatic. There are various pH which not-enzymatic hydrolyzation occur in different species. Starch grains in mature leaves do not consist only of polysaccharides. They are complex heterogenous substances with proteins, carbohydrates and lipids.

13 21

Do plants contain serum albumin?

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We have isolated serum albumin-like protein from a variety of plant tissues and plant species. The protein is recognized by the polyclonal antibody raised against rabbit serum albumin and its N-terminal 15 amino acids share 100 % sequence identity with the human serum albumin. However, the monoclonal antibody raised against the human serum albumin does not immunoreact with the plant protein. This protein is glycosylated and it is associated with cell membranes. Using Triton X-114 fractionation, we determined that the protein is present in the peripheral protein fraction in the microsomes. Chemical and physical properties of plant serum albumin-like protein are discussed.