Effect of S-methylmethionine as a protective compound on the metabolism of agricultural plants at low temperature

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ABSTRACT   The present research dealt primarily with a special protective mechanism which appears to be part of the defence responses of plants to low temperature. It was found that the metabolisation of S-methylmethionine (SMM) had a stimulatory effect on polyamine biosynthesis. The results proved that SMM acted via a special metabolic pathway to increase the quantities of certain polyamines (putrescine, agmatine, spermidine) known to be involved in the stabilisation of the cell environment, the macromolecules and the cell membranes. It was demonstrated that in wheat varieties, peas and maize, SMM treatment was able, to varying extents, to reduce low temperature-induced electrolyte leakage measured in terms of electrical conductivity.

Acta Biol Szeged 46(3-4):95-96 (2002)

S-methylmethionine (SMM) was first identified as a constituent of higher plants in cabbage leaves and shortly thereafter by Challanger and Hayward (1954) in asparagus. Subsequently, this sulphonium compound was found in a large number of plant sources. After the administration of radio-labelled methionine to a variety of intact plant preparations, substantial amounts of radiolabel have usually been found in SMM. The biosynthesis of this compound in cell-free preparations was first demonstrated by Sato at al. (1958) in homogenates of oat sections. Greene and Davis (1960) showed that the reaction catalysed by extracts of jack bean roots was dependent upon S-adenosyl-methionine. Methylated compounds also have an important place in plant metabolism. Our interest was chiefly aroused by the methylated form of methionine, SMM, since preliminary experiments had suggested that it could play an important role in reducing the unfavourable effects of abiotic stress factors. The aim of the present work was to clarify its connection with polyamine biosynthesis, and to examine its effect on electrolyte leakage.

Materials and Methods

The examinations were carried out when the plant species were in the two-leaf stage. Electrolyte leakage was studied in peas, maize and 8 wheat varieties with different levels of frost resistance. Wheat seedlings were grown for 8 days, maize for 10 days and peas for 14 days in $1/4$-strength Hoagland's solution. In the SMM treatments a 0.01% concentration of SMM was used, dissolved in the nutrient solution. The concentration of the specific inhibitor, methyl-glyoxal-bis-guanyl hydrazone (MGBG), was 0.55 M. The plants were raised in the phytotron under controlled conditions. Electrolyte leakage measurements were carried out according to Szalai et al. (1996). One g plant tissue (roots, leaves) were placed in 50 ml ultrapure water and shaken for 1 h in the dark. Conductivity was measured using an

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Automatic Seed Analyzer. The results were the means of 20 replications, and were statistically evaluated using the standard deviation and T-test methods. The low temperature treatments were carried out at 4°C for 24 h. The polyamines extracted from leaves and roots were separated by means of high performance liquid chromatography (Bencsik et al. 1998).

Results and Discussion

The primary aim of the experiments was to clarify the interaction of S-methylmethionine with polyamine biosynthesis, and to prove that SMM plays a role in reducing the effect of low temperature stress. It is well known that low temperature induces the synthesis of certain compounds (soluble sugars, amino acids, stress proteins, polyamines, etc.). These compounds stabilise the cell environment, thus eliminating or reducing the unfavourable effects. Polyamines are known to stimulate the activity of RNA polymerases, to inhibit the decomposition of RNA and the peroxidation of lipids, and to stabilise the cell membranes.

SMM can be found in many plants (Mudd and Datko 1990), but is only present in large quantities in brassicas, which are known to be tolerant of low temperatures. Premilinary experiments indicated that SMM is closely linked to the polyamine metabolism and plays a role in moderating the damaging effects of numerous stress factors. SMM arises from the methylation of methionine. The methyl donor is S-adenosylmethionine (SAM), which is an intermediary in the pathway of polyamine synthesis starting from methionine. The first question was whether the metabolisation of SMM took place in a manner similar to the metabolic pathway mentioned above. In order to determine this, the various pathways of SMM were investigated and it was found that SMM was converted to homoserine and dimethylsulphide with the aid of a hydrolase enzyme. It is interesting to note that SMM transformation may also take place spontaneously, without the presence of hydrolase, in an

alkaline medium. Spermidine is formed from homoserine and putrescine, via a number of labile intermediary products. With the aid of SMM labelled with ^{14}C it was proved that the carbon skeleton of SMM can be found in both homoserine and spermidine. When the specific inhibitor of the polyamine synthetic pathway starting from methionine, MGBG, was also used, no inhibitory effect was observed, thus proving that the alternative transformation route was followed. The induction of polyamine synthesis by SMM was examined in three plant species (pea, soybean, wheat). The results show that as the result of SMM treatment the quantities of polyamines, with the exception of spermine, rose to various extents. This accumulation was not reduced by MGBG, proving that the metabolisation of SMM did not follow the metabolic pathway starting from methionine.

Further experiments were aimed at determining the extent to which SMM was able to reduce the unfavourable effect of low temperature. It is well known that low temperature damages cell membranes. The loss of cytoplasm from the cell through the damaged membranes is known as electrolyte leakage, which can be measured by determining electrical conductivity. In the course of the experiments the electrolyte leakage was measured in the leaves and roots of control and SMM-treated plants for 8 winter wheat varieties, maize and peas. A comparison of the results obtained for the winter wheat varieties indicated that SMM reduced the extent of electrolyte leakage to various extents in the leaves and roots of treated plants at 4ºC (24 h, in the dark) compared to the control (25ºC). In peas, which are tolerant of low temperature, the differences in conductivity were lower than in maize, which is of subtropical origin.

The present studies involved a methylated methionine which preliminary experiments suggested might be closely linked with polyamine synthesis. The synthetic pathway of N-containing compounds are known to be extremely complicated and in many cases the interactions between these pathways have not yet been clarified. This is underlined by the results achieved with regard to the metabolisation of SMM, which show quite clearly that SMM is one of the initial/starting compounds for the synthesis of spermidine, which plays an important role in the protein synthesis taking place on the surface of the ribosomes. It should be noted that one of the by-products of SMM metabolisation is dimethyl-sulphide, a natural radical scavanger, which thus provides additional protection to the plant against the oxidative stress arising due to low temperature. Experiments were carried out on 3 crops to determine the extent of damage to cell membranes at low temperature. The results confirm that SMM treatment reduced electrolyte leakage to various extents, indicating that the compound plays a role in maintaining the integrity of cell membranes. It would appear from the experimental results that in genotypes sensitive to low temperature exogenous SMM contributes to the development of defence mechanisms in the plant by enhancing the intensity of polyamine biosynthesis.

The latest results (Hanson et al. 1994; Trossat et al. 1996; Pimenta et al. 1998) show that SMM is the initial compound for the synthesis of dimethyl-sulphoniopropionate (DSMP). SMM is synthesised in the cytosol and DSMP in the chloroplast. The whole of the synthetic pathway has not yet been clarified. DSMP is a strong osmoregulator and thus protects and stabilises the chloroplast membranes. It should also be mentioned that the dimethylsulphide which arises in the course of SMM metabolisation is an excellent radical scavanger.

On the basis of the above, the physiological and biochemical effects of SMM can be explained in three ways: 1) by stimulating polyamine biosynthesis it stabilises macromolecules (nucleic acids, proteins); 2) through the synthesis of DSMP it regulates the osmotic status of the cells; 3) the dimethylsulphide arising during the enzymatic and spontaneous decomposition of SMM is a natural radical scavanger which thus provides pretection against lipid peroxidation.

Acknowledgments

References

Bencsik K, Kremmer T, Boldizsár J, Tamás J, Mák M, Páldi E (1998) High-performance liquid chromatographic determination and standardization of agmatine. J Chromat A 824:175-180.

Challenger F, Hayward BJ (1954) The occurence of a methyl-sulphonium derivative of methionine-(α-aminodimethyl-γ-butyrothein) in aspargaus. Chemical Industry 729-730.

Giovanelli J, Mudd SH, Datko AH (1980) Sulfur amino acids in plants. In BJ Miflin, ed., The Biochemistry of Plants, Vol. 5, Academic Press, New York, pp 453-505.

Green RC, Davis NB (1960) Biosynthesis of S-methylmethionine in the jack bean. Biochim Biophys Acta 43:360-362.

Hanson D, Rivoal J, Paquet L, Gage DA (1994) Biosynthesis of 3-dimethylsulfonioproprionate in *Wallastonia biflora* (L.) DC. Plant Physiol 105:103-110.

Mudd SH, Datko AH (1990) The S-methylmethionine cycle in *Lemna paucicostata*. Plant Physiol 93:623-630.

Pimenta MJ, Kaneta T, Larondelle Y, Dohmae N, Kamiya Y (1998) S-adenosyl-L-methionine S-methyltransferase from germinating barley. Plant Physiol 118:431-438.

Sato CS, Byerrum RU, Albersheim P, Bonner J (1958) Metabolism of methionine and pectin esterification in a plant tissue. J Biol Chem 23:128-131.

Splittstoesser WE, Mazelis M (1967) The metabolism of methionine in higher plants. Catabolism of the methyl group by seedlings of various species. Phytochemisty 6:39-47.

Szalai G, Janda T, Páldi E, Szigeti Z (1996) Role of light in the development of post-chilling symptoms in maize. J Plant Physiol 148:378-383.

Trossat C, Nolte KD, Hanson AD (1996) Evidence that pathway of dimethyl sulfonioprionate biosynthesis begins in the cytosol and ends in the chloroplast. Plant Physiol 111:965-973.