Phosphoenolpyruvate Carboxylase Activity in Norway Spruce Needles: Effects of Air Pollutants under Controlled Conditions

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The effect of mixtures of air pollutants (SO_2, NO_2, O_3) on phosphoenolpyruvate carboxylase (PEPCase; EC 4.1.1.31) activities from needles of four-year-old Norway spruce (*Picea abies* [L.] Karst.) in closed fumigation chambers were analyzed. Three months exposure to high concentrations of O_3 (1030 µg/m^3) together with SO_2 (1030 µg/m^3) or NO_2 (615 µg/m^3) resulted in a dramatic increase in PEPCase activities.

Introduction

Some 20 years ago a novel type of tree damage in forest decline in the western parts of Germany was described and is now observed worldwide [1], see *e.g.* heavily damaged silver fir, Norway spruce and beech. Besides the well known symptoms, directly related to air pollutants from different sources including emission of individual industries, there are the so-called "clean-air" regions of forest decline which are far away from sources of pollutant emission. Attempts to explain the reasons for this phenomenon as well as to find reliable early indicators as signs for beginning tree damage before the appearance of obvious visual damage have so far failed. From a vast number of publications on this subject, many hypotheses have been discussed varying from exclusive effects of natural stress to different anthropogenic factors, to the exclusive effects of airborne pollutants [1–3].

The most important air pollutants include sulfur dioxide (SO_2), ozone (O_3) and nitrogen dioxide (NO_2). Whereas a single pollutant is usually below the threshold for signs of acute tree damage [4], in most areas of forest decline the presence of more than one pollutant might exhibit additively or synergistically strong effects. It is most desirable to find early biochemical responses of trees exposed to these pollutants. We report here on the results of enzymatic dose-effect studies with needles from Norway spruce trees exposed to various combinations of SO_2, O_3 and NO_2, applied under controlled climatic conditions, focussing on phosphoenolpyruvate carboxylase (PEPCase; EC 4.1.1.31).

Wild and coworkers [5, 6] found in their studies with trees from open-top chambers as well as from damaged trees from the field pollutant-dependent increases in PEPCase activity. Considering the various possible regulatory functions of PEPCase in the so-called C_3 plants [7], one would expect this enzyme to respond sensitively to pollutant-induced metabolic changes. Decrease in the C/N ratio due to changes in amino acid metabolism [8] and increase in nitrate reductase activity [9] may result in PEPCase activity increase in anaplerotic pathways [7, 10] and *via* malate in the involvement of reductant supply [11, 12], respectively.

Materials and Methods

The present work is part of the multidisciplinary GSF experiment No. 31 (planned by C. L.). For all studies four year-old generative spruce trees (*Picea abies* [L.] Karst.) were treated under controlled climatic and pollution programs in four phytotrons of the GSF (technical details: [13, 14]). Every phytotron chamber contained four fumigated subchambers one of which housing a control group with low-level air pollutants (57 µg/m^3 SO_2, 43 µg/m^3 O_3, 21 µg/m^3 NO_2). Fumigation values of the respective treatments are given in Fig. 1. For three months the trees were exposed to the elevated levels of pollutants for 10 h/day under a climate, simulating autumn regimes according to average daily values of Central Europe. At the end of the experiment, needles of the current year were detached in liquid nitrogen and stored in liquid nitrogen until processed. Enzyme extracts were prepared by grinding 1 g of needles in the presence of liquid nitrogen and quartz sand. After adding 2 g insoluble polyvinylpyrrolidone (PVP), the resulting powder was extracted with 50 mM potassium-phosphate buffer (pH 7.1) containing 1% Triton X-100, 2 mM

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ethylenediamine tetraacetate (EDTA) and 15 mM dithiotreitol (DTT). Protein precipitated between 35 and 80% ammonium sulphate saturation was filtered through Sephadex G-25 [15]. The eluate was used as source of PEPCase activity. Assay conditions and activity determination were according to Buchanan-Bollig *et al.* [16]. The assay contained 10 mM $NaHCO_3$, 200 mM bicine buffer (pH 8.0), 0.15 mM NADH, 1 mM DTT, 5 mM $MgCl_2$, 60 U malate dehydrogenase (Merck, Darmstadt), 3 mM PEP and 100 µl protein extract (equivalent to 200 µg protein). Activity was determined photometrically at 340 nm.

Results and Discussion

The present paper shows the first results of our studies on metabolic changes in Norway spruce needles treated with air pollutants under strictly controlled climatic conditions. Among measurements in the GSF experiment of various metabolites (pigments, carbohydrates, nucleotides, ions and amino acids [not documented]) and some enzymatic activities (glutamate synthase, glutamine synthetase, glutamate dehydrogenase, arginase, nitrate reductase [not documented] and PEPCase, the most striking results are the activity changes of two enzymes, nitrate reductase and PEPCase, which showed appreciable pollutant-dependent activity increases. The nitrate reductase responds with a marked NO_2-dependent activity increase [9]. The results on PEPCase activity measurements are summarized in Fig. 1. In particular the highest concentrations of O_3 tested in combination with NO_2 gave the highest activity values, in agreement with the theory of O_3 damage [17–19]. A small increase in

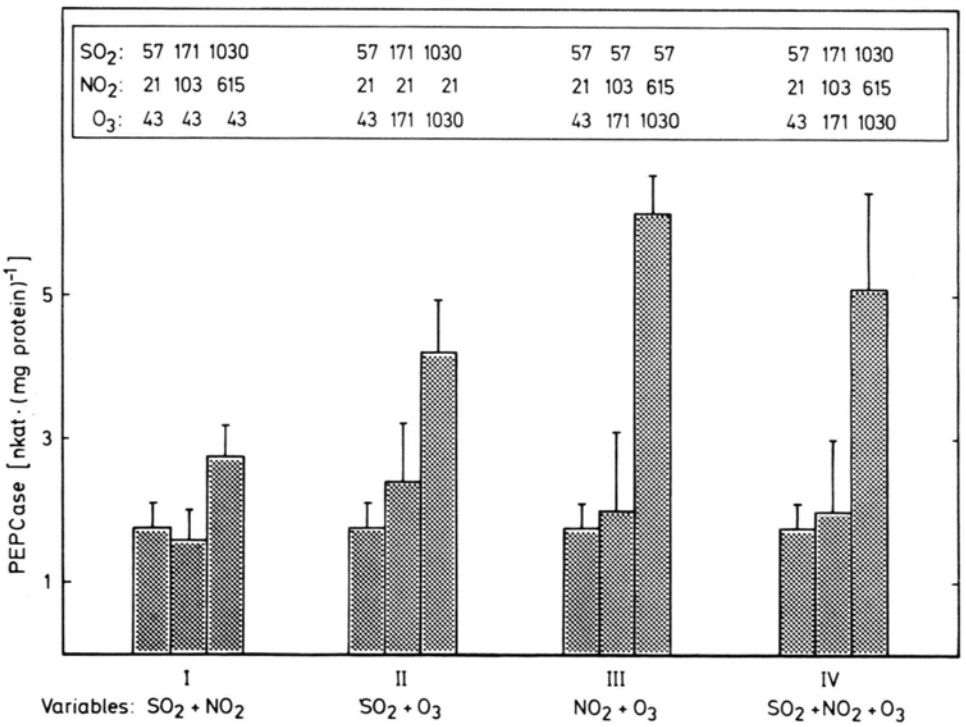

Fig. 1. Phosphoenolpyruvate carboxylase (PEPCase) activities in needles from four year-old Norway spruce trees exposed to various combinations of different amounts (µg/m³) of SO_2, NO_2 and O_3 in closed fumigation chambers. Values are expressed on the basis of extractable needle protein (specific activities). There were no significant differences in the protein contents, nor in the fresh or dry weights of the needles from the differently treated trees. The groups I through IV represent the four experimental protocols with the variable amounts of gases indicated. All trees in the control chambers (first bars) showed very similar PEPCase activities, which were pooled and presented as a common basis for the comparison with the elevated pollution programs. The activity values are the mean ± S.D. of six determinations from six individual trees. The experimental S.D. (protein extraction through activity determination with one needle population) was determined to be 8.6% (n = 7).

enzyme activity was already observed at the second level of pollutant concentrations (groups II–IV) with doses similar to those often observed in polluted areas in Germany. This study requires also application of high concentrations to generate effects in an appropriate time of exposure. It is important to note, that so far the strongest pollution protocols did not cause visual signs of damage. However, the time of exposure was three months only; thus long-term effects, occurring also at lower pollutant doses in the field, could not be assessed.

Besides the role of PEPCase in the so-called C_4 plants and those exhibiting the crassulacean acid metabolism (CAM), its possible function in other plants (C_3 plants) is poorly understood. Many different important functions have been discussed [7], including involvements in the supply of reductant for nitrate reductase [11, 12] or in nitrogen assimilation and amino acid synthesis [7] by way of 2-oxoglutarate and the glutamate synthase-glutamine synthetase system [20] or transamination with oxaloacetate [21].

In Norway spruce needles PEPCase will possibly initially be a site of fast regulation through metabolite inhibition and stimulation [22]. Changes of metabolic pool sizes and metabolic fluxes over longer time periods, however, could be reflected in changes of the extractable total PEPCase activity as seen in Fig. 1. Changes in the nitrogen metabolism [23], *e.g.* decrease in the C/N ratio [8], may result in PEPCase activity increase in anaplerotic pathways. However, also disturbance of translocation of assimilates [24] may cause severe alterations in the balance of metabolic concentrations in the needles. On the other hand, the observed PEPCase activity increase could be discussed in the context of an increased activity of the nitrate reductase, malate being a possible major source for hydrogen donors for this enzyme [11, 12].

Experiments have been initiated to study the role of PEPCase in Norway spruce and to screen for pollutant-dependent metabolic changes, connected to changes in PEPCase activity. In addition to changes in the reactions involved in the primary metabolism, changes in the highly complex secondary phenolic metabolism of Norway spruce needles [25, 26], *e.g.* O_3-induced early reactions [27], are also being taken into account.

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