SUBSTRATE SPECIFICITY OF GLUCOKINASE AND FRUCTOKINASE OF SEVERAL CONIFER SPECIES

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ABSTRACT

Annual plant species were found to have several distinct classes of hexokinases which are specific for different hexoses, such as glucose, fructose and mannose. In conifers one isozyme of hexokinase could be found in genetic studies if only glucose was employed as substrate. If fructose was substituted for glucose, another isozyme zone different from the common hexokinase could be observed in zymograms of extracts from seed tissues of Norway spruce, Scots pine and silver fir. Hence these three conifer species possess at least two different hexokinases, glucokinase and fructokinase.

KEY WORDS: hexokinase, glucokinase, fructokinase, substrate specificity, isozymes, *Pinus sylvestris*, *Picea abies*, *Abies alba*.

INTRODUCTION

Hexokinases (or hexose kinases) are important enzymes of the primary metabolism in plants since they are responsible for the phosphorylation of free hexoses, thus producing metabolites of higher energy content. The resulting hexose-6-phosphates are key substrates for several metabolic pathways, such as glycolysis or the oxidative pentose phosphate pathway. Studies on hexokinases in annual plant species showed that not only glucose but also fructose and mannose can serve as substrates for the phosphorylation process, however, the specificity of the respective enzymes (or isozymes) can vary in different plant species (for review, see Kruger 1990).

In conifer species the electrophoretic analysis of hexokinases mostly yielded only one isozyme (Beaulieu and Simon 1994; Niebling et al. 1987), since glucose was usually employed as a substrate (Wendel and Weeden 1989). Other hexoses were not used in these isozyme studies since the main goal was the identification of gene markers. In our study on genetic variation of enzymes functioning in the carbohydrate metabolism, also one hexokinase zone appeared in zymograms of tissue extracts from several conifer species (Bergmann and Mejnartowicz 2001). Since only glucose was used as substrate, it could not be established whether the same enzyme can also phosphorylate

fructose or whether additional kinases specific for other hexoses are functioning in conifer species.

The objective of this study was, therefore, to demonstrate the existence of different hexokinases in seed tissues of three conifer species, Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and silver fir (*Abies alba*).

MATERIAL AND METHODS

The plant material used in this study consisted of bulk seedlots from a Scots pine seed orchard (Wasserbett, south-western Germany), a Norway spruce crop stand (Westerhof, central Germany) and a native silver fir population (Rhodope Mts., Bulgaria). The seeds were germinated for a few days and then separated into the haploid endosperm (megagametophyte) and the diploid embryo. Both tissues were extracted with a Tris-HCl buffer pH 7.4 and the extracts were subjected to a horizontal starch-gel zone electrophoresis, using the Ashton-Bradon buffer system pH 8.1 for optimal resolution.

Following electrophoretic separation, the gel slabs were stained for glucokinase (EC 2.7.1.2) and fructokinase (EC 2.7.1.4). The staining solution for glucokinase consisted of 0.1 M Tris-HCl buffer pH 8.0 (50 ml) containing 150 mg D-glucose, 80 mg ATP, 5 mg NADP, 2 ml MgCl₂ (10%),

40 units of glucose-6-phosphate dehydrogenase and the common tetrazolium stain (PMS, MTT). For fructokinase the same stain composition was used but D-fructose was substituted for D-glucose and 60 units of phosphoglucose isomerase were additionally included in the staining solution (see also Wendel and Weeden 1989).

RESULTS AND DISCUSSION

The electrophoretic pattern of glucokinase (identical to the former hexokinase, HEK) of extracts from single seed endosperms and embryos of the three conifer species exhibits one activity zone migrating to the anode. Whereas this HEK zone consists of double-banded variants in haploid endosperm tissue of *P. abies* and *P. sylvestris*, (Fig. 1b and 1c) the corresponding zone of *A. alba* appears as a single band (Fig. 1a). In agreement with other studies (Goncharenko et al. 1995; Niebling et al. 1985) it was established that this zone is controlled by one gene locus which was designated HEK-A (Bergmann and Mejnartowicz 2001). Based on the banding patterns of haploid endosperms (sin-

gle and double bands) and diploid heterozygous embryos (double bands or four bands) the quaternary enzyme structure of HEK is assumed to be monomeric.

In order to check whether the glucokinase isozyme in the three conifer species is also specific for D-fructose, a second slice of the same starch gel was stained for fructokinase (FRK). Plant fructokinases are enzymes with a high, specific affinity to fructose and they catalyse the conversion of fructose to fructose-6-phosphate (Pego and Smeeckens 2000). As is shown in Fig. 1, a separate isozyme zones migrating more anodically than the glucokinase isozyme appeared in the zymograms of seeds from the three conifer species. The substrate specificity of the fructokinase, however, appears to be not as strong as that of the glucokinase, since some additional faint bands in the FRK zymograms of *A. alba* and *P. abies* resemble the HEK isozymes (Fig. 1). Although a genetic analysis of the FRK isozymes was not yet possible due to the lack of variation in the seed material studied so far, the presence of two distinct hexose kinases (fructokinase and glucokinase) suggests the genetic control of two separate loci, HEK-A and a putative FRK-A, in the three conifer species.

The results of this study clearly demonstrated that the hexose kinases in seed tissues of three conifer species consist of at least two different enzymes: glucokinases and fructokinases. The variety of kinases capable of phosphorylating different hexoses may be due, at least partly, to three features of carbohydrate metabolism in higher plants. One is the degree to which such metabolism is compartmented. The second is the variety of hexoses that can be phosphorylated and the third is the variable amounts with which the two dominant sugars, glucose and fructose, are produced in the primary metabolism (Kruger 1990). More detailed studies on this subject are needed to establish whether the phosphorylation of other hexoses, such as mannose and galactose, is also governed by separate classes of kinase.

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Fig. 1. Starch gel zymograms showing fructokinases (FRK) and glucokinases (= former hexokinases (HEK)) from seed endosperm extracts of *Abies alba* (a), *Picea abies* (b) and *Pinus sylvestris* (c). Each line represents of individual megagametophyte (endosperm).

SPECYFICZNOŚĆ SUBSTRATOWA GLUKOKINAZY I FRUKTOKINAZY U KILKU GATUNKÓW DRZEW SZPILKOWYCH

STRESZCZENIE

U niektórych roślin zielnych znaleziono kilka klas odrębnych heksokinaz, będących specyficznymi dla różnych heksoz, takich jak: glukoza, fruktoza i mannoza. W badaniach genetycznych drzew szpilkowych, znajdowano jeden izoenzym heksokinazy, jeśli tylko użyto glukozy jako substratu. Jeśli zamiast glukozy podstawiono jako substrat fruktozę, można było zaobserwować inną strefę aktywności izoenzymów, różną od wspólnej heksokinazy wyekstrahowanej z nasion świerka pospolitego, sosny zwyczajnej i jodły pospolitej. A zatem można stwierdzić, że te trzy drzewa szpilkowe posiadają co najmniej dwie odrębne heksokinazy: glukokinazę i fruktokinazę.

SŁOWA KLUCZOWE: heksokinaza, glukokinaza, fruktokinaza, specyficzność substratowa, izozymy, tkanki nasion, *Pinus sylvestris*, *Picea abies*, *Abies alba*.