Chloroplast pentose-5-phosphate 3-epimerase from potato: cloning, cDNA sequence, and tissue-specific enzyme accumulation

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Abstract **A cDNA clone encoding the chloroplast enzyme pentose-5-phosphate 3-epimerase (EC 5.1.3.1) in potato (*Solanum tuberosum*) was isolated and sequenced. The deduced sequence of 235 amino acids is similar to protein sequences of bacterial epimerases. Northern blot analysis showed the highest level of epimerase mRNA expression in potato leaves, whereas it was low in roots, tubers, and stems. Epimerase protein is mulated only in plant tissues possessing chloroplasts, i.e. in land to a lesser extent in stem. In contrast, transketolase, a sequential enzyme of epimerase in the reductive and oxidative pentose phosphate cycle, is accumulated in all plant tissues.**

Key words: *Solanum tuberosum*; Chloroplast; Pentose-5-phosphate 3-epimerase; cDNA sequence; Tissue-specific enzyme accumulation

1. Introduction

Carbon dioxide is fixed into organic carbon via the reductive pentose phosphate (Calvin) cycle involving thirteen enzyme reactions in higher plants, algae, and other photoautotrophic organisms. Some of the Calvin cycle enzymes or functionally equivalent components including pentose-5-phosphate 3-epimerase (EC 5.1.3.1) also take part in the oxidative pentose phosphate cycle localized to plant chloroplasts [1]. The epimerase represents an essential component of the sugar phosphate recycling sequence of the Calvin cycle and catalyzes the conversion of xylulose-5-phosphate into ribulose-5-phosphate. The latter metabolite then converted by ribulose-5-phosphate kinase into the CO_2-acceptor ribulose-1,5-bisphosphate. Until now, there are no reports in the literature on the purification, catalytic properties, and primary structures of epimerases from higher plants and algae. However, epimerases have been isolated and characterized from animal [2,3] and yeast cells [4]. Amino acid sequences deduced from cDNA sequences were reported for an epimerase encoded by the CO_2-fixing operon *cfx* of the purple bacterium *Alcaligenes eutrophus* [5] and two epimerase proteins from *E. coli* [6,7]. Purified epimerases from erythrocytes [2], calf liver [3], and *E. eutrophus* [5] were shown to be homodimeric enzymes with a molecular mass (M_r) of the subunits of about 23,000 [2,3].

We are seeking to determine the factors underlying the supramolecular organization of sequential enzymes with multiple functions in photosynthesis and other metabolic pathways confined to chloroplasts of higher plants. In this context, we are especially interested in the molecular structure and intermolecular interactions of soluble chloroplast enzymes and their isoforms contributing to the reductive and oxidative pentose phosphate cycle.

In this paper we report the primary structure of pentose-5-phosphate 3-epimerase from potato leaves. To our knowledge, this represents the first report of an epimerase sequence from higher plants. Evidence is provided indicating that in contrast to another sequential enzyme of the pentose phosphate pathways, i.e. transketolase, epimerase is expressed only in plant tissues containing chloroplasts.

2. Materials and methods

2.1. Materials

Potato plants (*Solanum tuberosum*) were grown in the greenhouse under natural day/night conditions. The potato cDNA library in lambda UniZap II was kindly provided by Dr. Uwe Sonnewald, Gatersleben, Germany. The *E. coli* strain XL 1 blue [8] was used for plasmid amplification and bacteria were grown on Lauria-Bertani medium [9].

2.2. cDNA cloning and sequencing

Pentose-5-phosphate 3-epimerase and transketolase were purified from spinach chloroplasts and antibodies against the enzymes were raised in rabbits as described previously ([10]; Teige, M. and Süss, K.-H., in preparation). About 10^5 p.f.u. of the potato leaf cDNA library were immunoscreened with monospecific epimerase antibodies. Expression of the fusion protein was induced by placing IPTG-saturated nitrocellulose filters on the agar plates overnight. Epimerase-producing clones were identified by Western blotting using the ECL Western Blotting Detection System as outlined by the supplier (Amersham, UK). Positive clones were purified and lenghts of cDNA inserts determined by PCR using flanking vector primers. The longest clones were subcloned into pBluescript II KS plasmids (Stratagene) by in vivo excission. Plasmid DNA was isolated using the Flexi-Prep Kit (Pharmacia, Sweden). The cDNA clones were completely sequenced on both strands with a set of nested deletions prepared by treatment with exonuclease III (Pharmacia) and oligonucleotide primers. DNA sequences were determined either on an A.L.F. DNA sequencer (Pharmacia) or with a Taquence Version 2.0 DNA sequencing kit (USB) using the dideoxynucleotide method [11]. Sequence data were analysed with the PCGENE software (Intelligenetics). The EMBL (release 42) and Swiss Prot (release 31) databases were screened to identify DNA and protein similarities, respectively.

2.3. mRNA analysis

Total RNA was isolated from plant organs by phenol extraction and selective precipitation [9]. Electrophoresis of RNA in presence of formaldehyde was performed essentially as described by Sambrook et al. [9]. RNA was transfered onto Hybond-N$^-$ nylon membrane and hybridized with ^{32}P-labelled total cDNA fragment according to the in-

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The novel sequence reported here has been deposited with the EMBL database and is available under accession number Z50098.

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structions of the supplier (Amersham, UK) with the final washing step being 0.2 × SSC, 0.1% SDS at 65°C. The RNA blots were quantified with a BAS 2000 Bio Imaging Analyser (Fuji).

2.4. Protein sequencing and Western blotting

Purified spinach chloroplast epimerase was separated by SDS-polyacrylamide gel electrophoresis (PAGE) [12] and blotted onto an Immobilon-P PVDF membrane (Millipore) in the presence of 5 mM thioglycolate. NH_2-terminal amino acid sequencing of the enzyme was performed essentially as described by LeGendre and Matsudaira [13] using an automated amino acid sequencer (Applied Biosystems). Protein concentrations were determined by the method of Bradford [14].

Protein extracts were prepared from potato leaves, stems, roots, and tubers by grinding equal amounts (fresh weight) of tissue in liquid nitrogen and extraction with 4 × (v/w) buffer containing 8 M urea, 4% SDS, 50 mM Tris-HCl, pH 8, 1 mM DTT. Equal amounts of protein (30 μg) were subjected to SDS-PAGE and electrotransferred onto nitrocellulose membrane (0.2 μm pore size; Schleicher and Schüll, Dassel, Germany) using a buffer of 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid/NaOH, pH 11.0/2 mM thioglycolate/10% methanol. The blots were developed with the ECL-Western Blotting Detection System. The films were light-scanned and quantified with the BAS 2000 Bio-Imaging Analyser.

3. Results and discussion

Pentose-5-phosphate 3-epimerase was isolated from spinach chloroplasts and its NH_2-terminal amino acid sequence determined by Edman degradation [10]. Monospecific antibodies were raised in rabbits and used to immunoscreen a cDNA library from potato leaves in order to obtain cDNA clones encoding epimerase. Three identical clones were purified and one of them was sequenced completely on both strands. The epimerase cDNA clone, 1056 base pairs (bp) long, contained an uninterrupted coding region of 843 bp, unfortunately not complete at its 5′-end (Fig. 1). All attempts failed to obtain the missing 5′ region of mRNA either by 5-RACE or repeated cDNA screening. The cDNA codes for 280 amino acids of a precursor protein that contains a stretch of amino acids starting at position 46 which matches the NH_2-terminal amino acid

```
CTTCTTTGGGTTCATCAACTCTGTTACAATCCCAAATTAGTGGATTTGGCGGGAGTCAAA   60
   S  L  G  S  S  T  L  L  Q  S  Q  I  S  G  F  G  G  S  Q     (19)
AGCTTCAAAAGATTTCTTTCTCCAACCCCAATTCACTCACTTTCACCAGGAGGAGAATTC  120
K  L  Q  K  I  S  F  S  N  P  N  S  L  T  F  T  R  R  R  I     (39)
AAACTGTGGTGAACGCTTCTTCTCGGGTGGATAAGTTCTCAAAAAGCGACATTATTGTTT  180
Q  T  V  V  N  A  S  S  R  V  D  K  F  S  K  S  D  I  I  V     (59)
CTCCGTCCATCCTTTCTGCTAACTTTTCTAAATTAGGAGAGCAGGTGAAAGCAATTGAGC  240
S  P  S  I  L  S  A  N  F  S  K  L  G  E  Q  V  K  A  I  E     (79)
AGGCAGGCTGTGACTGGATTCATGTAGATGTGATGGACGGTCGATTTGTTCCAAATATAA  300
Q  A  G  C  D  W  I  E  V  D  V  M  D  G  R  F  V  P  N  I     (99)
CTATTGGACCCCTTGTAGTTGATTCCTTGCGCCCTATCACAGATCTACCATTGGATGTGC  360
T  I  G  P  L  V  V  D  S  L  R  P  I  T  D  L  P  L  D  V    (119)
ATCTGATGATTGTCGAACCTGACCAGAGAGTACCTGACTTCATAAAAGCAGGTGCTGATA  420
H  L  M  I  V  E  P  D  Q  R  V  P  D  F  I  K  A  G  A  D    (139)
TTGTCAGTGTTCACTGTGAGCAATCTTCTACGATCCACTTGCATCGTACAATAAATCAGA  480
I  V  S  V  H  C  E  Q  S  S  T  I  H  L  E  R  T  I  N  Q    (159)
TTAAAAGTTTGGGAGCAAAAGCTGGGGTTGTCCTCAATCCTGGAACCCCTTTAACCGCAA  540
I  K  S  L  G  A  K  A  G  V  V  L  N  P  G  T  P  L  T  A    (179)
TTGAATATGTCCTTGATGCTGTTGATCTGGTGTTGATTATGTCTGTAAACCCTGGATTTG  600
I  E  Y  V  L  D  A  V  D  L  V  L  I  M  S  V  N  P  G  F    (199)
GGGGACAGAGCTTCATTGAGAGTCAGGTCAAGAAAATCTCGGACTTGAGAAAAATCTGCG  660
G  G  Q  S  F  I  E  S  Q  V  K  K  I  S  D  L  R  K  I  C    (219)
CTGAGAGGGGATTAAACCCTTGGATTGAAGTTGATGGTGGAGTTGGTCCCAAAAATGCTT  720
A  E  R  G  L  N  P  W  I  E  V  D  G  G  V  G  P  K  N  A    (239)
ACAAGGTCATTGAAGCTGGAGCCAATGCCTTGGTAGCTGGTTCTGCTGTCTTTGGAGCTC  780
Y  K  V  I  E  A  G  A  N  A  L  V  A  G  S  A  V  F  G  A    (259)
CTGATTATGCTGAAGCTATTAAAGGGATCAAGACGAGCAAAAGGCCTGAAGCAGTTGCTG  840
P  D  Y  A  E  A  I  K  G  I  K  T  S  K  R  P  E  A  V  A    (279)
TATGAGATTTCTGTATGACAGAGCGATACGTAGTAGCAAAGCACAAATAGTTGCGAGACT  900
V  ***                                                         (280)
GCAAGCATGGTTCACTCTGCAACCTACACGAAGAGGATAAATAAAATAACCCACCTCTCG  960
GAGGTTGTGTAATACTCTATATGTTGTATAACTTTTCTTGCATAGGGAACAAGATGAATC 1020
TGTACTGAATATCCAATTTTCAAGATGACATTTTA                          1055
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Fig. 1. cDNA sequence and deduced amino acid sequence of chloroplast epimerase from potato. The amino-terminus of the mature protein is indicated by the underlined Ser.

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                    ↓
potato  ...QTVVNASSRVDKFSKSDIIVSPSILSANFSK  75
                    ||||||||||||||||| ||||||
spinach         TSRVDKFSKSDIIVSPXILSANFA
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Fig. 2. A comparison of the deduced amino acid sequence of potato epimerase with the NH_2-terminal amino acid sequence of the isolated spinach chloroplast enzyme as determined by automated Edmann-degradation. The cleavage site is marked by an arrow.

sequence of the purified spinach chloroplast epimerase (Fig. 2). Therefore, the protein possesses a targeting peptide (TP) at least 45 amino acids long which is typical for nuclear-encoded chloroplast proteins [15]. Considering that the average length of chloroplast targeting peptides is about 50 amino acids, it is reasonable to assume that our epimerase cDNA clone is missing only 15–30 bp from its very 5′-end of the coding region. The Ser and Thr content of the TP is much higher as compared to the mature protein, i.e. 29% and 11%, respectively. The TP does not contain any Glu or Asp which represent 11% of all amino acids in the mature protein. The last 10 residues of the TP differ distinctly from the remainder of the sequence. Hydrophobicity is drastically increased and Arg is enriched in this region (30% vs. 3.4%) starting from an Arg at position −10 away from the cleavage site. All these features are typical for stroma-targeting chloroplast TPs [15].

A comparison of the deduced amino acid sequence of potato epimerase with the NH_2-terminal amino acid sequence of the isolated spinach chloroplast enzyme not only revealed the cleavage site of the targeting peptide, but also that the NH_2-terminal protein sequences from both plant species are almost completely identical. The only exception is the first amino acid which is a Ser in the potato enzyme and a Thr in the spinach enzyme. The amino acid at position 17 of the spinach epimerase sequence could not be determined by automated Edman-degradation presumably because of a secondary modification. The potato cDNA sequence codes for a Ser at this position thus pointing to the possibility that this residue might be phosphorylated in the mature enzyme.

The deduced amino acid sequence of epimerase from potato is 57.4% identical with an epimerase from the photosynthetic purple bacterium *Alcaligenes eutrophus* [5]. Moreover, the search in EMBL and SwissProt databases for homologous proteins revealed two proteins from *E. coli* and a partial cDNA from *Arabidopsis thaliana* (Fig. 3). The plant epimerases were found to be 78% and 89% identical in the nucleotide and protein sequence, respectively. The two *E. coli* epimerase proteins differ notably in their primary structures and also in similarity to the other published epimerase sequences (Table 1). They both appeared in the database as hypothetical proteins. Recently, however, Lyngstadaas et al. [7] have characterized the YHFD protein as a pentose-5-phosphate epimerase with a M_r of 24,000 which belongs to the *dam* operon containing 7 functionally unrelated genes. These authors also demonstrated that disruption of the epimerase gene did not influence the growth rate of bacteria on poor medium and lowered it by only about 20% on a rich one. This may indicate the presence in *E. coli* of another protein (YJCU) which is functionally equivalent to epimerase. It is remarkable to note, that other proteins of the oxidative pentose phosphate cycle, transketolase and ribose-5-phosphate isomerase, are also present as two isoforms in *E. coli* [16,17].

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EPIM_STUBE      SSRVDKFSKSDIIVSPSILSANFSKLQEQVKAIEQAGCDWI   41
EPIM_ALCEU   MHATEPNTGHGSQRAIRLAPSILSADFARLQEEVCAIEAGGADLV   45
YHFD_ECOLI   MK-----------QYLIAPSILSADFARLQEDTAKALAAGADVV   33
YJCU_ECOLI   MK--------------ISPSLMCMDLLKFKEQI-EFIDSHADYF   29
                          ..**... .. .. *..    .. *

EPIM_STUBE   HVDVMDGRFVPNITIGPLVVDSLRPIT-DLPLDVHLMIVEPDQRV   85
EPIM_ALCEU   HFDVMDNHYVSNLTIGPLVCEAIRPL-VSIPIDVHLMVEPVDALI   89
YHFD_ECOLI   HFDVMDNHYVPNLTIGPMVLKSLRNYGITAPIDVHLMVKPVDRIV   78
YJCU_ECOLI   HIDIMDGHFVPNLTLSPFFVSQVKKLA-TKPLDCHLMVTRPQDYI   73
             * *.**...*.*.*..*.  . ..    . *.* ***.   . .

EPIM_STUBE   PDFIKAGADIVSVHCEQSSTIHLHRTINQIKSLGAKAGVVLNPGT  130
EPIM_ALCEU   PMFAKAGANLISFHPEASR--HVDRTIGLIRDHGCKAGLVLNPAT  132
YHFD_ECOLI   PDFAAAGASIITFHPEASE--HVDRTLQLIKENGCKAGLVFNPAT  121
YJCU_ECOLI   AQLARAGADFITLNPETING-QAFRLIDEIRRHDMKVGLILNPET  117
             . .  ***.... * *    . * .  *.  . *.*...**.*

EPIM_STUBE   PLTAIEYVLDAVDLVLIMSVNPGFGGQSFIESQVKKISDLRKICA  175
EPIM_ALCEU   PLSWLDHTLDKLDLVLLMSVNPGFGGQAFIPGVLDKVRQARARID  177
YHFD_ECOLI   PLSYLDYVMDKLDVILLMSVNPGFGGQSFIPQTLDKLREVRRRID  166
YJCU_ECOLI   PVEAMKYYIHKADKITVMTVDPGFAGQPFIPEMLDKLAELKAWRE  162
             *.. ... .. * . .*.*.***.**.**   ..*. . .

EPIM_STUBE   ----ERGLNPWIEVDGGVGPKNAYKVIEAGANALVAG-SAVFGAP  215
EPIM_ALCEU   RQVAAGGRPVWLEIDGGVKADNITEIARAGADTFVAG-SAVFGAP  221
YHFD_ECOLI   ----ESGFDIRLEVDGGVKVNNIGEIAAAGADMFVAG-SAILTSQ  206
YJCU_ECOLI   ----REGLEYEIEVDGSCNQATYEKLMAAGADVFIVGTSGLFNHA  203
                 *   .*.**.    . ..  ***. ...* *.... .

EPIM_STUBE   DYAEAIKGIKTSKRPEAVAV                          235
EPIM_ALCEU   DADGGYRGILHRLREAATIT                          241
YHFD_ECOLI   TTKKSLMKCAVNWQR                               221
YJCU_ECOLI   ENIDEAWRIMTAQILAAKSEVQPHAKTA                  231
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Fig. 3. Alignment of the deduced amino acid sequences of potato epimerase with the deduced amino acid sequences of an epimerase from *Alcaligenes euthropus* [5], and two epimerases from *E. coli* [6,7]. Identical residues are indicated by stars, dots mark homologous amino acid substitutions.

Northern blot analysis revealed, that the amount of epimerase mRNA expressed in the organs of potato plants is different. A single band of 1.3 kb can be seen in both leaf and root RNA (Fig. 4). This epimerase transcript was also detected in the total RNA from stem and tubers (data not shown). As it was expected, the highest quantity of the mRNA is present in leaves; in stems, roots, and tubers the amount is 15%, 8%, and 3% of that found in leaves, respectively.

To compare the relative quantities of epimerase in different plant tissues, we have performed Western blot analysis of crude protein extracts from leaves, stems, tubers, and roots (Fig. 5, lower part). A strong protein signal was revealed in leaf extracts which corresponds to a protein with a M_r of about 24,000. In extracts from stems only a faint enzyme band could be detected even after very long film exposure of Western blots. Surprising, however, was the observation that epimerase could be observed neither in tuber nor in root tissue. It is tempting to conclude, therefore, that only cells possessing chloroplasts contain the enzymes catalyzing the reductive and oxidative pentose phosphate cycle. It is generally assumed, that, besides the oxidative pentose phosphate cycle localized to chloroplasts, plant cells possess the same metabolic pathway also in the cytosol. However, the lack of epimerase in root and tuber tissue seems to contradict this assumption. Recently, Schnarrenberger et al. [18] have observed that in spinach leaves the enzymes of the oxidative pentose phosphate cycle are predominantly, if not exclusively, confined to chloroplasts. These authors were also unable to detect compartment-specific isoforms of the enzymes involved in this pathway including pentose-5-phosphate 3-epimerase. We are aware of the fact that our antibodies against the spinach chloroplast epimerase might be unable to recognize possible cytosolic isoform of this enzyme, but these isoenzyme forms should be distinguishable by ion-exchange chromatography and this seems not to be the case [18]. Accordingly, plants very probably contain only a chloroplast-specific form of epimerase. Since epimerase mRNA is also present in plant organs devoid of green chloroplasts, although at much lower levels as compared to leaves, the tissue-specific expression and accumulation of this enzyme appears to be regulated at translational and/or posttranslational levels.

To prove our conclusion on the tissue-specific accumulation of enzymes participating in the pentose phosphate cycles, we have analyzed the accumulation of transketolase, i.e. a sequential enzyme to epimerase of the same pathways, in potato organs by Western blotting (Fig. 5, upper part). To our great surprise, transketolase could be detected in all plant tissues at approximately the same amount on a protein basis. Transketolase from all potato organs forms only one distinct band after SDS-PAGE with a M_r of 74,000. Since roots and tubers do not contain chloroplasts, transketolase must be present either in plastids or cytosol or both cell compartments. The reason why

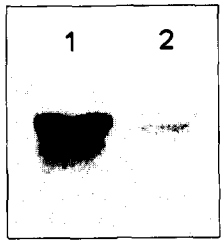

Fig. 4. Northern blot analysis of RNA from leaves (1) and roots (2) of potato. Twenty μg of total RNA were electrophoresed in formaldehyde-containing 1% agarose gel, transferred onto Hybond N^+ membrane, and hybridized with ^{32}P-labelled epimerase total cDNA probe.

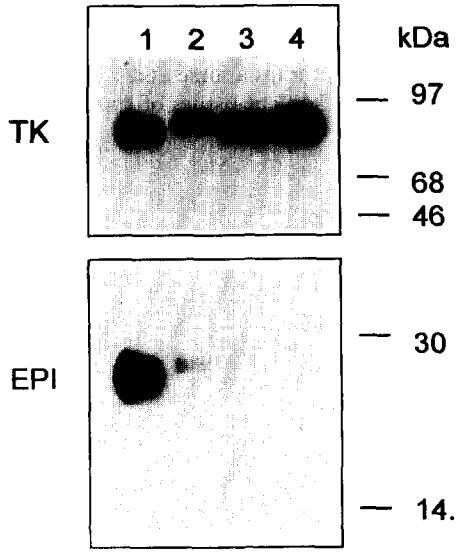

Fig. 5. Accumulation of pentose-5-phosphate 3-epimerase and transketolase in potato leaf (1), stem (2), tuber (3), and root tissue (4). Proteins were isolated and separated by SDS-PAGE. Samples were loaded on the basis of equal quantities (30 μg) of protein in extracts obtained from plant tissues. Transketolase (upper part) and epimerase (lower part) were detected using specific antibodies as described in section 2. The molecular mass markers are indicated on the right-hand side.

Table 1
Comparison matrix for amino acid sequences of epimerase from *Solanum tuberosum*, *Alcaligenes eutrophus* [5], and *Escherichia coli* [6,7]

	1	2	3	4
(1) EPIM_STUBE	–	46.9	47.5	31.6
(2) EPIM_ALCEU		–	61.5	32
(3) YHFD_ECOLI			–	36.2
(4) YJCU_ECOLI				–

Results are given in percentage identity.

two enzymes involved in the same pathways are expressed so differently in plant tissue remains to be elucidated. It is possible, however, that transketolase plays additional metabolic roles besides to catalyze essential steps in the two pentose phosphate cycles. The apparent absence of the oxidative pentose phosphate pathway in cell compartments other than chloroplasts could be attributed to the absence of other sequential enzyme components, namely epimerase. Screening and immunocytochemical localization of pentose phosphate cycle components in cells of different plant organs could help to understand more about compartmentalization, supramolecular organization, and regulation of enzymes in situ.

4. Conclusions

We have isolated and sequenced the first eucaryotic epimerase cDNA clone from a potato leaf cDNA library. By comparing the deduced amino acid sequence of the cDNA with the NH_2-terminal amino acid sequence of the purified spinach chloroplast epimerase, the cleavage site of a chloroplast targeting peptide was identified. The primary structure of the plant enzyme is similar to those of epimerases from bacteria. Evidence is provided, that epimerase is accumulated specifically in plant tissues containing chloroplasts, i.e. in leaves and stem. Since mRNA, but not the enzyme was detected in roots and tubers, we conclude that the expression and accumulation of epimerase is regulated at translational and/or posttranslational levels. The expression in plant tissues of transketolase, which represents a sequential enzyme of the epimerase in the pentose phosphate cycles, appears to be independent of pentose-5-phosphate 3-epimerase.

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