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Cloning and Sequencing of a cDNA Encoding 2,3-Bisphosphoglycerate-independent Phosphoglycerate Mutase from Maize

POSSIBLE RELATIONSHIP TO THE ALKALINE PHOSPHATASE FAMILY*

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The primary sequence of maize 2,3-bisphosphoglycerate-independent phosphoglycerate mutase was deduced from cDNAs isolated from maize cDNA libraries by screening with specific antibodies to the cofactor-independent enzyme and from a maize genomic clone. The genomic clone provided the 5′-nucleotide sequence encoding the N-terminal amino acids which could not be obtained from the cDNA. Confirmation that the nucleotide sequence was for the cofactor-independent phosphoglycerate mutase was obtained by sequencing the peptides generated from cyanogen bromide cleavage of the purified protein. This is the first report of the amino acid sequence of a 2,3-bisphosphoglycerate cofactor-independent phosphoglycerate mutase, which consists of 559 amino acids and is twice the molecular size of the mammalian cofactor-dependent enzyme subunit. Analysis of the cofactor-independent phosphoglycerate mutase amino acid sequence revealed no identity with the cofactor-dependent mutase types. Northern blot analysis confirmed this difference since the maize cofactor-independent phosphoglycerate mutase cDNA did not hybridize with mRNA of the cofactor-dependent mutase. The lack of amino acid identity between cofactor-dependent and -independent enzymes is consistent with their different catalytic mechanisms and suggests that both enzymes are unrelated evolutionarily and arose from two independent ancestral genes. However, a constellation of residues which are involved in metal ion binding in various alkaline phosphatases is conserved in the maize cofactor-independent phosphoglycerate mutase, which suggests that the enzyme is a member of the alkaline phosphatase family of enzymes.

The phosphoglycerate mutase (PGAM, EC 5.4.2.1.)[1] is an enzyme of the glycolytic pathway that catalyses the interconversion of 3-phosphoglycerate and 2-phosphoglycerate. There are two types of phosphoglycerate mutases: one that requires 2,3-bisphosphoglycerate as a cofactor and the other that does not. Both types are widely distributed. The cofactor-dependent enzyme (PGAM-d) is present in all vertebrates, most invertebrates, and some fungi and bacteria. The cofactor-independent enzyme (PGAM-i) is present in all plants, algae, and some invertebrates, fungi, and bacteria. The two PGAM types also differ in their structure, reaction mechanism and kinetic properties (see Ref. 1 for a review).

Cofactor-dependent PGAM has been extensively studied. In mammalian tissues the native enzyme is a dimer with a subunit molecular mass of 30 kDa. A muscle-type subunit and a brain-type subunit have been identified and the cDNAs of the two different subunits have been isolated and sequenced (2–4). The enzyme shows a tissue-specific distribution consisting of two homodimeric and one heterodimeric forms (1). The human muscle gene has also been isolated (5, 6) and the x-ray crystallographic structure of yeast PGAM has been determined (1). However, there is a paucity of information for the cofactor-independent enzyme which is less abundant than the mammalian enzyme and has been recalcitrant to purification. Plant PGAM-i is a monomeric enzyme with a molecular mass of approximately 60 kDa. Its kinetic and immunological properties have been studied in wheat germ (7–9), rice germ (10), castor oil seeds (11), and maize (12), but no primary sequence of any cofactor-independent enzyme has been determined.

The cofactor-dependent PGAM family has been shown to be evolutionarily related to a family of acid phosphatases and Fru-2,6-P_2ase (13, 14). Since both the cofactor-dependent and -independent types catalyze the same reaction, it is possible that the cofactor-independent form also belongs to this PGAM-d/acid phosphatase/Fru-2,6-P_2ase enzyme family. In order to compare the structures and possible common evolutionary origin of the two PGAM forms, we present here the amino acid sequence of cofactor-independent PGAM deduced from a cloned cDNA of the maize enzyme. There was no homology between the sequences of the two PGAM types, which strongly suggests that although they catalyze the same reaction, they arose independently during evolution. Surprisingly, there was a partial identity with metal binding site residues of alkaline phosphatases, which suggests that the cofactor-independent type is a divergent form of the alkaline phosphatase family of enzymes.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) M80912.

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[1] The abbreviations used are: PGAM, phosphoglycerate mutase; PGAM-i, 2,3-bisphosphoglycerate-independent phosphoglycerate mutase; PGAM-d, 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase; bp, base pair(s); kb, kilobase(s); HPLC, high-performance liquid chromatography.

EXPERIMENTAL PROCEDURES

Materials—Enzymes were from Boehringer Mannheim, Pharmacia, and New England Biolabs. Radioactive materials were purchased from Amersham. Antibodies to PGAM-i were obtained as described previously (12). Diaminobenzidine was from Sigma and peroxidase-conjugated swine anti-rabbit IgG was from Dakopatts. Maize embryo cDNA λ-ZAP library (W64) was a generous gift from L. Ruiz (Centro de Investigación y desarrollo, Consejo Superior de Investigaciones Cientificas, Barcelona, Spain). Maize λgt11 library (W22) was from Clontech and λ Charon 35 genomic library (W64) was a kind gift from J. Rigau (Centro de Investigación y desarrollo, Consejo Superior de Investigaciones Cientificas, Barcelona, Spain). All other reagents were analytical grade.

Protein Analysis and Sequencing—Maize PGAM (E41) was purified as described previously (12). The protein was precipitated with trichloroacetic acid (20%), centrifuged, and the pellet was dissolved in 500 μl of 6.9 M guanidine HCl. Dithiothreitol was added to a final concentration of 2 mM, and the tube was kept at 50 °C for 1 h. Iodacetamide was added to a final concentration of 10 mM and kept at 30 °C for 1 h, and the protein solution was dialyzed against 0.1% β-mercaptoethanol. The precipitated denatured and alkylated protein was centrifuged and the pellet redissolved in 88% formic acid. Water was added to give a 70% formic acid final concentration, and cyanogen bromide (CNBr) was added to a concentration of 100 mg/ml. The reaction mixture was flushed with N_2 and left overnight at room temperature. The sample was then dried and redissolved in 88% formic acid.

HPLC separation of the CNBr-digested peptide was performed with a reverse phase column, using a Beckman model 344 HPLC system, equipped with a Beckman model 344 HPLC detector with a 214-nm lamp. Fractions corresponding to a peak in the profile were sequenced by automatic Edman degradation in an Applied Biosystems 470A protein sequencer (15). HPLC of the phenylthiohydantoin-derivatives was performed in the Applied Biosystems phenylthiohydantoin analyzer (120A).

cDNA Library Screening—A maize embryo cDNA library made in λ-ZAP was screened with maize PGAM antibodies prepared as described (12). Phage plates from 10^5 recombinants were transferred to nitrocellulose filters and were hybridized with antibodies (16). The filters were incubated with peroxidase-conjugated swine anti-rabbit IgG and developed with diaminobenzidine by the method of Davis (17). A second maize cDNA library in λgt11 was screened with random-primed labeled clones isolated from the λ-ZAP screening.

Oligonucleotide Synthesis—Using an Applied Byosystems model 380A DNA synthesizer the following oligonucleotides were synthesized: 1) GACACCACCATCACTCAACAACCCAA (26-mer, residues 392–417); 2) CTCAGGAGCACCATTCTTTAACG (21-mer, residues 152–174); 3) GAGTCTGAGCGACATGGAT (19-mer, residues 121–139); 4) CAGCACCAAGTGCATTGT (18-mer, residues 124–141).

Sequencing—*Exo*III/S1 deletions of the clones PGMA1 and PGMA2 were constructed by the method of Henikoff (18) and sequenced by the dideoxy method of Sanger (19) using the forward and reverse M13 universal primers. Oligonucleotides and M13 primers were used to partially sequence cDNA clone PGAM3 and the genomic clones λ-Charon PGAM-g and PGAM-g1. The sequences were determined from both strands at 99%.

Primer Extension and S1 Nuclease Mapping—Poly(A) RNA was isolated from maize embryos as described previously (12), using oligo(dT)-cellulose affinity chromatography. Primer extension analyses were performed according to Ausubel *et al.* (20). Oligonucleotide 3 was end-labeled with [γ-^{32}P]ATP and polynucleotide kinase and incubated with 8 μg of poly(A) RNA. The extension reaction was started by adding 40 units of Moloney murine leukemia virus reverse transcriptase. After incubation at 37 °C for 90 min, the products were precipitated with ethanol and analyzed on a gel containing 6% polyacrylamide and 7 M urea. S1 nuclease mapping was performed as described by Ausubel *et al.* (20), using the double-stranded genomic PGAM-g1 clone as template and [γ-^{32}P]ATP end-labeled oligonucleotide 3 as primer. After elongation of the primer by the Klenow fragment of *Escherichia coli* DNA polymerase I, the product was digested with 40 units of *Bgl*II, and the single-strand probe was isolated by electrophoresis in a denaturing 5% polyacrylamide gel containing 7 M urea. The radioactive probe was excised from the gel and eluted as described by Sambrook *et al.* (16). After resuspension in water, 2.5×10^5 cpm of the probe were added to 21 μg of poly(A) RNA in hybridization buffer, denatured by heating 15 min at 65 °C, and then hybridization was carried out for 15 h at 30 °C. The hybrids were digested with 300 units of S1 nuclease for 90 min at 30 °C, precipitated with ethanol, and analyzed in a polyacrylamide sequencing gel.

Northern Blot Analysis—Total maize embryo RNA was prepared as described by Dean *et al.* (21). After 1.5% agarose gel electrophoresis, RNA was transferred to a Hybond N nylon sheet. PGAM1 and PGAM3 random primer labeled cDNAs were used as probes. Hybridization was performed essentially following the manufacturer's instructions. Filters were washed at 55 °C with 0.1 × SSC (SSC: 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 0.1% sodium dodecyl sulfate for 80 min (four changes).

Southern Blot Analysis—Genomic DNA was digested with *Eco*RI, *Hin*dIII, *Xba*I, *Sac*I, and *Sph*I. After electrophoresis on 0.8% agarose gel, the digested DNA was transferred to a nylon sheet (GeneScreen) according to Sambrook *et al.* (16). The filters were treated under the same conditions used in the Northern blotting experiments except that they were washed with 0.2 x SSC for 120 min at 42 °C (four changes).

RESULTS AND DISCUSSION

Immunologic Screening of Maize cDNA Expression Libraries for PGAM-i cDNA—A maize cDNA expression library was screened with a specific PGAM-i antibody (12). Two clones, PGAM1 and PGAM2, approximately 1.7 and 1.6 kb long, respectively (Fig. 1), were obtained from a λ-ZAP library and sequenced. Neither of the two clones were full-length, since the cDNA of a protein subunit with a mass of 60 kDa would be expected to be at least 1.8 kb in length. As the cDNA lacked the 5′ region, a second maize cDNA library was

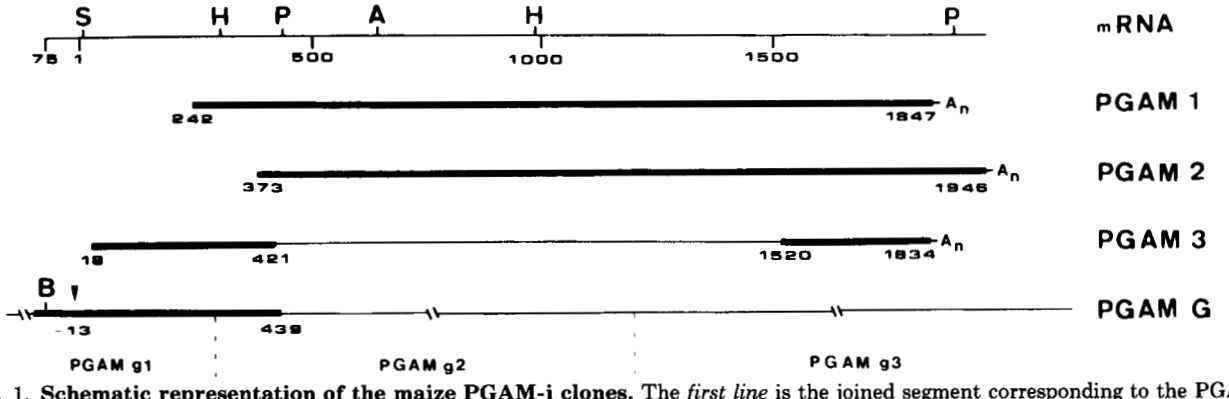

FIG. 1. **Schematic representation of the maize PGAM-i clones.** The *first line* is the joined segment corresponding to the PGAM-i. Four clones with their respective overlaps are shown. PGAM1 and PGAM2 were cloned from the λ-ZAP cDNA library, PGAM3 from the λgt11 cDNA library and PGAMg clones from the λ Charon 35 genomic library. *Thick lines* represent the sequenced regions. *Uppercase letters* represent restriction enzymes (*H*, *Hin*dIII; *P*, *Pst*I; *S*, *Sac*I; *B*, *Bgl*II, and *A*, *Ava*II). The *arrow* indicates the presence of an intron in the PGAMg clones. Nucleotide numbering starts with the adenosine of the translation initiator ATG.

screened with a 683-bp *Hin*dIII 5′ fragment derived from the PGAM1 clone (Fig. 1). A new 1.84-kb clone (PGAM3) which overlapped PGAM1 and PGAM2 was isolated and, although longer than PGAM1 and PGAM2, the translation product from the first Met had an estimated relative molecular mass of only 55 kDa. This cDNA had an open reading frame of 1650 bases, but still did not include the 5′ region. The clone did contain a stop codon following the open reading frame and a putative polyadenylation signal AATAAT 55 bases downstream from the termination codon.

Isolation of a λ Charon Maize Genomic Clone for PGAM-i— In order to obtain the missing 5′ region of the clone, a λ Charon maize genomic library was screened with the *Eco*RI/*Hin*dIII 5′ fragment of clone PGAM3 as a probe (Fig. 1) and another new clone was obtained (PGAM-g). A Southern blot of an *Eco*RI digestion of the PGAM-g clone showed that a 5-kb band (PGAM-g1 clone) hybridized with the PGAM3 *Eco*RI/*Hin*dIII 5′cDNA probe and that two additional bands of 1.3 kb (PGAM-g2) and 2.8 kb (PGAM-g3), respectively, hybridized with the complete cDNA clone PGAM3 (data not shown). The 5-kb *Eco*RI fragment (PGAM-g1) was subcloned into Bluescript and sequenced. The sequence obtained agreed with that obtained from cDNA libraries and, in addition, it contained the 5′ region encoding the N-terminal amino acid sequence, lacking in the cDNA (18 bp) (Fig. 2).

To rule out the possibility that the sequence obtained from the genomic clone actually represents intronic regions, S1 nuclease protection experiments were performed. As shown in Fig. 3*A*, the pattern is relatively complex yielding two groups of protection fragments. One band of the upper group of bands coincides with an intron 3′ consensus sequence AG, which suggests that there is an intron in the 5′-untranslated region beginning 13 bp upstream from the initiator codon. However, none of the bands of the lower group coincides with AG sequences, thus ruling out the presence of introns in the 5′ region encoding the N-terminal amino acids. The bands of

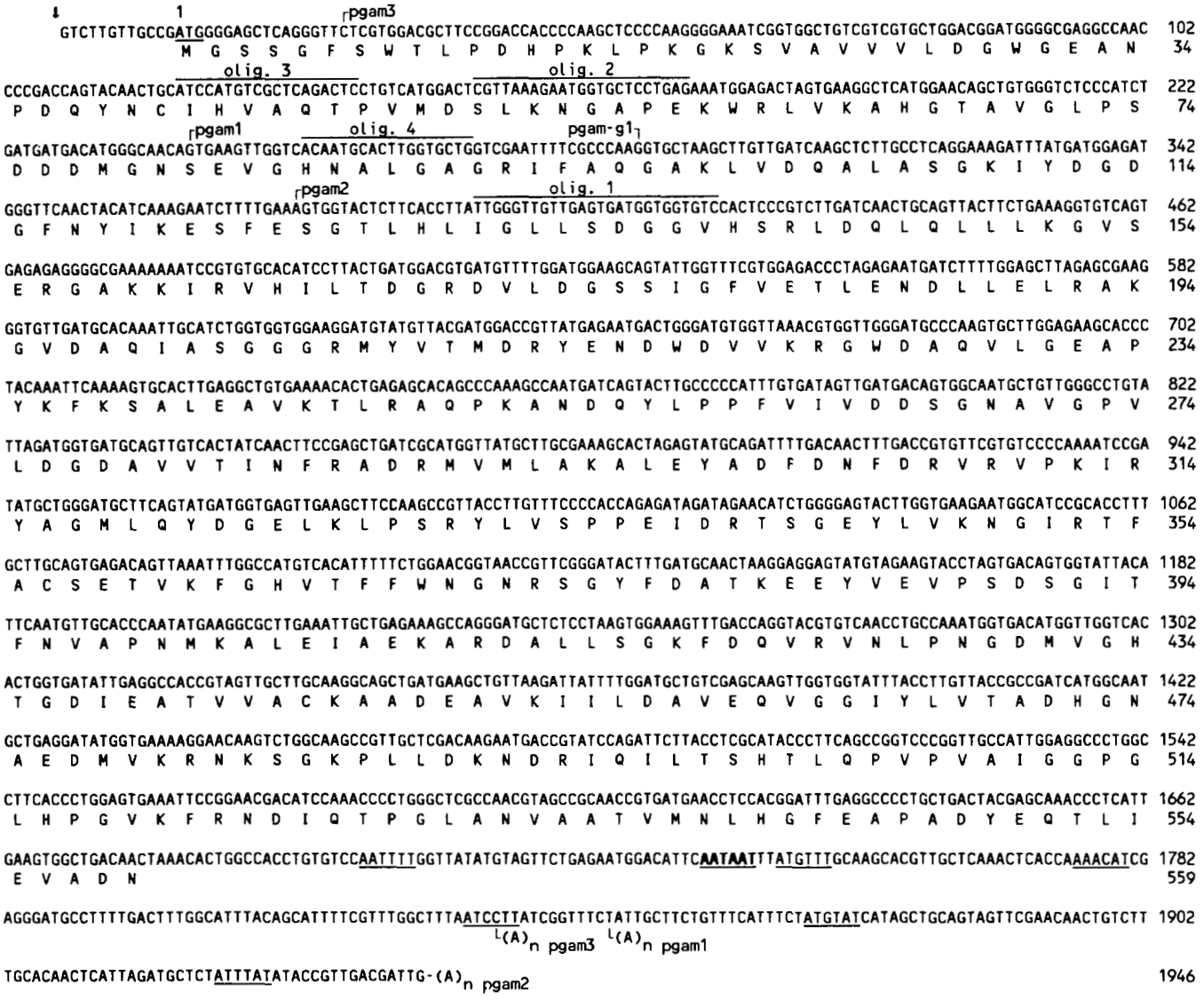

FIG. 2. **Nucleotide sequence of the PGAM-i cDNA and the deduced protein sequence.** Nucleotide numbering begins with the first base of the ATG start codon. The nucleotide sequences which coincide with the plant poly(A) signal consensus sequences are *underlined*. The most conserved plant signal is represented in *bold type*. The initiator codon is also *underlined*. The *arrow* indicates the beginning of the intron detected 13 bp upstream from the start codon in the PGAM-g1 clone. The 5′ end of the different cDNA clones (⌈) and the 3′-coding end of PGAM-g1 clone (⌉), together with the positions of the poly(A) tails, are also indicated. The position of primers used for sequencing, S1-nuclease mapping, and primer extension are *overlined*.

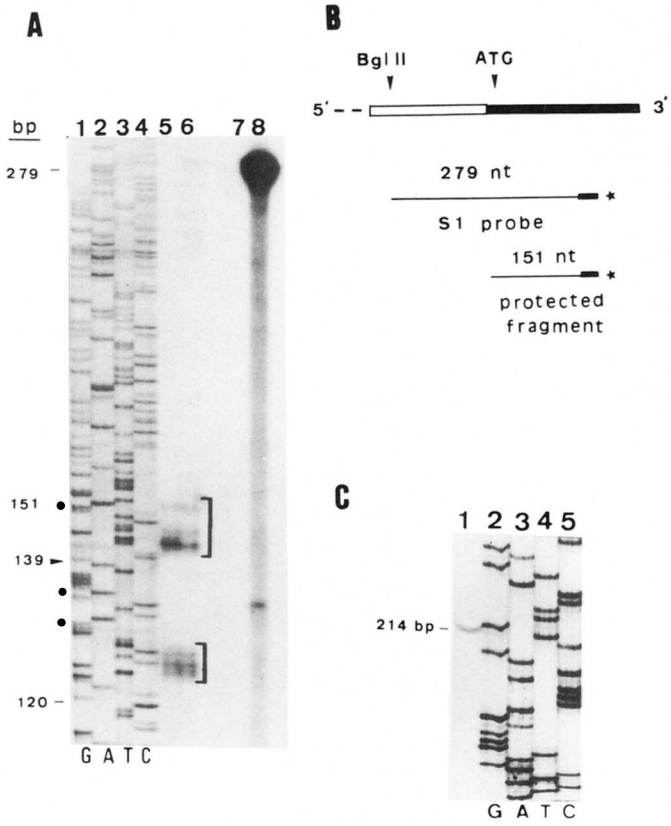

FIG. 3. **S1 protection and primer extension of PGAM-i.** *A*, S1 protection. *Lanes 1–4* represent the sequence of the genomic clone PGAM-g1 using oligo 3 as a primer (same oligo used in S1 and primer extension experiments). *Lanes 5* and *6* represent the products of S1 digestion in the presence of 60 μg of total RNA and of 21 μg of poly(A) RNA, respectively. *Lane 7* corresponds to the control with 20 μg of yeast tRNA. *Lane 8* contains the undigested probe. The *arrow* shows the position of the initiator ATG while AG sequences are indicated by *asterisks*. *Numbers* on the *left* of the figure correspond to sequence location markers. *B*, schematic representation of S1 protection experiment. *Boxes*: *white*, intron; *black*, exon. The intron begins 13 nucleotides upstream from the initiator codon ATG. *C*, primer extension. *Lane 1* shows the primer-extended product using oligo 3 as a primer and poly(A) RNA as template. *Lanes 2–5* represent sequencing reaction used as size markers.

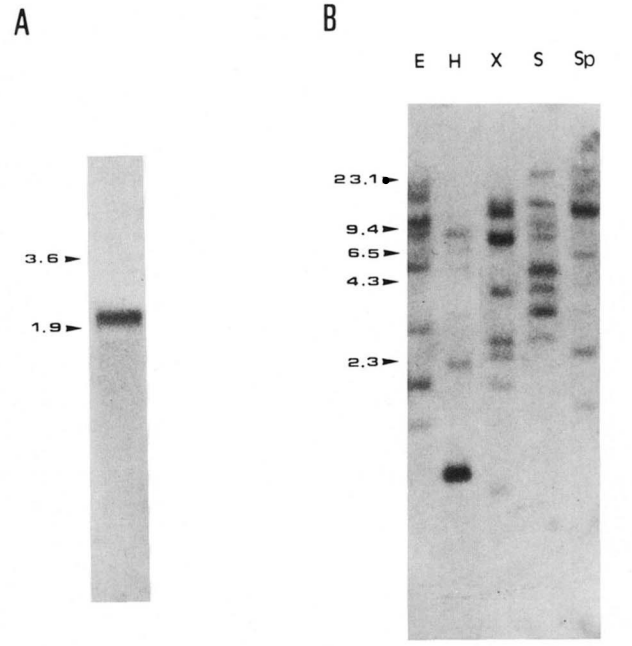

FIG. 4. **Northern and Southern blot analyses.** *A*, Northern blot. The embryos were germinated for 48 h. Only a single band of an estimated size of 2.1 kb, using the whole cDNA PGAM3 as a probe is detected. *Arrows* indicate the mobility of the ribosomal RNA. *B*, Southern blot. The analysis was carried out with genomic maize digested with *Eco*RI (*E*), *Hin*dIII (*H*), *Xba*I (*X*), *Sac*I (*S*), and *Sph*I (*Sp*). The PGAM3 cDNA was used as a probe. Markers size (*left*) are derived from *Hin*dIII digested λ-DNA.

the lower group probably are a result of polymorphism among different PGAM-i genes. Primer extension analyses produced a 214-nucleotide fragment (Fig. 3*C*), which indicated that transcription begins 75 bp upstream from the start codon. The differences between the lengths of the S1 protection and primer extension products support the presence of an intron upstream from the initiator ATG.

A Southern blot of maize genomic DNA digested with several restriction enzymes and probed with the cDNA (Fig. 4*B*) revealed several bands in each enzyme digestion, which suggest that several copies of the PGAM gene may be present in the maize genome. This is consistent with the results of the S1 nuclease protection. Rat DNA, similarly digested, restriction was not recognized by PGAM-i cDNA (data not shown), which suggests that no PGAM-i silent genes are present in the rat genome.

Nucleotide Sequence of cDNA of PGAM-i—The length of the transcript without the poly(A) tail is 2021 bp, which is approximately the size of the mRNA on Northern blots (Fig. 4*A*), and the molecular weight of the translated product are also the same as those of the native protein. Furthermore, Northern blot analyses also demonstrated that cofactor-independent PGAM3 cDNA did not hybridize with cofactor-dependent mammalian RNA (data not shown).

The coding sequences of the cDNA possess a low G+C content (49%) which is slightly lower than the corresponding values of the cofactor-dependent mutase cDNAs (51–62%). The frequency of G or C present in the third position of codons is 46%, which is significantly lower than that of the dependent enzyme (67–83%). The frequency of A or T in the third position of codons (54%) is higher than that of many maize proteins (22) owing to the high T levels, which represents 37% of the third-position bases. This value is unusual in maize where A and T are present in the third position of codons only in a low percentage (22).

The 3′-nontranslated regions of the three cDNA clones had identical sequences and differed only in length. Thus, PGAM1 possesses 97 bp, PGAM2, 384 bp, and PGAM3, 110 bp between the stop signal and the poly(A) site, which is in agreement with the presence of several polyadenylation signal sites in plants, and similar results have been described in RNA from potatoes, barley, maize, and other plants (23–26). The putative plant poly(A) signal consensus sequence is A A/T N A/C/T A/T T/A (23), but the most conserved signal is AATAAT, which is also the polyadenylation signal most closely resembling the animal consensus sequence. This signal is detected in all maize cDNAs, but other signals which agree with the consensus are also present and could be responsible for the different lengths of the maize cDNA clones.

Amino Acid Sequence of CNBr Peptides of PGAM-i—Fig. 5 shows a comparison between the partial amino acid sequence (50%) of the purified protein and the corresponding amino acid sequence deduced from the cDNA. All CNBr peptides, with the exception of one that was blocked, were sequenced either completely or for up to 32 cycles. Since the native protein could not be directly sequenced, the blocked peptide most likely corresponds to the N-terminal CNBr peptide. The

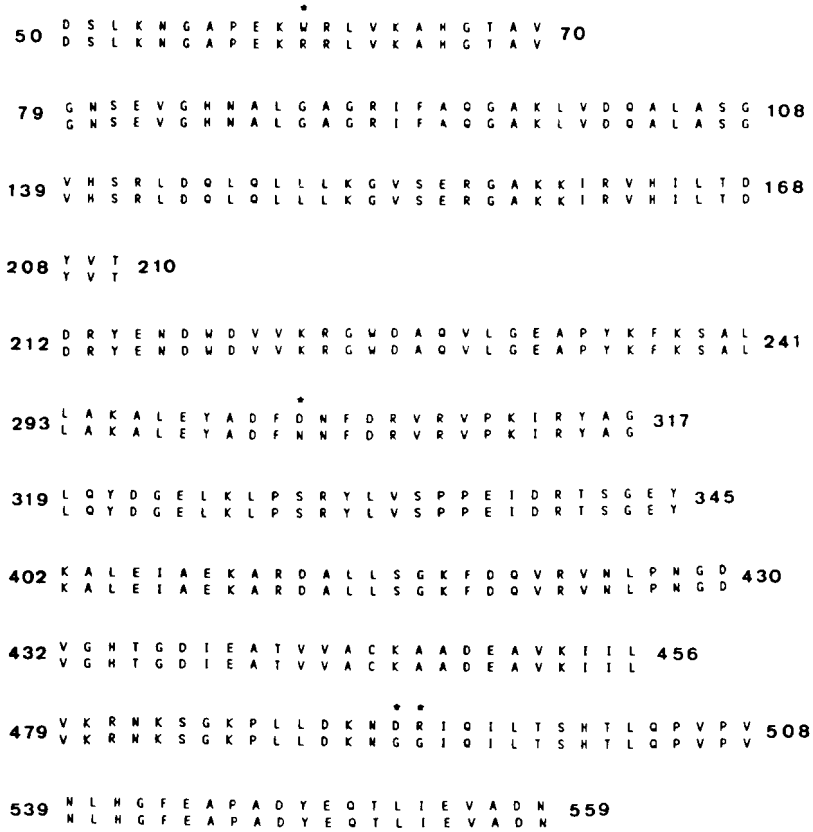

FIG. 5. **Comparison of amino acid PGAM-i sequences deduced from cDNA and from peptides obtained by CNBr digestion.** The amino acid sequence deduced from PGAM-i cDNA is given in the *upper lines*, whereas the sequences obtained from CNBr peptides are shown in the *lower lines*. The four differences detected are indicated by *asterisks*. *Numbers* indicate the position of the first and last amino acid of the sequence.

amino acid sequence deduced from the cDNA agrees with that obtained from the protein and confirms that these clones corresponded to cofactor-independent PGAM. The four differences observed (Fig. 5) are probably due to the differences in maize varieties used for protein purification and cloning. Other polymorphisms are observed between the sequence of the PGAM1, PGAM2, and PGAM-g clones and the PGAM3 clone, but all changes involved third-position codons and none resulted in changes in the amino acid sequence.

The Deduced Amino Acid Sequence of Maize Cofactor-independent PGAM—The deduced amino acid sequence of PGAM-i is shown in Fig. 2. PGAM-i consists of 559 amino acids, and it is reasonable to assume that translation begins at the methionine indicated in Fig. 2 for several reasons: 1) the molecular mass deduced from the amino acid sequence is approximately 61 kDa, which is consistent with the molecular mass of the pure protein (9, 11, 12); 2) the length of the transcript is also consistent with the size of the mRNA observed by Northern blot analysis; 3) as noted above, all CNBr peptides were sequenced with the exception of the one beginning with the proposed initiator Met.

The pI value calculated on the basis of the amino acid sequence is 6.03, a value which is lower than the calculated values of cofactor-dependent enzymes (6.15–6.26) and which may explain the greater anodic migration with respect to mammalian isozymes on cellulose acetate (12).

The hydropathy profile of maize PGAM (data not shown) possesses a homogeneous pattern with regularly alternating hydrophobic and hydrophylic zones which suggest a globular structure. As expected by comparison of amino acid sequences, the profile differs from that of PGAM-d. The predicted secondary structure of PGAM-i shows a lack of helicity in the N-terminal end of the enzyme which in contrast is very rich in β strands and turn structures. The central core of the molecule and the C-terminal end present six long sequences with possible α-helix structures, surrounded by stretches of β and turn structures, which is typical of many enzymes and globular proteins.

Comparison of the known sequences of PGAM-d with the maize PGAM-i sequence revealed that there was only a very low level of identity. Furthermore, searches of the protein data base did not reveal any obvious identity of the entire PGAM-i sequence to any known protein sequences. Although the amino acid sequence of maize PGAM is not homologous to any of the cofactor-dependent enzymes, an intriguing partial identity with the metal binding site of alkaline phosphatase (27–29) was detected (Fig. 6). Six residues which corresponded to the alkaline phosphatase metal binding sites, and the regions surrounding them, are conserved. These are Glu^{320}, Asp^{325}, His^{329}, Asp^{368}, His^{369}, and His^{410} of the *E. coli* alkaline phosphatase (29). Although the metal ion dependence of the PGAM-i-catalyzed reaction is controversial (30, 31), the conservation of alkaline phosphatase metal binding sequences in the primary sequence of PGAM-i suggests that the latter enzyme may be a metalloprotein.

It is also noteworthy that in addition to the partial amino acid identity between PGAM-i and the alkaline phosphatases there is strong support for the hypothesis that PGAM-d and acid phosphatases are evolutionarily related (13, 14). The relationship of both PGAM types to phosphatase families suggests that they both evolved from an ancestral gene(s) which coded for a protein possessing phosphohydrolase activity. Interestingly, both the acid phosphatase/Fru-2,6-P_2ase/PGAM-d family and the alkaline phosphatase family catalyze their reactions via a phosphoenzyme intermediate, but the phosphoacceptor is histidine in the former and serine in the latter (1). Cofactor-dependent PGAM catalysis involves the formation and hydrolysis of this phosphoenzyme intermediate with the participation of 2 histidine residues at positions 10 and 185. These active site histidines are conserved in the acid

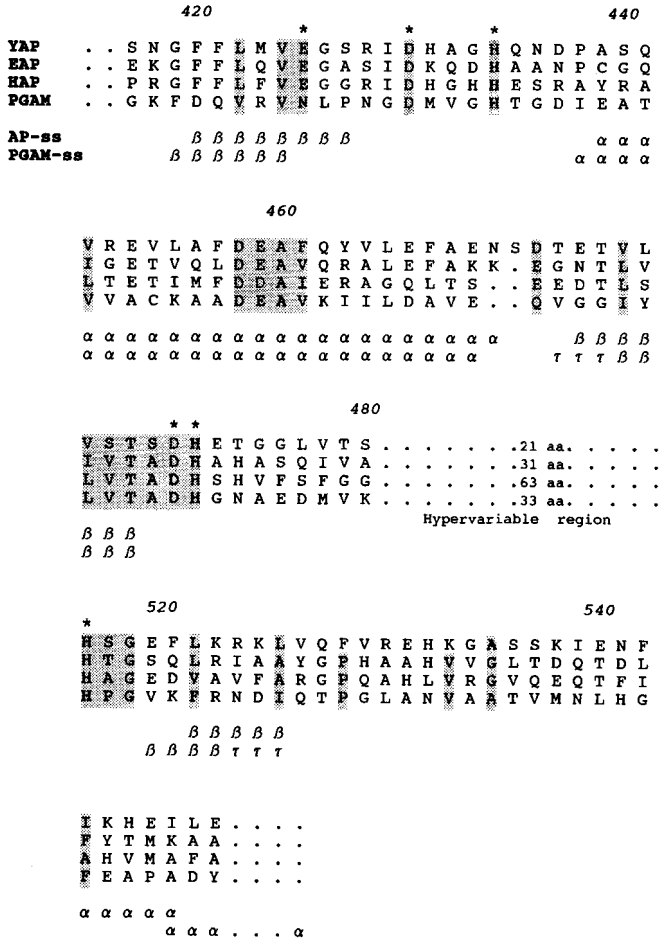

FIG. 6. **Comparison of the amino acid sequences of yeast (*YAP*), *E. coli* (*EAP*), and human (*HAP*) alkaline phosphatases with those of maize phosphoglycerate mutase (*PGAM*).** *Shaded* sequences represent homologous regions, and asterisks represent the metal binding sites of alkaline phosphatases. The predicted secondary structures (*ss*) of alkaline phosphatases (*AP*) are conserved in most of the mutase sequence and are represented by: α, α-helix, β, β-strands; τ, turn structures.

phosphatase and Fru-2,6-P_2ase (13) but are not present in the PGAM-i sequence. Although a phosphoenzyme intermediate has not been detected for PGAM-i sequence, studies with ^{18}O-labeled substrate indicated that a phosphoenzyme is involved (7, 32, 33). Although it is possible that a histidine also serves as the phosphoacceptor in the PGAM-i reaction, the partial identity of the maize enzyme with alkaline phosphatases suggests that serine may serve this role in PGAM-i.

The active site of all alkaline phosphatases contains a conserved phosphorylation site triad (Asp-Ser-Ala/Gly). There are several phosphorylation site candidates for this phosphorylation site triad in the maize PGAM-i sequence, including Asp^{266}-Ser-Gly^{268}. However, additional structure/function studies employing site-directed mutagenesis will be necessary to establish this other regions as the phosphorylation site of the cofactor-independent enzyme. If it is demonstrated that PGAM-i and alkaline phosphatases derive from a common ancestral gene, PGAM-i can be considered to be the most divergent member of the alkaline phosphatase family (29).

Evolution of the PGAM Enzyme Families—Glycolytic enzymes are ancient widely distributed proteins which have evolved slowly and are particularly suitable for the study of the evolution of enzyme and metabolic pathways. Moreover, they are very well characterized structurally (1) and present in large amounts. The PGAM family provides convincing illustrations of some of the main ways in which enzymes are thought to have evolved. As proposed by Fothergill and Watson (1), PGAM comprises at least four kinetically and structurally distinct enzyme forms which, nevertheless, have many common features. First, they all catalyze the interconversion of 2-phosphoglycerate and 3-phosphoglycerate. This family comprises PGAM-d, PGAM-i, Mn^{2+}-dependent PGAM, and bisphosphoglycerate mutase. This enzyme family catalyzes three distinct reactions. The major reaction of the first three enzymes is the mutase. The predominant activities of bisphosphoglycerate mutase are 2,3-bisphosphoglycerate synthesis and degradation via its phosphatase activity, whereas the mutase represents a minor activity.

Comparison of PGAM-d and bisphosphoglycerate mutase amino acid sequences revealed approximately 50% homology (4). It has been proposed that both enzymes are related and arose early in evolution from a single ancestral gene (3), probably by gene duplication followed by divergence and changes in the active site that gave rise to the catalytic differences between PGAM-d and bisphosphoglycerate mutase. In contrast, the PGAM-i reported here shows a total lack of homology with PGAM-d, which suggests that both enzymes are quite evolutionarily unrelated and arose from two independent ancestral genes. This lack of homology confirms the different immunological properties previously reported (12).

The only known sequence of PGAM-i is that from maize which is reported here, but immunological studies suggest that there are different forms of this enzyme since antibodies to maize PGAM-i recognize the wheat germ PGAM-i but not the enzyme from mushrooms or from arachnids which also possess PGAM-i (12). Elucidation of the amino acid sequences of other PGAM-i from these and other plant tissues will be required in order to compare the evolutionary pattern of this class of enzymes with that of the PGAM-d class and to establish further the relationships of the branch of the PGAM family to the alkaline phosphatase family.

Definitive elucidation of the reaction pathway mechanism, including the identification of the putative phosphoenzyme intermediate, should now be possible since a coding length cDNA is available and the protein can be expressed in *E. coli*. This will provide a means for obtaining large amounts of enzyme needed for the x-ray crystallographic analysis as well as for investigating structure/function relationships, including the identification of catalytic, substrate, and metal ion binding site residues by site-directed mutagenesis. Such studies have already begun for some enzymes of the PGAM family (34), and comparison of binding site and catalytic residues of the two types of PGAM should provide insight into how these different forms have evolved to catalyze the same reaction.

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