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The complete amino acid sequence of yeast phosphoglycerate kinase

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The complete amino acid sequence of yeast phosphoglycerate kinase, comprising 415 residues, was determined. The sequence of residues 1–173 was deduced mainly from nucleotide sequence analysis of a series of overlapping fragments derived from the relevant portion of a 2.95-kilobase endonuclease-*Hin*dIII-digest fragment containing the yeast phosphoglycerate kinase gene. The sequence of residues 174–415 was deduced mainly from amino acid sequence analysis of three CNBr-cleavage fragments, and from peptides derived from these fragments after digestion by a number of proteolytic enzymes. Cleavage at the two tryptophan residues with *o*-iodosobenzoic acid was also used to isolate fragments suitable for amino acid sequence analysis. Determination of the complete sequence now allows a detailed interpretation of the existing high-resolution X-ray-crystallographic structure. The sequence -Ile-Ile-Gly-Gly-Gly- occurs twice in distant parts of the linear sequence (residues 232–236 and 367–371). Both these regions contribute to the nucleoside phosphate-binding site. A comparison of the sequence of yeast phosphoglycerate kinase reported here with the sequences of phosphoglycerate kinase from horse muscle and human erythrocytes shows that the yeast enzyme is 64% identical with the mammalian enzymes. The yeast has strikingly fewer methionine, cysteine and tryptophan residues.

Phosphoglycerate kinase (ATP:3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3) is active in all cells during glycolysis, and catalyses the formation of ATP by the transfer of a phospho group from 1,3-bisphosphoglycerate to ADP. There is a requirement for bivalent cations (Mn^{2+} or Mg^{2+}), since the metal-ion complexes of the nucleotides are the true substrates. The enzyme is a monomer of M_r 44500 (Merrett, 1981).

High-resolution X-ray-diffraction data for yeast (Bryant *et al.*, 1974) and horse (Banks *et al.*, 1979) phosphoglycerate kinases show that the enzyme consists of two lobes, each containing about 200 amino acids, which correspond to the *N*-terminal and *C*-terminal halves of the polypeptide chain. The major link between the two lobes involves residues 186–200. In addition, residues 395–408 cross back into the *N*-terminal lobe, and the two ends of the polypeptide chain are close together. Difference electron-density maps have shown that the MgADP-binding site is located in the *C*-terminal lobe. Density corresponding to 3-phosphoglycerate has been found near the *N*-terminal lobe (Watson *et al.*, 1982). The location of the two ligand-binding sites in the two different lobes requires a substantial relative movement of the two lobes during catalysis. There is evidence that this is the case for yeast phosphoglycerate kinase from kinetic (Scopes, 1978; Larsson-Raznikiewicz & Jansson, 1973), n.m.r.-spectroscopic (Tanswell *et al.*, 1976), small-angle X-ray scattering (Pickover *et al.*, 1979) and hydrodynamic studies (Roustan *et al.*, 1980).

Chemical-modification studies on yeast phosphoglycerate kinase have implicated a number of different residues as being important for activity. These include arginine (Rogers & Weber, 1977; Philips *et al.*, 1978), tyrosine (Hjelmgren *et al.*, 1976; Roustan *et al.*, 1976; Bacharach *et al.*, 1977), glutamic acid (Desvages *et al.*, 1980) and lysine (Markland *et al.*, 1975) residues. Similar studies have shown that cysteine (Markland *et al.*, 1975) and histidine (Brevet *et al.*, 1973) residues can be modified without loss of activity.

The amino acid sequences of phosphoglycerate kinase from human erythrocytes (Huang *et al.*, 1980*a*,*b*) and horse muscle (Hardy *et al.*, 1981; Merrett, 1981) have been determined. These two enzymes are nearly identical and show only 15 differences. A human phosphoglycerate kinase variant associated with erythrocyte enzyme de-

ficiency has been characterized as involving an Asp→Asn replacement at residue 268 (Fujii *et al.*, 1980).

The yeast phosphoglycerate kinase gene has been shown to be located on a 2.95-kilobase endonuclease-*Hin*dIII fragment (Hitzeman *et al.*, 1980; Dobson *et al.*, 1982). Recently the gene has been mapped within this fragment, and the nucleotide sequence of the 5′-flanking region determined (Dobson *et al.*, 1982).

The present paper reports the determination of the complete amino acid sequence of yeast phosphoglycerate kinase by bringing together both amino acid sequence and nucleotide sequence studies.

Materials and methods

Materials

Dried baker's yeast (*Saccharomyces cerevisiae*) from Distillers Co. was used for the purification of phosphoglycerate kinase. *Saccharomyces cerevisiae* strain X2180-1B (=α *suc 2 mal gal 2 cup 1*) was used for the isolation of the phosphoglycerate kinase gene. Trypsin (3× crystallized), chymotrypsin (3× crystallized) and pepsin (2× crystallized) were from Worthington Biochemical Corp.; thermolysin (3× crystallized) was from Calbiochem; staphylococcal proteinase (from *Staphylococcus aureus* V8) was from Miles Laboratories; carboxypeptidase Y was from Pierce Chemical Co.; restriction endonucleases were from Bethesda Research Laboratories. Reagents for cloning and DNA sequencing were as described previously (Dobson *et al.*, 1982). Reagents and solvents for amino acid sequence analysis were from Rathburn Chemicals, except that Quadrol/trifluoroacetic acid buffer (0.25 M, stabilized with aminoethyl-cellulose) was from Fluka. CNBr was supplied by Eastman Kodak Co., and *o*-iodosobenzoic acid was from Pierce Chemical Co. All other chemicals were from BDH Chemicals and were AnalaR or AristaR grade. AnalaR pyridine was redistilled after refluxing with ninhydrin (2g/l) for 1h.

Purification of phosphoglycerate kinase

The purification procedure was based on that of Scopes (1971) and Fifis & Scopes (1978), and involved ammonia autolysis, $(NH_4)_2SO_4$ fractionation and affinity elution from CM-cellulose with 3-phosphoglycerate (Fig. 1). The phosphoglycerate kinase fractions eluted with 3-phosphoglycerate (fraction VI, tubes 52–73) frequently contained various amounts of a contaminating protein (subunit M_r approx. 34000 by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis), and an additional gel-filtration step was necessary. Solid $(NH_4)_2SO_4$ was added (final concn. 600g/l) to fractions 52–73, and left overnight at room temperature. The suspension was stirred for 30min and then centrifuged at 10000*g* for 35min, and the precipitate was dissolved in a minimum volume of 1% (w/v) NH_4HCO_3 (final volume 8–12ml). The sample was loaded on to a Sephadex G-75 column (2.6cm × 92cm) and eluted with 1% (w/v) NH_4HCO_3 (Fig. 2). Phosphoglycerate kinase was detected by assay of its activity (Scopes, 1975) and by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis.

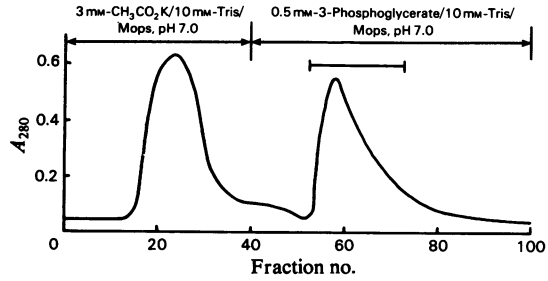


Fig. 1. *Affinity elution of yeast phosphoglycerate kinase from CM-cellulose*

Partially purified phosphoglycerate kinase (total protein 16.4g) in 10mM-Tris adjusted to pH6.0 with 1M-Mes (4-morpholine-ethanesulphonic acid) (final volume 500ml) was loaded on to a column (17cm² × 5cm) equilibrated with 0.2mM-Na_2EDTA/10mM-Tris/Mes buffer, pH6.0. The column was washed with 1 litre of 3mM-potassium acetate/10mM-Tris adjusted to pH7.0 with 1M-Mops (4-morpholinepropanesulphonic acid), and a peak of protein was eluted. The eluting buffer was changed to 0.5mM-3-phosphoglycerate/10mM-Tris/Mops buffer, pH7.0, and a peak of phosphoglycerate kinase was eluted (1.5g; specific activity 620units/mg). Fractions 52–73 were pooled as indicated. The fraction size was 5ml, and the flow rate approx. 100ml/h.

Overall strategy and methods of cleavage

Fragments and smaller peptides of phosphoglycerate kinase were generated as indicated in Fig. 3, and were purified by gel filtration, covalent chromatography, ion-exchange chromatography, high-pressure liquid chromatography and high-voltage paper electrophoresis. CNBr cleavage was done at room temperature for 24h in 70% (v/v) formic acid at a 100-fold molar excess of CNBr over methionine residues, and the resulting fragments were separated on Sephadex G-100 (Fig. 4). The largest CNBr-cleavage fragment, CN1 (173 residues), was further purified by covalent chromatography on activated-thiol–Sepharose 4B (Fig. 5).

Fragment CN2, which contains the only two tryptophan residues in yeast phosphoglycerate kin-

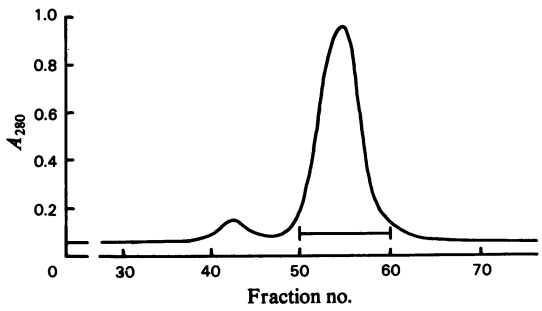


Fig. 2. *Purification of yeast phosphoglycerate kinase on Sephadex G-75*

Concentrated fraction VI (total protein 250mg) was loaded on to a column (2.5cm × 180cm) of Sephadex G-75 and eluted with 1% (w/v) NH_4HCO_3. Fractions 50–60 were pooled as indicated. The fraction size was 5ml, and the flow rate 25ml/h. The purified phosphoglycerate kinase (200mg) had a specific activity of 650units/ml.

ase, was cleaved with *o*-iodosobenzoic acid for 24h at room temperature in the dark (Mahoney & Hermodson, 1979). The reagent was preincubated with *p*-cresol, as recommended by Mahoney *et al.* (1981), to destroy contaminating iodoxybenzoic acid. The resulting fragments were separated on Sephadex G-50 (Fig. 6).

The CNBr-cleavage or *o*-iodosobenzoic acid-cleavage fragments were digested with proteolytic enzymes as described previously (Fothergill & Harkins, 1982).

Peptide purification

Peptide mixtures derived from fragment CN2 were fractionated by cation-exchange chromatography on Technicon P resin as described previously (Fothergill & Harkins, 1982). Further purification was done, when necessary, by high-voltage electrophoresis at pH6.5 or 2 (Fothergill & Fothergill, 1970). At pH6.5, peptide mobility ($m_{6.5}$) was expressed relative to aspartic acid, with valine as a neutral marker. At pH2, mobility (m_2) was measured relative to valine, with 5-dimethylaminonaphthalene-1-sulphonic acid as a neutral marker. Peptides were detected on paper by staining with cadmium/ninhydrin reagent (Heilmann *et al.*, 1957).

The amide content of a peptide was determined by correlating its electrophoretic mobility at pH6.5 and its molecular mass (Offord, 1966), or, where appropriate, by identification of the phenylthiohydantoin derivatives of the amino acid residues.

Peptide mixtures derived from fragments CN3 and CN4 were fractionated by high-voltage electrophoresis or by reversed-phase high-pressure liquid chromatography. A linear gradient of 0.1% trifluoroacetic acid and propan-2-ol (0–100% propan-2-ol in 60min, with a flow rate of 1ml/min) was used with a Waters Associates C_{18} μBondapak column (4mm × 30cm). Peptides were detected by monitoring the effluent at 214nm.

Peptide nomenclature

The following abbreviations indicate that the peptide was derived from digestion or cleavage by: C, chymotrypsin; CN, CNBr; P, pepsin; S, staphylococcal proteinase; T, trypsin; Th, thermolysin; W, *o*-iodosobenzoic acid. Numerals that immediately follow these letters refer to the peak number from the ion-exchange, gel-filtration or high-pressure-liquid-chromatographic fractionations. Lower-case letters refer to the electrophoretic mobility at pH6.5, with 'a' being the most acidic. Numerals refer to electrophoretic mobility at pH2, with '1' being the most acidic.

Amino acid analysis

Hydrolysis was done in evacuated tubes with 0.5ml of 5.7M-HCl (AristaR HCl diluted with an equal volume of water) at 110°C for 20, 48 or 96h. Quantitative analyses were done with a Locarte amino acid analyser with a single column. Tryptophan was determined after hydrolysis with 3M-mercaptoethanesulphonic acid (Penke *et al.*, 1974).

Amino acid sequence determination

Amino acid sequences were determined manually by the dansyl–Edman procedure as described previously (Fothergill & Harkins, 1982), or automatically with a Beckman 890C liquid-phase sequencer equipped with the Beckman cold-trap accessory. The details of the sequencer operation and the identification of the amino acid phenylthiohydantoin derivatives have been described previously (Smith *et al.*, 1982).

Nucleotide sequence determination

The nucleotide sequence corresponding to the *N*-terminal 207 residues of phosphoglycerate kinase was derived from an overlapping series of endonuclease-*Eco*RI/endonuclease-*Bam*HI-digest DNA fragments generated as previously described (Dobson *et al.*, 1982). Nucleotide sequence data corresponding to residues 284–415 were obtained by first subcloning appropriate restriction fragments from plasmid pMA3a-PGK (Dobson *et al.*, 1982) into the single-stranded DNA bacteriophages M13mp701 (constructed by D. Bentley, University of Oxford, Oxford, U.K.) or M13mp9. The DNA was then sequenced by the dideoxy chain termination technique (Sanger *et al.*, 1977) with the use of [α-^{32}P]dTTP (Amersham International). The nucleotide sequence information corresponding to residues

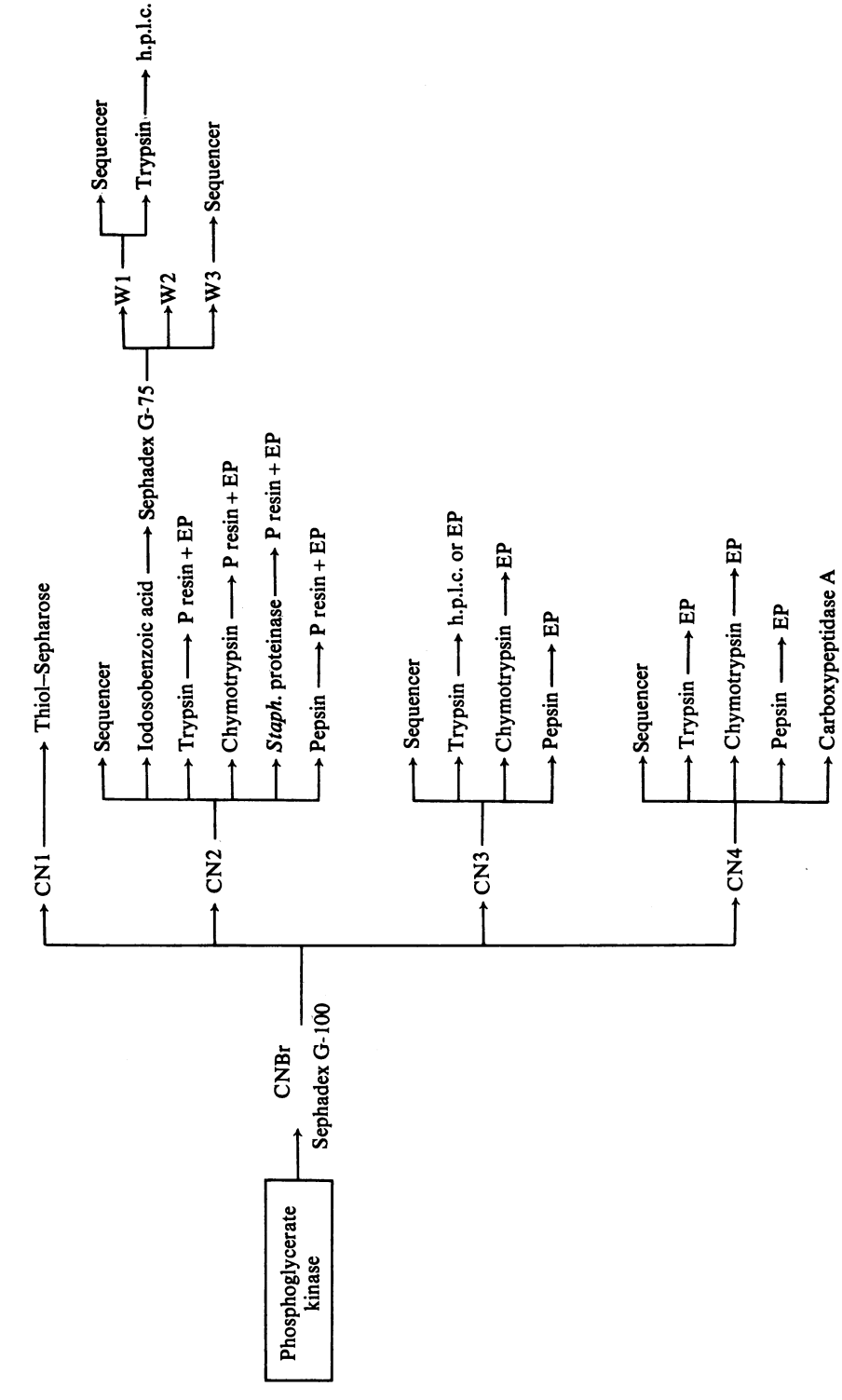


Fig. 3. *Fragmentation of yeast phosphoglycerate kinase*

The cleavage and digestion procedures and the methods of purification are described in the text. Abbreviations: EP, electrophoresis; h.p.l.c., high-pressure liquid chromatography.

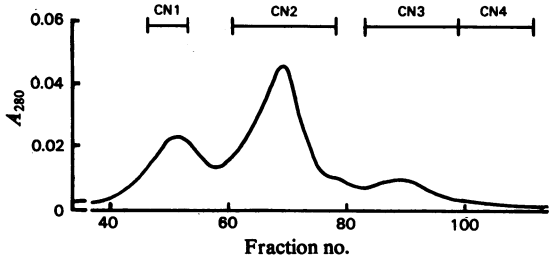


Fig. 4. *Gel filtration of CNBr-cleavage fragments of yeast phosphoglycerate kinase*

Approx. 50mg of phosphoglycerate kinase was dissolved in 5ml of 70% (v/v) formic acid, and approx. 35mg of CNBr was added. The mixture was left for 24h at room temperature, and then loaded on to a column (2.6cm x 92cm) of Sephadex G-100 and eluted with 50% (v/v) formic acid. Fractions containing fragments CN1, CN2, CN3 and CN4 were pooled as indicated. The fraction size was 5ml.

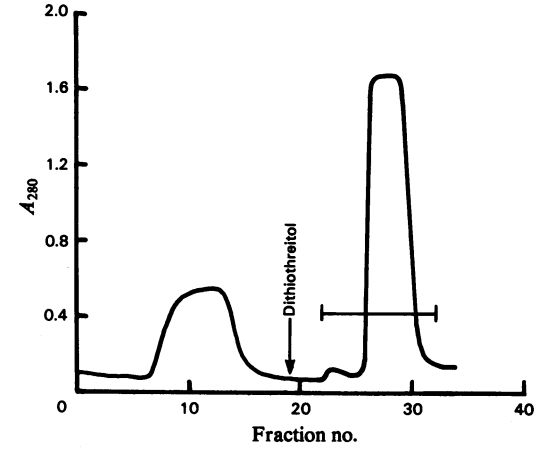


Fig. 5. *Purification of fragment CN1 of yeast phosphoglycerate kinase on activated thiol–Sepharose 4B*

Fractions containing fragment CN1 were freeze-dried and dissolved in 1mM-Na_2EDTA/0.5M-Tris/HCl buffer, pH8.8, containing 8M-urea. A 5-fold molar excess of dithiothreitol over cysteine residues was added, and the mixture was incubated under N_2 for 90min at room temperature. The protein was transferred into 1mM-Na_2EDTA/0.1M-NaCl/0.1M-sodium phosphate buffer, pH8.6, containing 8M-urea by passage down a Sephadex G-10 column (2.5cm x 35cm). The sample was then loaded on to a column (2.5cm x 9cm) of activated thiol–Sepharose 4B and washed with 2 column volumes of the same buffer. The column was then eluted with 20mM-dithiothreitol in the same buffer, and fractions containing fragment CN1 were pooled as indicated. The fraction size was 6ml.

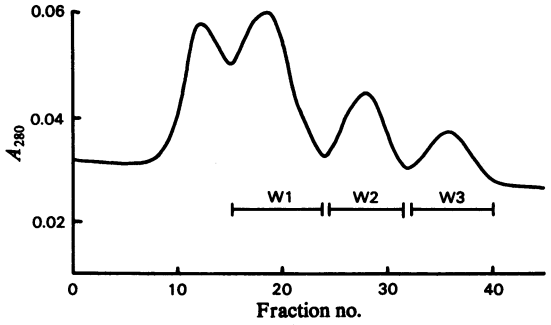


Fig. 6. *Gel filtration of o-iodosobenzoic acid-cleavage fragments from fragment CN2 of yeast phosphoglycerate kinase*

Fragment CN2 (6mg) was dissolved in 2ml of 80% (v/v) acetic acid/4M-guanidinium chloride, and 15mg of *o*-iodosobenzoic acid (that had been preincubated wtih 1mg of *p*-cresol) was added. The mixture was incubated for 24h at room temperature in the dark, and then loaded on to a column (1.5cm x 124cm) of Sephadex G-50 and eluted with 50% (v/v) formic acid. Fractions containing peptides W1, W2 and W3 were pooled as indicated. The fraction size was 3ml.

284–415 places the endonuclease-*Eco*RI site around residue 343, and an endonuclease-*Pvu*II site at residue 306 [instead of residues 306 and 287 as inferred previously from preliminary amino acid sequence data (Dobson *et al.*, 1982)].

Results and discussion

Purification and characterization of yeast phosphoglycerate kinase

An affinity elution purification procedure based on that devised by Fifis & Scopes (1978) was found to be a convenient and rapid method for purifying phosphoglycerate kinase in high yield (1.5g from 500g of dried yeast). However, the final product frequently contained a single additional protein, sometimes in significant amount. It seemed likely that this contaminating protein was another glycolytic enzyme, possibly glyceraldehyde 3-phosphate dehydrogenase or phosphoglycerate mutase. Since phosphoglycerate kinase is the only monomeric glycolytic enzyme, and therefore has a substantially smaller native molecular weight, gel filtration on Sephadex G-75 was used as an additional purification step (Fig. 2). The phosphoglycerate kinase purified in this way was homogeneous by sodium dodecyl sulphate / polyacrylamide - gel electrophoresis (M_r approx. 45000), and had a specific activity of 650 units (μmol/min)/mg at 30°C,

Table 1. *Amino acid compositions of phosphoglycerate kinase and its CNBr-cleavage fragments*

The results are expressed as mol of residue/mol of fragment, and the values for serine and threonine are corrected by extrapolation to zero time. Other values are averages for the three different times of hydrolysis. The values in parentheses are those found in the sequence.

Amino acid	Phosphoglycerate kinase	Sum of CNBr fragments	Fragment CN1	Fragment CN2	Fragment CN3	Fragment CN4
Cm-Cys	1.0 (1)	1.0 (1)	1.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)
Asp / Asn	39.2 (25) / (14)	39.3 (25) / (14)	17.9 (11) / (7)	11.9 (8) / (4)	6.9 (5) / (2)	3.1 (2) / (1)
Thr	18.3 (18)	18.0 (18)	4.1 (4)	10.8 (11)	1.2 (1)	1.9 (2)
Ser	26.3 (27)	26.4 (27)	15.5 (16)	8.2 (8)	1.2 (1)	1.2 (1)
Hse	0.0 (0)	3 (3)	+ (1)	− (0)	+ (1)	+ (1)
Glu / Gln	36.5 (29) / (8)	37.3 (29) / (8)	17.2 (12) / (5)	12.0 (11) / (1)	5.2 (3) / (2)	2.9 (3) / (0)
Pro	17.3 (17)	17.1 (17)	7.2 (7)	5.8 (6)	3.1 (3)	1.0 (1)
Gly	37.2 (37)	37.5 (37)	9.2 (9)	18.0 (18)	8.1 (8)	2.2 (2)
Ala	43.0 (43)	43.0 (43)	14.0 (14)	20.0 (20)	6.0 (6)	3.0 (3)
Val	37.5 (38)	37.4 (38)	17.7 (18)	14.7 (15)	3.1 (3)	1.9 (2)
Met	3.0 (3)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Ile	22.6 (23)	21.2 (23)	6.9 (7)	6.5 (7)	5.5 (6)	2.3 (3)
Leu	41.3 (42)	41.5 (42)	18.7 (19)	10.4 (10)	10.1 (10)	2.1 (2)
Tyr	7.1 (7)	6.9 (7)	4.6 (5)	1.2 (1)	1.1 (1)	0.0 (0)
Phe	18.8 (19)	18.4 (19)	5.2 (5)	6.7 (7)	3.9 (4)	2.6 (3)
His	8.3 (8)	7.7 (8)	6.7 (7)	1.0 (1)	0.0 (0)	0.0 (0)
Lys	40.9 (41)	40.9 (41)	15.2 (15)	16.8 (17)	5.9 (6)	4.0 (4)
Arg	13.1 (13)	13.2 (13)	9.7 (10)	1.3 (1)	2.2 (2)	0.0 (0)
Trp	2.0 (2)	2.0 (2)	0.0 (0)	2.0 (2)	0.0 (0)	0.0 (0)
Total	413.4 (415)	411.8 (415)	171.8 (173)	147.3 (148)	64.5 (64)	29.2 (30)
N-Terminus	Blocked		Blocked	Glu	Val	Ala
Position in sequence			1–173	268–415	174–237	238–267

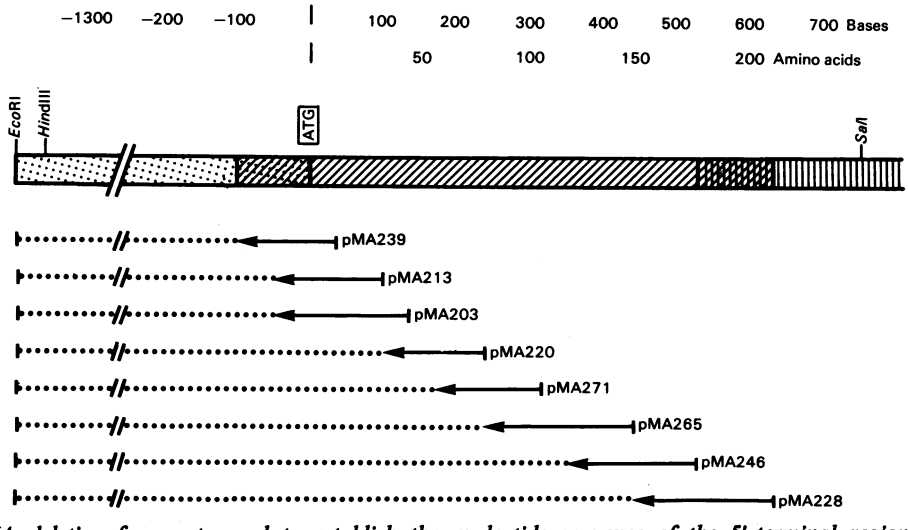


Fig. 7. *DNA deletion fragments used to establish the nucleotide sequence of the 5′-terminal region of the yeast phosphoglycerate kinase gene*

The relationship of the eight deletion fragments (pMa239 etc.) to the 5′-end of the endonuclease-*Hin*dIII-digest fragment containing the phosphoglycerate kinase gene is shown. The stippled portion (▨) represents the non-coding region, the hatched portion (▨) shows the region for which the nucleotide sequence has been determined, and the portion with the vertical lines (▮) the region for which the amino acid sequence has been determined. The sequenced portion of each deletion fragment is indicated by the solid line, and the unsequenced portion by the dotted line.

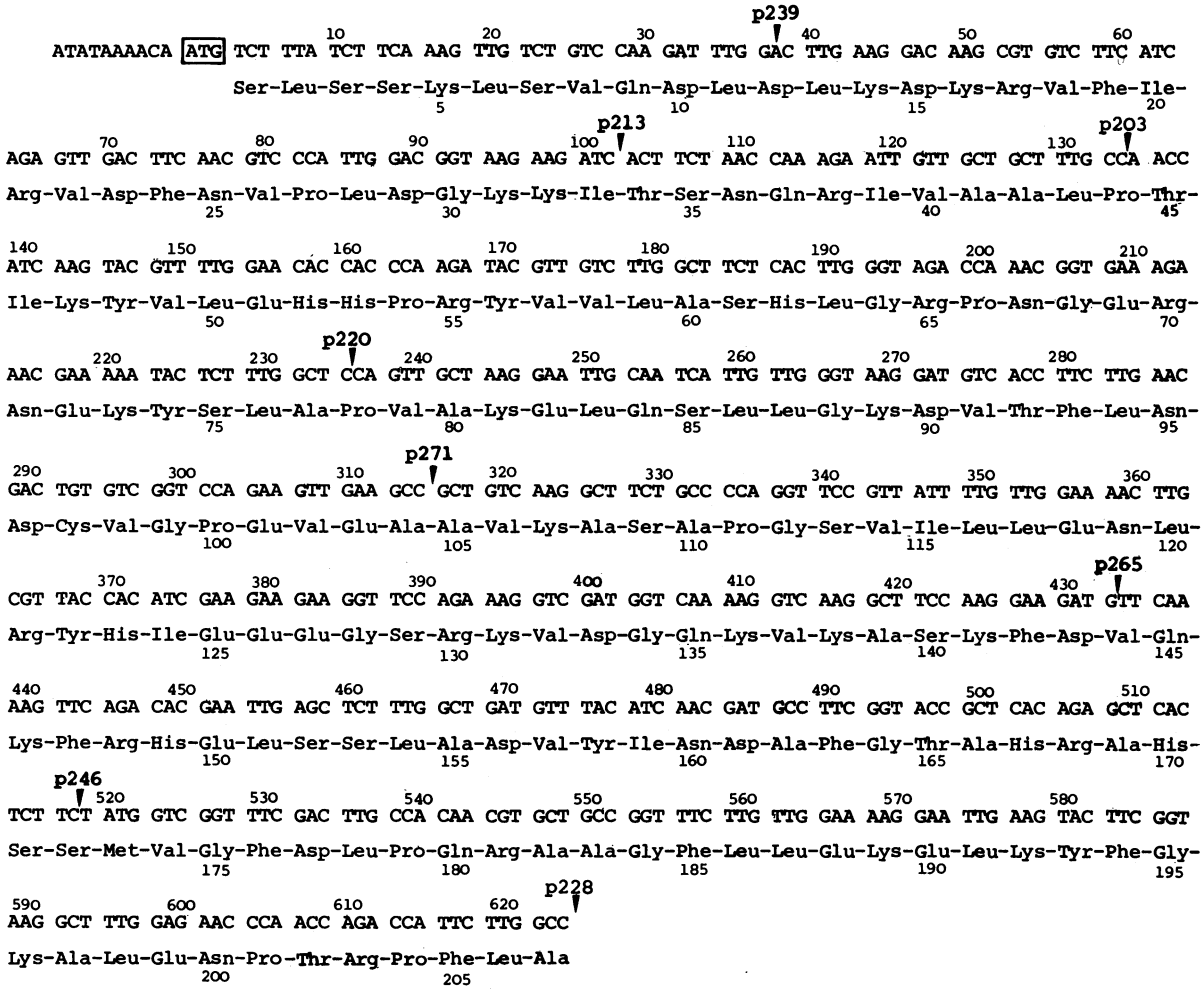


Fig. 8. *Nucleotide sequence corresponding to residues 1–207 of yeast phosphoglycerate kinase*
The initiation codon is shown boxed in. The 3'-ends of the eight deletion fragments are indicated by the arrowheads. Amino acid residue 174 corresponds to the *N*-terminus of CNBr-cleavage fragment CN3.

assuming a value of 5.0 for $A^{1\%}_{1cm,280}$. The amino acid composition is given in Table 1.

CNBr-cleavage fragments

Cleavage of phosphoglycerate kinase with CNBr at the three methionine residues yielded the expected four fragments. The three smaller fragments, CN2, CN3 and CN4, were well separated by gel filtration on Sephadex G-100 (Fig. 4), but fragment CN1 required further purification. Yeast phosphoglycerate kinase has only one cysteine residue, which is located in fragment CN1, and this property was exploited to purify fragment CN1 by covalent chromatography with activated thiol–Sepharose 4B (Fig. 5). Most of the contaminating material was not bound to the column, and the fragment CN1 that was eluted with dithiothreitol was homogeneous by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (M_r approx. 18500).

Amino acid analyses of the CNBr-cleavage fragments (Table 1) revealed the presence of a homoserine residue in fragments CN1, CN3 and CN4 but not in fragment CN2, thus establishing fragment CN2 as the *C*-terminal fragment. Fragments CN2, CN3 and CN4, but not fragment CN1, showed free *N*-terminal residues after reaction with dansyl (5-dimethylaminonaphthalene-1-sulphonyl) chloride, indicating that fragment CN1 had a

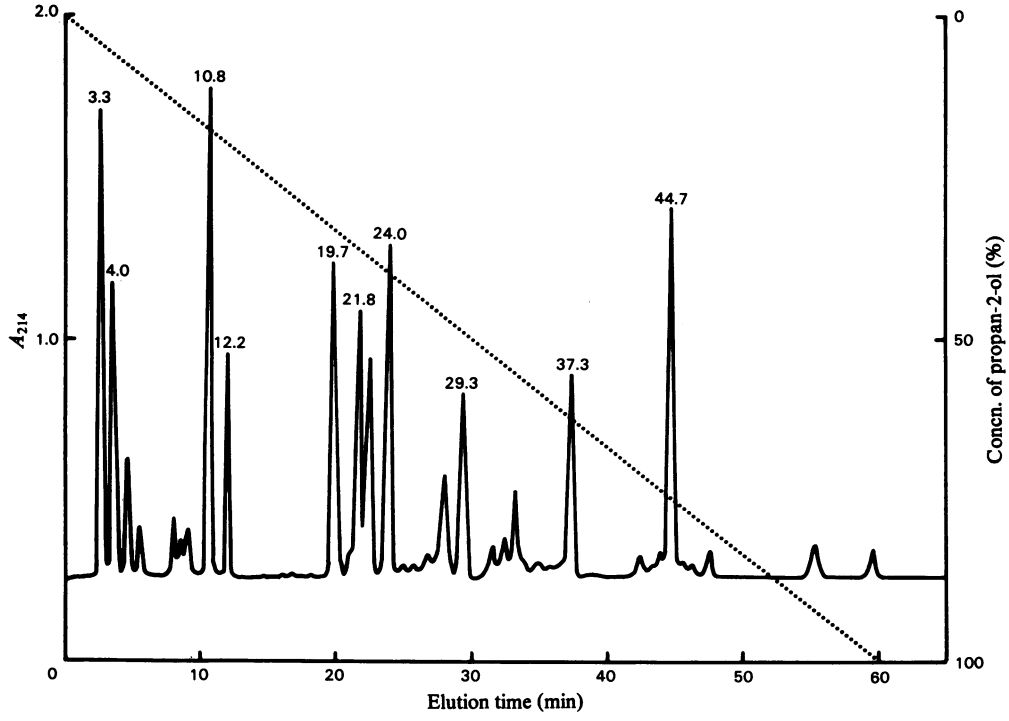


Fig. 9. *Separation of tryptic peptides from fragment CN3 of yeast phosphoglycerate kinase by reversed-phase high-pressure liquid chromatography*

Fragment CN3 (450nmol) was dissolved in 1.5ml of 1% (w/v) NH_4HCO_3 and digested with trypsin (0.3mg) for 12h at 37°C, and freeze-dried. The digestion mixture was dissolved in 200μl of 0.1% trifluoroacetic acid, and peptides in a 12μl sample (27nmol) were separated as described in the text. Fractions corresponding to each major peak were collected. ——, A_{214}; ·····, concn. of propan-2-ol.

blocked *N*-terminus and was thus the *N*-terminal fragment. The compositions of the fragments account for the total composition of phosphoglycerate kinase, and the actual numbers of residues found in the sequence are in good agreement with the composition.

Sequence of fragment CN1

The sequence of the *N*-terminal 173 residues of phosphoglycerate kinase (corresponding to CNBr-cleavage fragment CN1) was deduced by DNA sequencing of a series of endonuclease-*Eco*RI/endonuclease-*Bam*HI-digest fragments encompassing the region 100 bases upstream of the initiation codon to 628 bases into the coding sequence. Eight of the deletion fragments, and the region of each fragment that was sequenced, are shown in Fig. 7. The sequenced portions show considerable overlap, and were sufficient to establish an unequivocal sequence. The longest deletion fragment, pMA228, provided a substantial overlap into the portion of the sequence determined by amino acid sequence analysis.

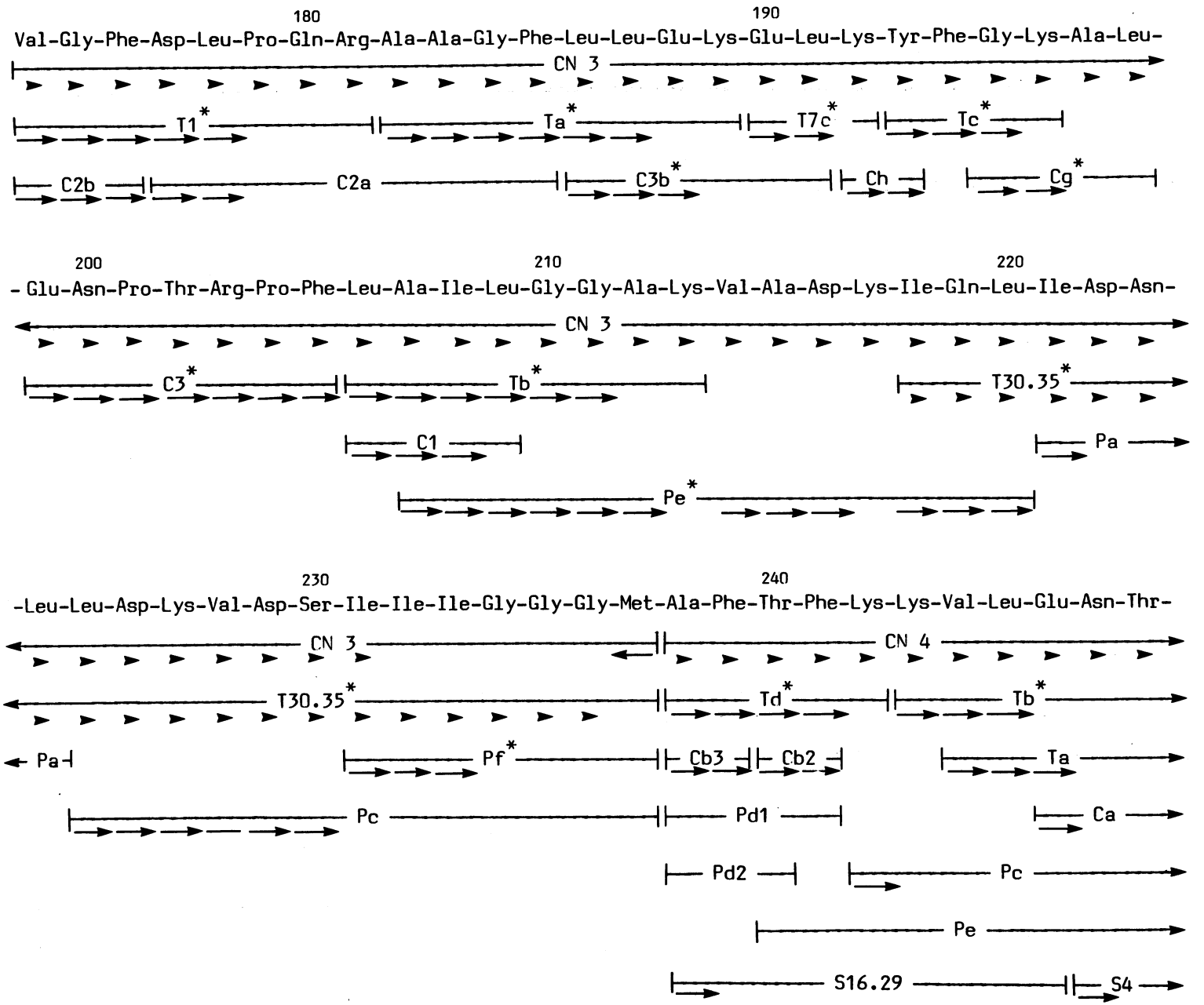
The nucleotide sequence extending 624 bases into the 5′-end of the coding sequence is shown in Fig. 8. The corresponding amino acid sequence (extending to residue 207) is also shown.

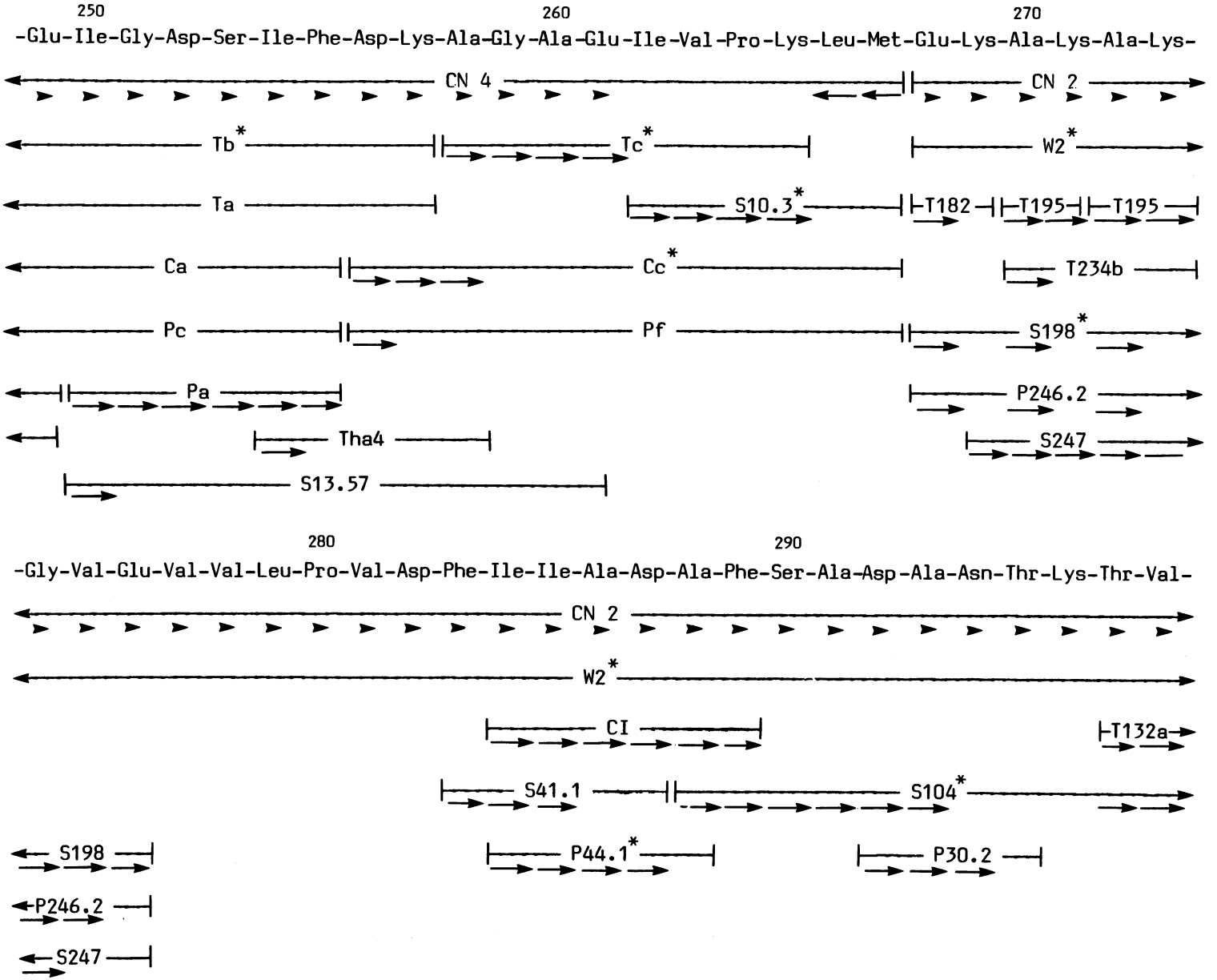
The *N*-terminus of yeast phosphoglycerate kinase has been identified as *N*-acetylserine (Yoshida, 1972). Two different *N*-terminal dipeptide sequences have been reported: *N*-acetylserylaspartic acid (Kulbe *et al.*, 1976) and *N*-acetylserylglycine (Fattoum *et al.*, 1981). The nucleotide sequence results show that the second residue is leucine.

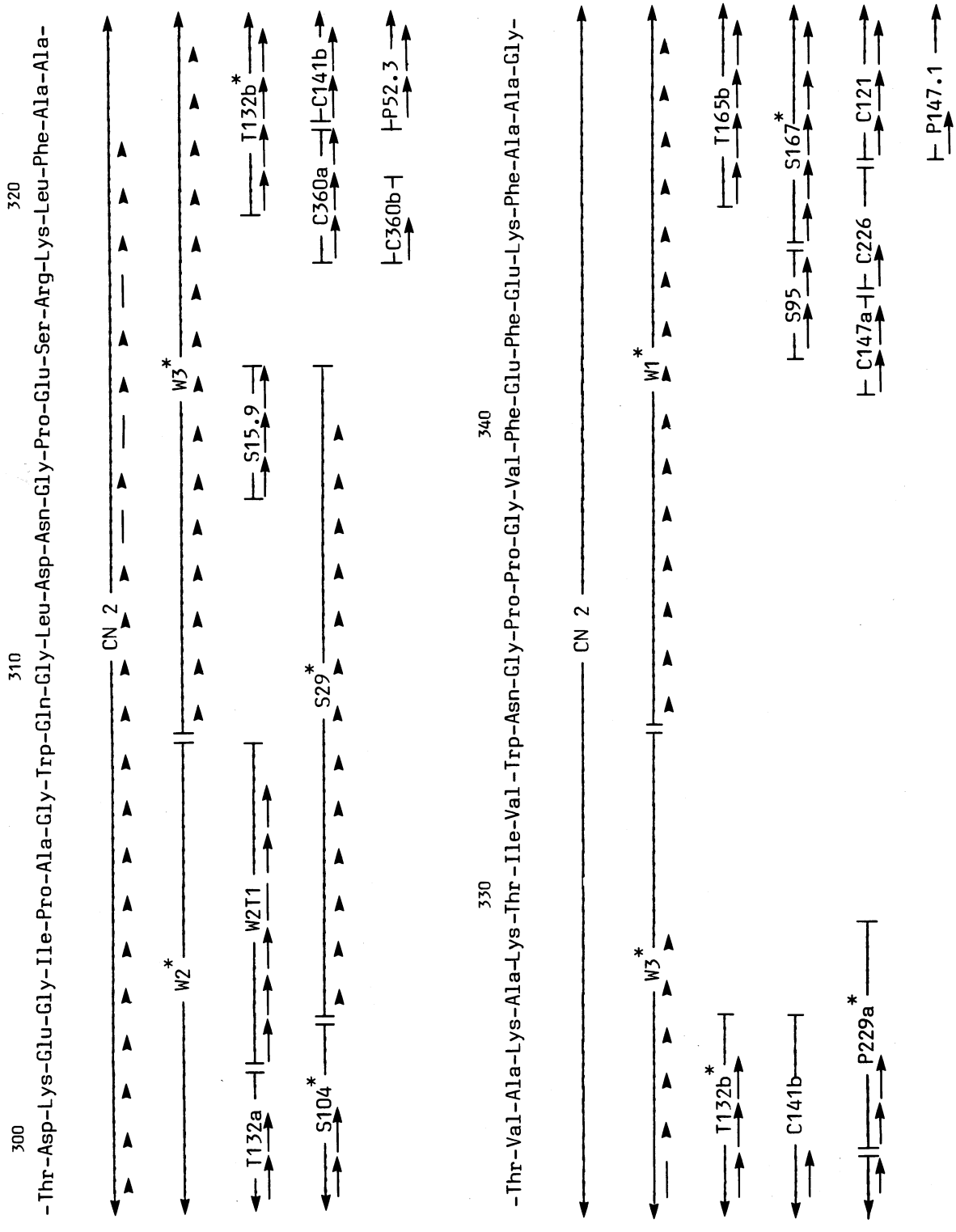
The nucleotide sequence corresponding to amino acid residues 174–207 was in complete agreement with the results of amino acid sequence analysis of these residues.

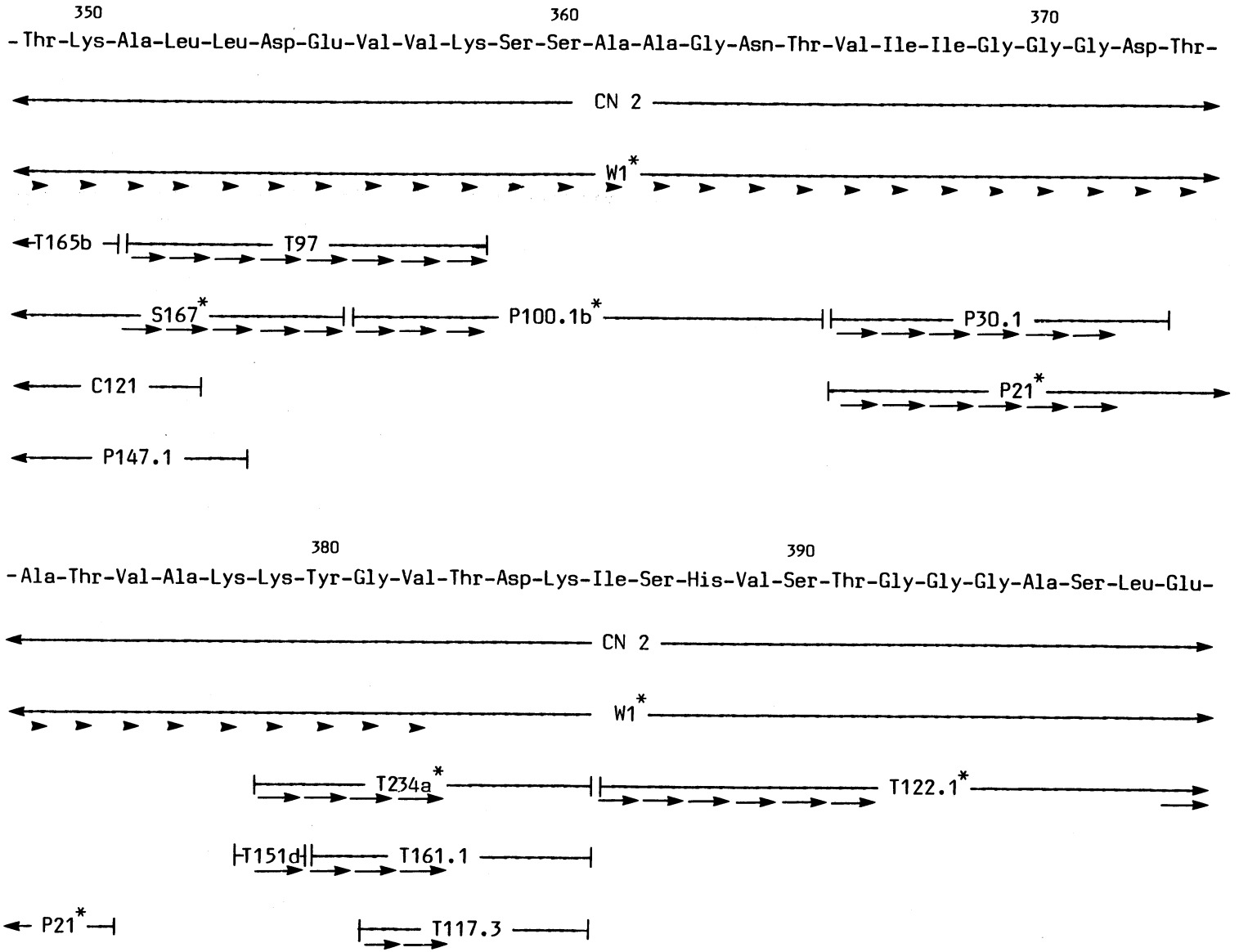
Sequence of fragment CN3

The sequence of fragment CN3, comprising 64 residues, was determined by automatic *N*-terminal sequence analysis, and by manual sequencing of peptides derived by digestion with trypsin, chymotrypsin and pepsin. The peptides were purified by


180
190
Val-Gly-Phe-Asp-Leu-Pro-Gln-Arg-Ala-Ala-Gly-Phe-Leu-Leu-Glu-Lys-Glu-Leu-Lys-Tyr-Phe-Gly-Lys-Ala-Leu-
CN 3
T1*
Ta*
T7c*
Tc*
C2b
C2a
C3b*
Ch
Cg*
200
210
220
- Glu-Asn-Pro-Thr-Arg-Pro-Phe-Leu-Ala-Ile-Leu-Gly-Gly-Ala-Lys-Val-Ala-Asp-Lys-Ile-Gln-Leu-Ile-Asp-Asn-
CN 3
C3*
Tb*
T30.35*
C1
Pa
Pe*
230
240
-Leu-Leu-Asp-Lys-Val-Asp-Ser-Ile-Ile-Ile-Gly-Gly-Gly-Met-Ala-Phe-Thr-Phe-Lys-Lys-Val-Leu-Glu-Asn-Thr-
CN 3
CN 4
T30.35*
Td*
Tb*
Pa
Pf*
Cb3
Cb2
Ta
Pc
Pd1
Ca
Pd2
Pc
Pe
S16.29
S4


250
260
270
-Glu-Ile-Gly-Asp-Ser-Ile-Phe-Asp-Lys-Ala-Gly-Ala-Glu-Ile-Val-Pro-Lys-Leu-Met-Glu-Lys-Ala-Lys-Ala-Lys-
CN 4
CN 2
Tb*
Tc*
W2*
Ta
S10.3*
T182
T195
T195
Ca
Cc*
T234b
Pc
Pf
S198*
Pa
P246.2
Tha4
S247
S13.57
280
290
-Gly-Val-Glu-Val-Val-Leu-Pro-Val-Asp-Phe-Ile-Ile-Ala-Asp-Ala-Phe-Ser-Ala-Asp-Ala-Asn-Thr-Lys-Thr-Val-
CN 2
W2*
CI
T132a
S41.1
S104*
S198
P44.1*
P30.2
P246.2
S247


300
310
320
-Thr-Asp-Lys-Glu-Gly-Ile-Pro-Ala-Gly-Trp-Gln-Gly-Leu-Asp-Asn-Gly-Pro-Glu-Ser-Arg-Lys-Leu-Phe-Ala-Ala-
CN 2
W2*
W3*
T132a
W2T1
S104*
S29*
S15.9
T132b*
C360a
C141b
C360b
P52.3
330
340
-Thr-Val-Ala-Lys-Ala-Lys-Thr-Ile-Val-Trp-Asn-Gly-Pro-Pro-Gly-Val-Phe-Glu-Phe-Glu-Lys-Phe-Ala-Ala-Gly-
CN 2
W3*
W1*
T132b*
C141b
P229a*
T165b
S95
S167*
C147a
C226
C121
P147.1


350
360
370
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CN 2
W1*
T165b
T97
S167*
P100.1b*
P30.1
C121
P21*
P147.1
380
390
-Ala-Thr-Val-Ala-Lys-Lys-Tyr-Gly-Val-Thr-Asp-Lys-Ile-Ser-His-Val-Ser-Thr-Gly-Gly-Gly-Ala-Ser-Leu-Glu-
CN 2
W1*
T234a*
T122.1*
T151d
T161.1
P21*
T117.3

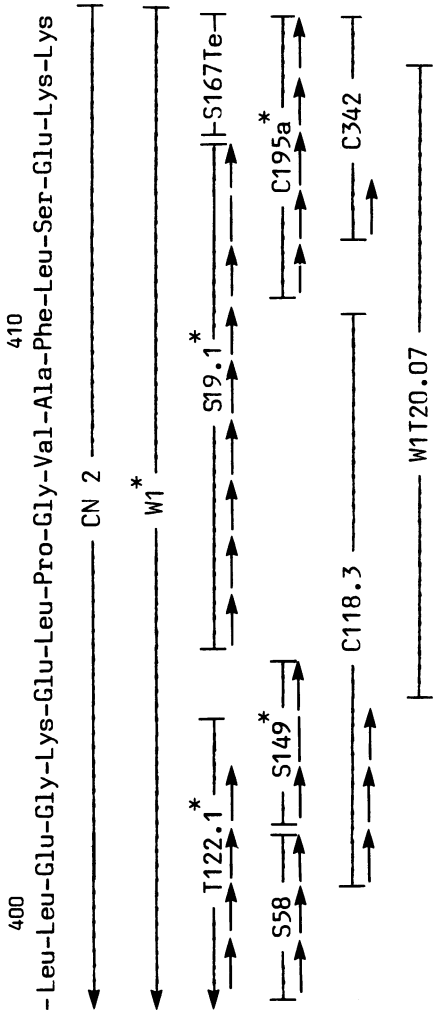

Fig. 10. *Amino acid sequence of residues 174–415 of yeast phosphoglycerate kinase*
Residues were identified by automatic Edman degradation (▬), by the manual dansyl-Edman procedure (→) or by carboxypeptidase Y digestion (←). Rounds in which no residue was identified are indicated by ——. The amino acid compositions and peptide mobilities of those peptides indicated by * are given in Table 2. Peptide nomenclature and details of the methods are given in the text.

high-voltage electrophoresis or by high-pressure liquid chromatography, and Fig. 9 shows the elution profile of tryptic peptides from a C_{18} μ-Bondapak column. All the peptides recovered in significant yield from fragment CN3 were examined, and the results of sequence analysis are shown in Fig. 10. Amino acid compositions and electrophoretic mobilities of important peptides are given in Table 2.

The automated sequence analysis of fragment CN3 penetrated 55 residues. The initial yield from the sequencer was 38 nmol, and the repetitive yield was 97.1% (from Phe-3 to Phe-12). Ample evidence to establish the sequence of fragment CN3 was obtained from the proteolytic digests and from the sequencer results. The amide assignments were made by direct identification of the amino acid phenylthiohydantoin derivatives. The *N*-terminus of fragment CN3 corresponds to residue 174 of phosphoglycerate kinase.

Sequence of fragment CN4

Fragment CN4 comprises 30 residues, and the sequence was determined by automated and manual sequence analysis. Tryptic, chymotryptic, peptic, thermolytic and staphylococcal-proteinase digests were done. The results of sequence analysis are shown in Fig. 10, and the amino acid compositions and electrophoretic mobilities of important peptides are given in Table 2.

The initial yield from the sequencer was 40 nmol, and the repetitive yield was 96.7% (from Phe-2 to Phe-18). The amide assignments were made by direct identification of the amino acid phenylthiohydantoin derivatives. The *N*-terminus of fragment CN4 corresponds to residue 238 of phosphoglycerate kinase.

Sequence of fragment CN2

Fragment CN2, comprising 148 residues, was significantly larger than fragments CN3 and CN4, and several additional fragmentation procedures were required to establish its sequence. Automated *N*-terminal sequence analysis of the whole fragment penetrated 54 residues. The initial yield from the sequencer was 80 nmol, and the repetitive yield was 95.8% (from Glu-1 to Glu-9).

Fragment CN2 contains the only two tryptophan residues in phosphoglycerate kinase, and chemical cleavage at these residues was particularly useful. The purification of the resulting three fragments is shown in Fig. 6. Fragment W1 corresponds to residues 334–415, fragment W2 to residues 268–308 and fragment W3 to residues 309–333. Fragments W1 and W3 were degraded in the liquid-phase sequencer; the initial yields were 8 nmol and 7 nmol, and the repetitive yields were 99.0% (from Gly-5 to Gly-36) and 97.7% (from Asp-4 to Ala-18) respectively.

Table 2. *Data for peptides establishing the amino acid sequences of residues 174–415 of phosphoglycerate kinase*

Experimental details and peptide nomenclature are given in the text. Values are molar ratios, and are corrected for destruction or partial hydrolysis. The symbol — means insignificant amount in the composition, or not done for mobility and *N*-terminal determinations. The total number of residues in each peptide corresponds to that found in the sequence.

	Fragment CN3											Fragment CN4				
Peptide ...	T1	Ta	C3b	T7c	Tc	Cg	C3	Tb	Pe	T30.35	Pf*	Td	Tb	Cc	Tc	S10.3
$m_{6.5}$ ...	0	+0.09	−0.24	+0.08	+0.40	+0.50	0	+0.33	+0.21	—	+0.39	+0.42	−0.26	+0.08	+0.07	—
m_2 ...	+0.84	—	—	+1.39	—	—	+0.90	—	—	—	—	—	—	—	—	—
Asx	1.0	—	—	—	—	—	1.0	—	1.0	4.3	—	—	3.0	0.7	—	—
Thr	—	—	—	—	—	—	0.8	—	—	—	—	1.0	1.0	—	—	—
Ser	—	—	—	—	—	—	—	—	—	0.8	—	—	1.3	—	—	—
Hse	—	—	—	—	—	—	—	—	—	+	+	—	—	+	—	+
Glx	1.0	1.1	2.0	1.1	—	—	1.0	—	1.3	1.3	—	—	2.1	1.1	1.1	—
Pro	1.0	—	—	—	—	—	2.0	—	—	—	—	—	—	—	0.8	1.1
Gly	0.8	1.0	—	—	1.0	1.2	—	2.2	2.0	3.0	3.0	—	1.4	1.1	1.1	—
Ala	—	1.8	—	—	—	1.0	—	2.0	2.7	—	—	1.0	—	2.0	2.0	—
Val	0.9	—	—	—	—	—	—	—	0.8	0.9	—	—	1.0	0.8	0.9	1.1
Ile	—	—	—	—	—	—	—	1.0	1.5	3.7	2.3	—	1.7	0.6	0.8	0.8
Leu	1.0	1.9	2.8	1.0	—	0.9	—	1.8	2.0	2.5	—	—	1.0	0.9	—	1.0
Tyr	—	—	—	—	0.7	—	—	—	—	—	—	—	—	—	—	—
Phe	1.0	1.0	—	—	0.9	—	0.9	—	—	—	—	2.0	0.9	—	—	—
His	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lys	—	0.6	0.9	1.0	1.0	1.1	—	1.1	2.0	0.7	—	1.0	1.5	1.8	1.0	1.1
Arg	1.0	—	—	—	—	—	0.9	—	—	—	—	—	—	—	—	—
Trp	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	8	8	6	3	4	4	7	8	14	20	6	5	15	12	8	6
N-Terminus	Val	Ala	Leu	Glx	Tyr	Gly	Glx	Leu	Ala	Ile	Ile	Ala	Lys	Asx	Ala	Ile

	Fragment CN2																
Peptide ...	W1	W2	W3	S198	P44.1	S104	S29	T132b	P229a	S167	P100.1b	P21	T234a	T122.1	S149	S19.1	C195a
$m_{6.5}$ ...	—	—	—	+0.34	−0.30	−0.03	−0.16	+0.31	+0.71	+0.10	+0.36	−0.26	+0.36	0	+0.13	—	+0.23
m_2 ...	—	—	—	+1.42	+0.78	+0.89	+0.29	+0.77	+1.51	+1.00	+0.79	+0.48	+1.28	+0.83	+1.44	+0.38	+1.44
Asx	4.8	4.2	1.9	—	1.2	2.3	2.2	—	—	1.0	1.2	1.0	0.9	—	—	—	—
Thr	5.5	2.9	2.1	—	—	2.7	—	1.0	0.6	1.0	1.1	1.8	0.9	1.1	—	—	—
Ser	5.2	1.9	0.9	—	—	1.3	—	—	—	—	1.9	—	—	2.5	—	0.8	1.0
Hse	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Glx	7.1	3.3	2.1	2.0	—	1.1	1.9	—	—	1.1	—	—	—	1.9	0.9	1.0	1.2
Pro	3.2	2.2	1.1	—	—	—	1.6	—	—	—	—	—	—	—	—	1.0	—
Gly	13.1	3.1	2.2	1.2	—	—	3.6	—	—	1.3	1.0	3.0	1.0	3.8	1.0	1.0	—
Ala	9.0	7.0	4.0	2.0	2.0	2.8	1.1	3.0	2.0	3.0	2.0	1.1	—	1.0	—	0.8	—
Val	7.5	4.8	1.8	0.9	—	1.1	—	1.2	0.6	—	1.3	0.6	0.9	0.9	—	0.9	—
Ile	3.1	2.8	0.9	—	1.6	—	0.8	—	—	—	—	1.4	—	1.0	—	—	—
Leu	6.6	1.1	2.9	—	—	—	0.9	1.2	—	1.9	—	—	—	3.1	—	1.8	1.0
Tyr	—	—	—	—	—	—	—	—	—	—	—	—	0.4	—	—	—	—
Phe	3.9	1.7	1.0	—	—	0.8	—	0.8	—	1.1	—	—	—	—	—	1.1	—
His	1.1	—	—	—	—	—	—	—	—	—	—	—	—	1.1	—	—	—
Lys	9.2	4.9	2.2	2.6	—	1.8	—	1.0	0.9	1.9	0.8	—	1.6	1.0	0.9	—	2.0
Arg	—	—	0.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Trp	—	+	+	—	—	—	1.0	—	—	—	—	—	—	—	—	—	—
Total	82	41	25	9	5	15	14	8	5	12	10	10	7	18	3	9	5
N-Terminus	Asn	Glu	Gln	Glx	Ile	Ala	Gly	Leu	Val	Lys	Val	Val	Lys	Ile	Gly	Leu	Leu

* The composition of peptide Pf after 72 h hydrolysis was: Hse, +; Gly, 3.0; Ile, 2.7.

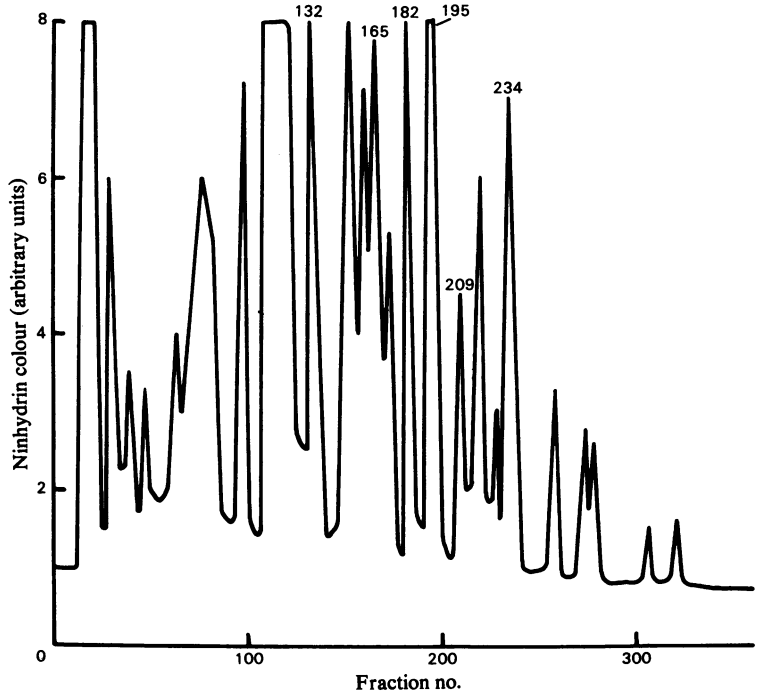

Fig. 11. *Cation-exchange fractionation of tryptic peptides from fragment CN2 of yeast phosphoglycerate kinase*
Digestion and elution conditions are given in the text. Some peak numbers are indicated.

Fragment CN2 was digested with trypsin, chymotrypsin, staphylococcal proteinase or pepsin. The resulting peptides were separated by cation-exchange chromatography, and Fig. 11 shows the elution profile for the tryptic peptides. Similar results were obtained with the other digests.

The results of automated and manual sequence analysis are shown in Fig. 10, and the amino acid compositions and electrophoretic mobilities of important peptides are given in Table 2. The amide assignments were made by direct identification of the amino acid phenylthiohydantoin derivatives, except for Asp-384, Glu-398, Glu-401, Glu-404 and Glu-413. These were all identified as acidic residues by peptide mobilities and by staphylococcal-proteinase specificity. The *N*-terminus of fragment CN2 corresponds to residue 268 of phosphoglycerate kinase.

Sufficient evidence from amino acid sequence analysis was obtained to establish unambiguously almost all of the sequence of fragment CN2. In a few regions, however, because of the nature of the sequence, and the specificity of the proteolytic enzymes, relatively limited information was available. In these regions (residues 329–332, 383–384 and 392–397) results from nucleotide sequence studies were used to establish the sequence. Fig. 12 gives the nucleotide sequence corresponding to amino acid residues 281–415.

Complete sequence of yeast phosphoglycerate kinase

The entire sequence of the enzyme, comprising 415 residues, is summarized in Fig. 13. The relative molecular mass calculated from the sequence is 44556; this value is close to that estimated by physical methods. Several different lines of evidence were used to establish the order of the CNBr-cleavage fragments. Fragment CN1 is *N*-terminal, since it has a blocked *N*-terminus. This was confirmed by the nucleotide sequencing results, which also established the overlap between fragments CN1 and CN3. Fragment CN2 contains no homoserine residue and is therefore *C*-terminal. Fragment CN4 can thus be placed unambiguously. Comparison of the yeast phosphoglycerate kinase sequence with those of the horse (Merrett, 1981) and human (Huang *et al.*, 1980*b*) enzymes confirms that this order is the correct one.

There are a number of examples of non-random distribution of residues that may be significant with respect to structure and function. The *N*-terminal lobe of phosphoglycerate kinase has 11 of the 13 arginine residues, seven of the eight histidine residues and six of the seven tyrosine residues. The *C*-terminal lobe, in contrast, has the two tryptophan residues and 23 of the 36 glycine residues. Very

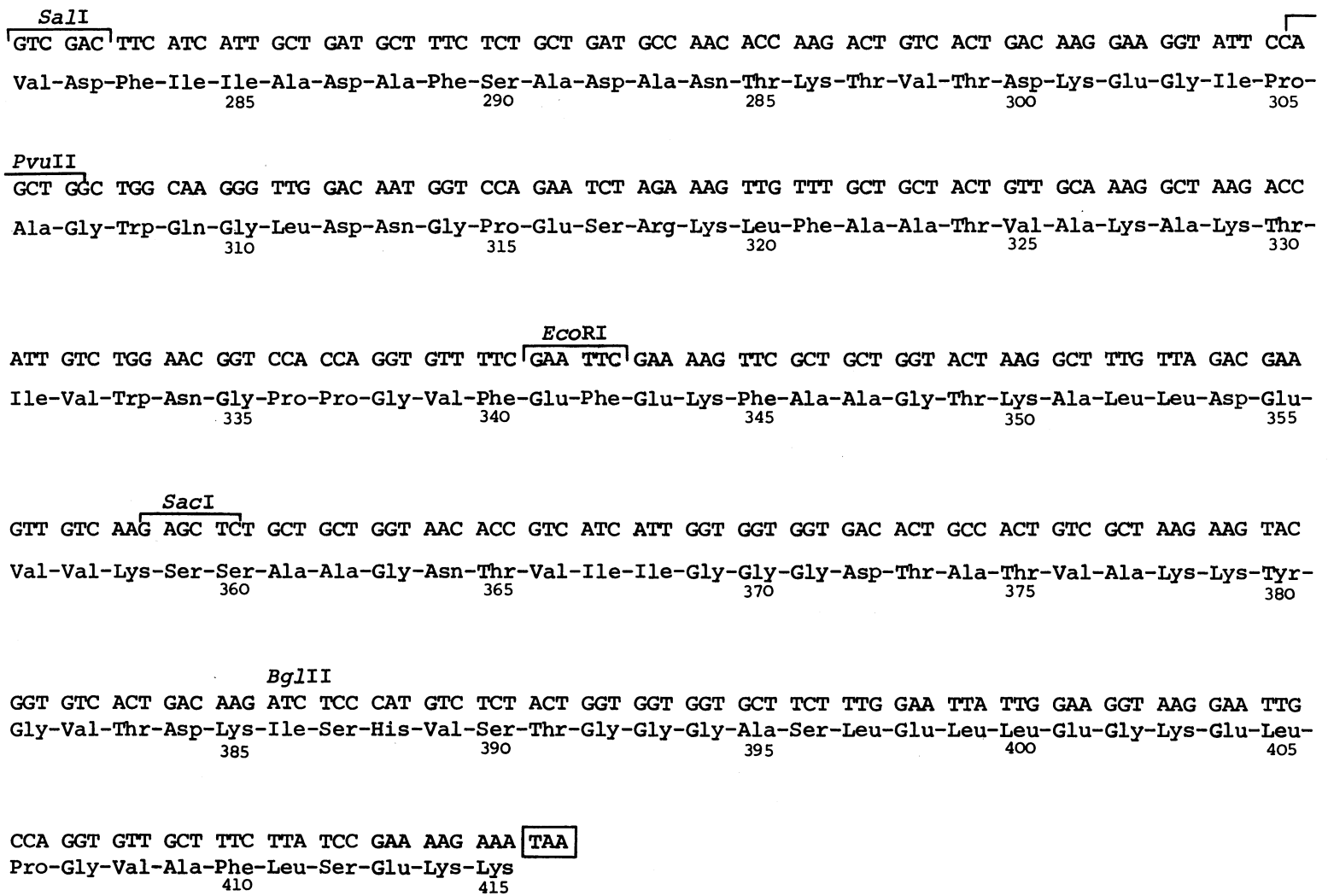

Fig. 12. *Nucleotide sequence corresponding to residues 281–415 of yeast phosphoglycerate kinase*
The sites of digestion by the restriction endonucleases *Sal*I, *Pvu*II, *Eco*RI, *Sac*I and *Bgl*II are indicated. The stop codon is shown boxed in.

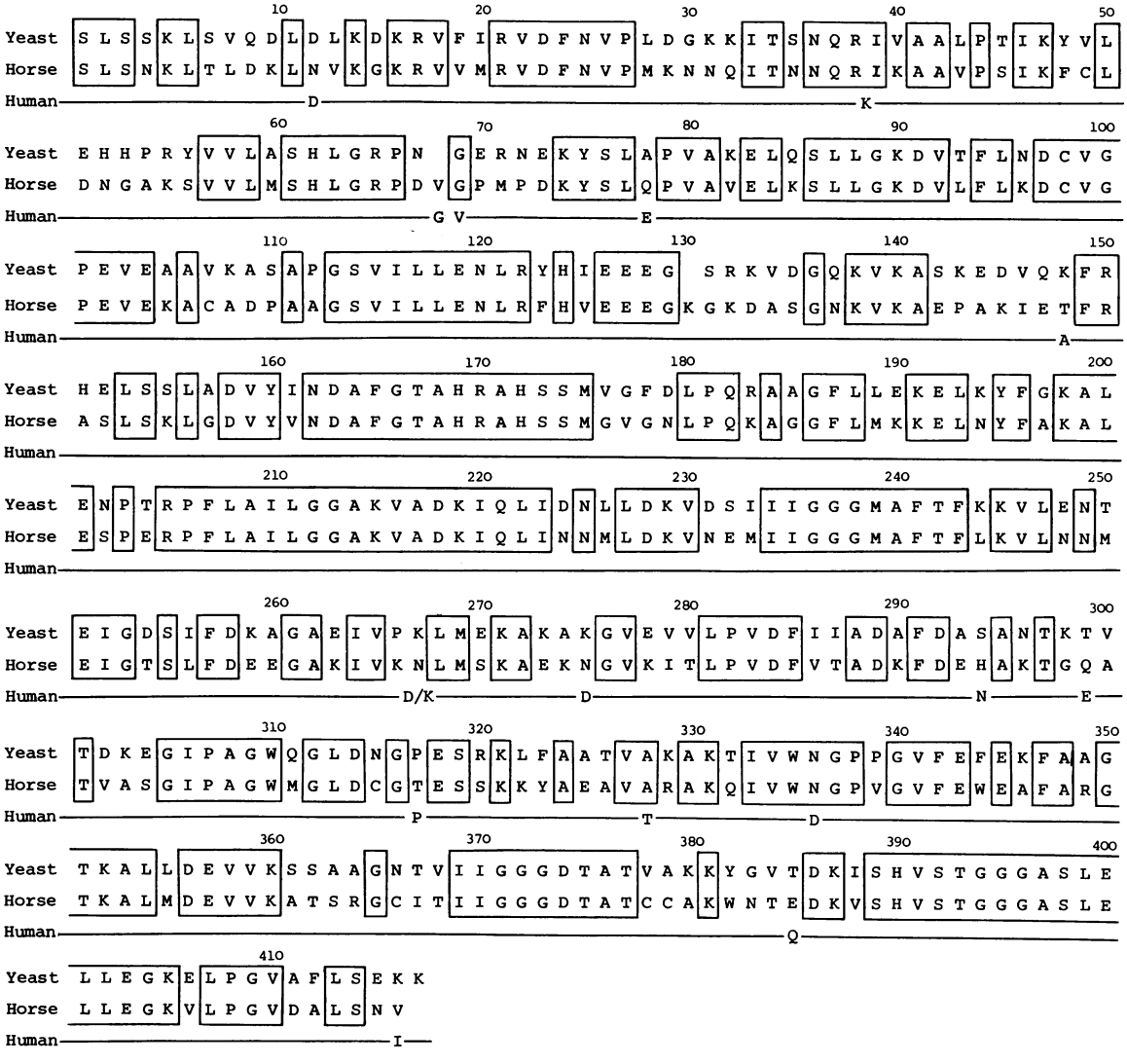

Fig. 13. *Comparison of the complete amino acid sequence of yeast phosphoglycerate kinase with those of the horse and human enzymes*

The sequence of the horse enzyme is taken from Merrett (1981), and that of the human enzyme from Huang *et al.* (1980*b*). The residues are numbered in accordance with the sequence of the horse enzyme. The sequence of the human enzyme is identical with that of the horse enzyme as indicated by the continuous line except where a different amino acid is shown. The single-letter code for amino acids is used: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

strikingly, there are two -Ile-Ile-Gly-Gly-Gly- sequences, at residues 232–236 and 367–371. It is known from the high-resoluton X-ray-crystallographic structure that arginine and histidine residues are clustered to form a possible substrate-binding site in the *N*-terminal lobe, and that the phosphate part of the ATP-binding site in the *C*-terminal lobe is lined with glycine residues (including residues 234–236). A fuller discussion of the structure, and its significance with respect to ligand binding and the mechanism of action are presented elsewhere (Watson *et al.*, 1982).

Chemical modification of yeast phosphoglycerate kinase with tetranitromethane leads to loss of activity with the concomitant nitration of one tyrosine residue, that can be protected by the presence of MgATP (Markland *et al.*, 1975; Hjelmgren *et al.*, 1976). A small chymotryptic peptide containing a nitrotyrosine residue was purified, and the sequence was established as Lys-Tyr(NO_2)-Phe-Phe-Lys (Bacharach *et al.*, 1977). This peptide is clearly homologous with residues 192–196, -Lys-Tyr-Phe-Gly-Lys-, apparently with a Phe→Gly replacement. This suggests that Tyr-193 is the reactive tyrosine residue modified by tetranitromethane.

Fragmentary pieces of evidence for the sequence of certain regions of yeast phosphoglycerate kinase have been published. Our results are in broad agreement with those reported by Fattoum *et al.* (1978) on the isolation of CNBr-cleavage fragments, except that fragment CN2 differs substantially in size (86 residues from their results instead of 148). Desvages *et al.* (1980) have reported that a reactive glutamic acid residue (*), thought to be near the *C*-terminus, is located in the sequence

-Asp(Ala,Val,Asp)Glu*-Gly-Glu-Leu-Phe-Val-Ala-Lys

This peptide does not appear to correspond to any portion of the sequence reported in the present paper. Fattoum *et al.* (1981) have determined the *N*-terminal sequences of CNBr-cleavage fragments CN3 (28 of the 64 residues) and CN4 (30 residues). Our results are substantially in agreement, although there are a few discrepancies. Fattoum *et al.* (1981) also reported the isolation and partial characterization of tryptic peptides from a citraconylated fragment, apparently corresponding to residues 1–97. These peptides, however, do not seem to correspond to any portion of the sequence of phosphoglycerate kinase determined in the present work.

Comparison with horse and human phosphoglycerate kinase

The amino acid sequences of phosphoglycerate kinase isolated from horse skeletal muscle and from human erythrocytes have been determined (Hardy *et al.*, 1981; Merrett, 1981; Huang *et al.*, 1980*a*,*b*). The three phosphoglycerate kinase sequences are compared in Fig. 13. The two mammalian sequences differ at only 15 positions. The yeast sequence is 64% identical with the mammalian sequences (268 of 417 residues for the horse, and 270 of 418 residues for the human). The three enzymes differ slightly in length (at positions 38–39, 68, 130 and 417). It is apparent that the sequence identities are not distributed randomly, nor do they correlate well with regular elements of secondary structure. For example, residues 142–151 show considerable sequence variation, but comprise helix IV in the *N*-terminal lobe (Watson *et al.*, 1982). As might be expected, most of the residues implicated in ligand-binding or as being important for catalysis are conserved.

A genetic variant of human erythrocyte phosphoglycerate kinase associated with enzyme deficiency and thermal instability has been shown to contain an Asp→Asn replacement at position 267 (Fujii *et al.*, 1980). That this change could be associated with a difference in properties seems remarkable when it is noted that horse phosphoglycerate kinase normally has an asparagine in this position, and that the yeast enzyme has a quite different residue, lysine.

Yeast phosphoglycerate kinase has been the subject of extensive study because of its catalytic importance and because it can be readily purified. There are many publications dealing with purification and characterization, kinetics, 'essential' residues and mechanism of action. Its high-resolution crystallographic structure has been solved. The yeast enzyme appears to be particularly suitable for spectroscopic studies (see, e.g., Tanswell *et al.*, 1976; Burgess & Pain, 1977), owing to the fortuitous distribution of aromatic residues. The determination of the amino acid sequence now permits the detailed interpretation of the crystallographic structure, and will contribute significantly to the interpretation and understanding of the many studies of the structure and function of phosphoglycerate kinase.

Structural features of phosphoglycerate kinase that are important for activity can now be investigated by site-specific mutagenesis experiments. It is possible to introduce a plasmid containing the phosphoglycerate kinase gene back into yeast under conditions where 50% of the total cell protein is the product of this gene (M. J. Dobson, M. F. Tuite, A. J. Kingsman & S. M. Kingsman, unpublished work). This will allow the production of large quantities of site-specific mutant phosphoglycerate kinase molecules whose structure and function can be compared with the wild-type enzyme.

We thank Dr. H. C. Watson for his generous co-operation in making the results of X-ray-crystallographic studies on yeast phosphoglycerate kinase freely available to us. The high-resolution X-ray structure was of considerable assistance during the course of the sequence work in suggesting the order of peptides and in independently checking sequences. We are grateful to Dr. J. E. Fothergill and Dr. H. C. Watson for many helpful discussions. We thank Mrs. J. Bathgate and Mr. I. Davidson for excellent technical assistance, and the Science and Engineering Research Council, Celltech and the Medical Faculty of the University of Aberdeen for financial assistance.

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