conclusion from this work. The study confirms that commercial preparations of Cibacron Blue are heterogeneous and demonstrates that by difference spectroscopy various subfractions differ in their abilities to discriminate between various conformational states of GS. It is evident that further studies along these lines should be deferred until dye preparations of high purity can be obtained.

Registry No. GS, 9023-70-5; ADP, 58-64-0; Cibacron Blue F_3GA, 12236-82-7.

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Glucose-6-phosphate Dehydrogenase from *Saccharomyces cerevisiae*: Characterization of a Reactive Lysine Residue Labeled with Acetylsalicylic Acid†

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ABSTRACT: Glucose-6-phosphate dehydrogenase from *Saccharomyces cerevisiae* (bakers' yeast) reacts with acetylsalicylic acid, and this is accompanied by inactivation and modification of essentially one lysine residue per subunit. The amino acid sequence of an 11-residue tryptic peptide containing the reactive lysine residue of the yeast enzyme is given and establishes the existence of different subgroups of glucose-6-phosphate dehydrogenases. Thus, the labeled yeast structure has few similarities to the known structure around the reactive lysine residue of the enzyme from *Leuconostoc mesenteroides*, although it has extensive similarities with a structure in the human enzyme. It is further shown that amino acid sequences around reactive lysine residues of dehydrogenases in general vary, even though similarities occur around reactive lysine residues in 6-phosphogluconate, glutamate, and glyceraldehyde-3-phosphate dehydrogenases.

The importance of glucose-6-phosphate dehydrogenase as an enzyme of the pentose phosphate pathway has been recognized for almost 50 years [cf. Horecker (1976)], but relatively few structural studies have been reported [cf. Levy (1979)]. For the "classical" enzyme from human erythrocytes, a tentative, 495-residue primary structure showing most of the subunit amino acid sequence has been published (Beutler, 1983) without experimental evidence. The sequence determined for a C-terminal heptapeptide (Descalzi-Cancedda et al., 1984) does not agree with this sequence regarding the actual C-terminal structure (Lys-Leu replacing Gly) and possibly another position (a tryptic cleavage site replacing Leu).

The enzyme from the prokaryotic organism *Leuconostoc mesenteroides* has been labeled with pyridoxal 5′-phosphate, and an eight-residue sequence including a labeled lysine residue has been established (Haghighi et al., 1982). The enzyme from this source has also been crystallized in a form suitable for X-ray crystallographic determination of the three-dimensional structure (Adams et al., 1983). The *Leuconostoc* enzyme is one of the glucose-6-phosphate dehydrogenases that utilizes either NAD^+ or $NADP^+$, in contrast to the enzymes from eukaryotic and most prokaryotic cells, which are $NADP^+$ specific or $NADP^+$ preferring (Levy, 1979). It is not known whether this difference in functional behavior occurs despite clearly related primary structures, as in the case of the

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"classical" and "allosteric" lactate dehydrogenases (Hensel et al., 1983) (which both use NAD^+), or whether the *Leuconostoc* glucose-6-phosphate dehydrogenase is a highly different protein from those requiring $NADP^+$, a possibility exemplified by the largely dissimilar glutamate dehydrogenases with requirements for NAD^+ and $NADP^+$, respectively (Austen et al., 1977).

The human glucose-6-phosphate dehydrogenases of medical importance (Luzzatto, 1979) require $NADP^+$. The same is true of the enzyme from *Saccharomyces cerevisiae* (bakers' yeast), and this enzyme has been reported to be inactivated by acetylsalicylic acid (Han et al., 1980). Such inactivation could provide a means of identifying a reactive nucleophile involved in the activity of the yeast enzyme, and could also provide a means of characterizing glucose-6-phosphate dehydrogenases from other sources. We now describe specific labeling of the yeast enzyme, characterization of the labeled residue as a modified lysine residue, and determination of the surrounding amino acid sequence.

Experimental Procedures

Materials. Crystalline glucose-6-phosphate dehydrogenase, sp act. 300–400 units/mg of protein (Sigma Chemical Co.), requires little or no purification. When necessary, it can be purified by ion-exchange (Lindblom, 1983) or affinity chromatography [cf. Hey & Dean (1983) and Craney & Goffredo (1983)]. [1-^{14}C]Acetylsalicylic acid (sp act. 26.2 Ci/mol) (Amersham International) was made up freshly by addition of nonradioactive acetylsalicylic acid sodium salt to give 100 mM and sp act. of 2 Ci/mol.

Enzyme Assay. Glucose-6-phosphate dehydrogenase activity was determined spectrophotometrically at 340 nm, in a Beckman Model Acta M-VI double-beam instrument, with cells of 1-cm light path and maintained at 37 °C. Reaction was initiated by the addition of enzyme giving a total volume of 3 mL, comprising 86 mM triethanolamine–HCl buffer, pH 7.6, 6.7 mM magnesium chloride, 1.2 mM glucose 6-phosphate, and 0.4 mM $NADP^+$. The reference cell contained the same mixture without enzyme.

Polyacrylamide Gel Electrophoresis. Proteins were denatured in 3% sodium dodecyl sulfate, 0.1% dithiothreitol, and 0.5 mM ethylenediaminetetraacetic acid (EDTA) at 95 °C, and were run on slab gels (10–16% polyacrylamide) in tris(hydroxymethyl)aminomethane (Tris)–glycine buffer, pH 9.3, containing 0.1% sodium dodecyl sulfate [cf. Laemmli (1970)], at 50 V for 30 min and then 100 V overnight. Staining was with Coomassie Brilliant Blue R-250.

Carboxymethylation. Freeze-dried protein (0.34 mM) in 6 M guanidine hydrochloride, 2 mM EDTA, 100 mM Tris, pH 8.1, and 2.2 mM dithiothreitol was incubated under nitrogen for 3 h at 37 °C. Sodium iodoacetate was then added to 8.3 mM, and anaerobic incubation was continued for 1.5 h at 37 °C, followed by dialysis and freeze-drying.

Amino Acid Analysis. Carboxymethylated glucose-6-phosphate dehydrogenase (100 nmol) was dissolved in 70% (v/v) formic acid (500 μL) and chromatographed on a column (1.6 × 200 cm) of Sephadex G-50 fine, in 30% (v/v) acetic acid. Early fractions (void volume), containing the only peak of material absorbing at 280 nm, were pooled, and samples were hydrolyzed in evacuated sealed tubes, with 6 M HCl and 0.5% (w/v) phenol at 110 °C. Amino acid analysis was then performed with a Beckman Model 121M analyzer. Peptides purified by reverse-phase high-performance liquid chromatography were hydrolyzed and analyzed without further purification.

Cyanogen Bromide Cleavage. Cyanogen bromide (0.4 g/mL) was added to the carboxymethylated protein (12 mg/mL) in 70% (v/v) formic acid, and the mixture was allowed to stand, protected from light, for 24 h at 21 °C. The solution was then concentrated to a small volume by rotary evaporation (fume hood) and separated as below.

Size Separation of Cyanogen Bromide Fragments. The products of cyanogen bromide cleavage were chromatographed on Sephadex G-50 fine (1.6 × 200 cm) in 30% acetic acid. Fractions were pooled according to absorbance at 280 nm and ^{14}C content and concentrated by rotary evaporation.

Tryptic Digestion. Material from the pool containing the largest cyanogen bromide fragments was incubated with trypsin (10 μg/mg) in 0.1 M ammonium bicarbonate, pH 8, for 4 h at 37 °C. The solution was then freeze-dried, and the tryptic peptides obtained were separated on Sephadex G-50 fine, as for the cyanogen bromide fragments above.

Purification of Labeled Tryptic Peptide. Peptides of the pooled fractions containing the ^{14}C label were purified by reverse-phase high-performance liquid chromatography on a μBondapak C_{18} column (Waters Associates) (3.9 mm × 250 mm) with linear gradients of acetonitrile in 0.1% trifluoroacetic acid. Detection was by absorbance at 214 nm and liquid scintillation counting of small samples.

Sequence Analysis. Samples of the peptide were first characterized by amino acid analysis and by manual Edman degradation for a few cycles by the (dimethylamino)azobenzene isothiocyanate double-coupling method (Chang et al., 1978; von Bahr-Lindström et al., 1982). Sequencer degradations in a Beckman 890D instrument were carried out in the presence of glycine-precycled Polybrene with a 0.1 M Quadrol peptide program (Jörnvall & Philipson, 1980). Phenylthiohydantoin derivatives were identified by reverse-phase high-performance liquid chromatography (Zimmerman et al., 1977), supplemented when appropriate with thin-layer chromatography and with liquid scintillation counting for ^{14}C.

Results

Homogeneity and Lysine Content. The enzyme gave one band on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. In some preparations, traces of two faster moving bands were also discernible. Comparison with bovine serum albumin, liver catalase, and liver glutamate dehydrogenase indicated a subunit size close to M_r 58 000. The amino acid composition of the enzyme is given in Table I and shows that the lysine content is about 40 residues per subunit.

Inactivation and Labeling with Acetylsalicylic Acid. Small-scale incubations showed that suitable rates of inactivation were achieved at pH 7–9, with 2–20 mM acetylsalicylic acid in Tris-HCl buffers at 20–25 °C. The amounts of enzyme used were small, and these acetylsalicylic acid concentrations therefore correspond to large molar excesses.

Preparative inactivation was carried out by using 68 μM enzyme with an initial concentration of 1.5 mM acetylsalicylic acid, labeled with ^{14}C in the acetyl group. After 30 min, further labeled acetylsalicylic acid was added to a final concentration of 3 mM. Decline of enzyme activity was followed as shown in Figure 1. When 6% of the original activity remained, an equal volume of 150 mM ethanolamine acetate at pH 6.0 was added to stop the reaction. Reagents were removed by dialysis against 1 mM HCl. These conditions provided about 27 ethanolamine amino groups to compete with every enzyme lysine ε-amino group and, together with the lowering of the pH, limited further labeling of the protein. The total acetylsalicylic acid added corresponded to 44 mol/mol of subunit. The inactivated, freeze-dried enzyme was dissolved, reduced with dithiothreitol, and carboxymethylated in guanidine hydrochloride as given under Experimental Procedures.

Table I: Amino Acid Composition of Bakers' Yeast Glucose-6-phosphate Dehydrogenase[a]

amino acid	mol % from acid hydrolysis	estimated no. per subunit
asparagine/aspartic acid	12.0	61
threonine	4.1	21
serine	5.9	30
glutamine/glutamic acid	10.6	54
proline	5.8	30
glycine	7.4	38
alanine	5.8	29
valine	8.0	40
methionine	1.6	8
isoleucine	4.9	25
leucine	9.3	47
tyrosine	4.2	22
phenylalanine	5.4	27
lysine	8.0	40
histidine	2.0	10
arginine	5.0	25
sum	100.0	507

[a] Values are molar ratios after hydrolysis with HCl for 20, 48, and 72 h and correction for slow release (Val, Ile) or destruction (Ser, Thr). Estimated numbers of each residue per subunit were calculated for an assumed size of 507 residues excluding cysteine and tryptophan, which were presently not determined.

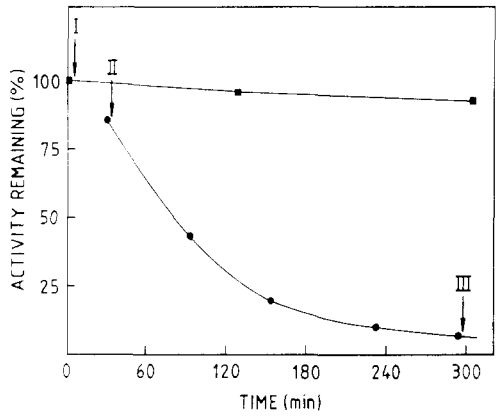

FIGURE 1: Inactivation of bakers' yeast glucose-6-phosphate dehydrogenase by acetylsalicylic acid in Tris-HCl, pH 8.5, at 21 °C. Activity was assayed at zero time (100%), and a sample was withdrawn to serve as control (■). At I, acetylsalicylic acid (1.5 mM) was added to the enzyme (68 μM), and at II, a further addition was made (to a total of 3 mM acetylsalicylic acid). Activity remaining (●) reached about 6% of initial activity after 4–5 h. Reaction was stopped (at III) by addition of excess ethanolamine acetate, pH 6.0, and dialysis.

Samples of the product were removed to determine the incorporation of label; the ^{14}C content was measured by scintillation counting and the amount of protein by amino acid analysis after hydrolysis, showing an incorporation of 1.4 acetyl moieties per subunit. Therefore, inactivation of the enzyme by excess acetylsalicylic acid was associated with a strictly limited modification of the protein.

Cleavage of Labeled Protein. To distinguish whether the limited modification resulted from high reactivity of a single residue or low reactivity of many residues, the protein was cleaved with CNBr in 70% formic acid, and the labeled peptides were purified by Sephadex G-50 chromatography in 30% acetic acid. As shown in Figure 2A, almost all of the labeled material was eluted in one peak. However, this peak, containing large peptides, also corresponds to most of the protein. Therefore, material from this pool was digested with trypsin, and the resulting peptides were similarly chromatographed on Sephadex G-50. As shown in Figure 2B, label was found in essentially only one region, corresponding to peptides of about

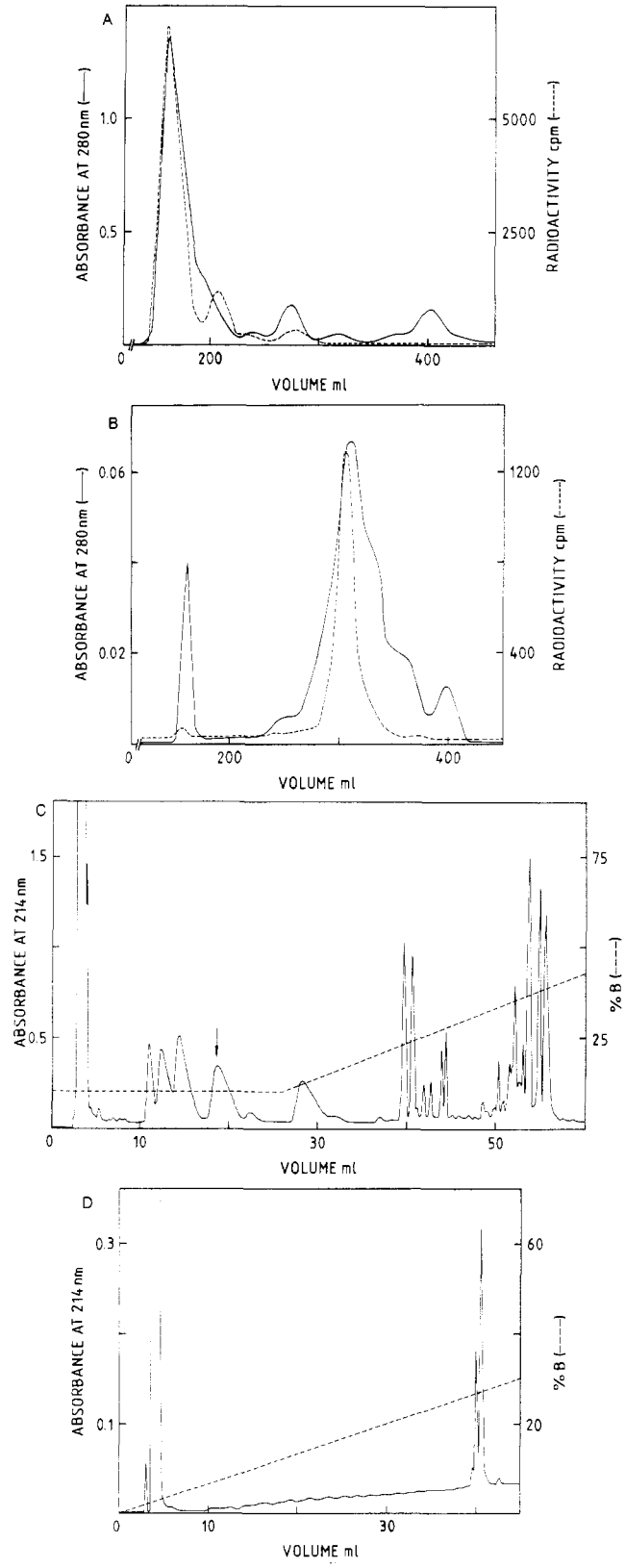

FIGURE 2: (A) Chromatography on Sephadex G-50 (1.6 × 200 cm) in 30% acetic acid of CNBr fragments from carboxymethylated bakers' yeast glucose-6-phosphate dehydrogenase that had first been selectively labeled with [1-^{14}C]acetylsalicylic acid. (B) Chromatography [as in (A)] of a tryptic digest of the main peak shown in (A). (C) HPLC of the main radioactive peak shown in (B) A total of 95.4% of the radioactive material was located in the peak marked with an arrow. (The remaining 4.6% was recovered in the peak eluting close to 30 mL.) Column was a μBondapak C_{18}; solvent A was 0.1% trifluoroacetic acid and solvent B was acetonitrile. (D) HPLC [as in (C)] of material from the peak marked in (C). Under these conditions, the radioactive peptide (large peak at right of figure) eluted slightly later than a nonradioactive impurity, allowing collection of pure material.

Table II: Bakers' Yeast Glucose-6-phosphate Dehydrogenase: Data for Composition and Amino Acid Sequence of Tryptic Peptide Containing Selectively Labeled Lysine Residue[a]

amino acid	molar ratio	amino acid	molar ratio
Asx	1.2 (1)	Leu	1.9 (2)
Glx	1.2 (1)	Tyr	0.9 (1)
Gly	1.3 (1)	Lys	1.6 (2)
Val	1.0 (1)	His	0.8 (1)
Ile	1.0 (1)		

1	2	3	4	5	6	7	8	9	10	11
Ile	Asp	His	Tyr	Leu	Gly	Lys	Glu	Leu	Val	Lys
HT	HT	HT	HT	HT	HT	HT	HT	HT	HT	HT
						RA				

[a] For the composition, values are given from acid hydrolysis without corrections for impurities, slow release, or destruction and (within parentheses) as estimated from the sequence determined. The amino acid sequence was determined with residue identification by HPLC (H), TLC (T), radioactivity (R), or amino acid analysis after back-hydrolysis (A), as indicated. Incorporation of label ([^{14}C]acetyl) into the peptide corresponded to 1.0 acetyl group per molecule, and this was present in the peptide at position 7 as ϵ-acetyllysine.

10 residues in size. These were purified further by reverse-phase high-performance liquid chromatography. Elution with acetonitrile in 0.1% trifluoroacetic acid gave almost all (95.4%) of the radioactive material in one peak, indicated by an arrow in Figure 2C; only a small amount of the radioactivity (remaining 4.6%) was recovered in one later peak (cf. legend to Figure 2C). Rechromatography of the main radioactive peak, with a shallow linear gradient of acetonitrile, removed a small amount of nonradioactive impurity, as shown in Figure 2D. These results establish that the limited modification with acetylsalicylic acid is highly specific.

Structural Analysis of Labeled Peptide. The labeled material (Figure 2D) was found to correspond to a single pure peptide, which was completely analyzed as shown in Table II. The incorporation of label corresponded to 1.0 mol of acetyl group/mol of peptide. Radioactivity in the extracts from the Edman degradations showed that the labeled residue occupied position 7 in the peptide as shown in Figure 3. Upon reverse-phase high-performance liquid chromatography, the corresponding modified phenylthiohydantoin derivative was found to elute close to the internal standard of methionine sulfone phenylthiohydantoin (relative elution time 0.99 in relation to the Met derivative). The nature of the labeled residue was established by back-hydrolysis of the thiohydantoin with $HCl/SnCl_2$, giving lysine as shown by amino acid analysis. The results define the presence of labeled N^{ϵ}-acetyllysine in position 7 of the structure characterized in Table II.

Discussion

Modification and Inactivation. The results show that reaction of bakers' yeast glucose-6-phosphate dehydrogenase with acetylsalicylic acid causes inactivation accompanied by specific labeling of a single lysine residue. The position of the label and the structure surrounding this "essential lysine" have been characterized (Figures 1 and 3). A previous report, based on kinetic findings, suggested that the inactivation by acetylsalicylic acid may result from the acetylation of the functional group essential for catalytic activity (Han et al., 1980). However, the loss of activity could be mediated directly or indirectly. Thus, the ϵ-amino group of the lysine residue might be required for the catalytic process; or, the altered charge/size of the modified group might be incompatible with productive complex formation or necessary conformational changes. This study therefore establishes a functional importance of the segment containing the lysine residue without prejudice to the assignment of the precise role.

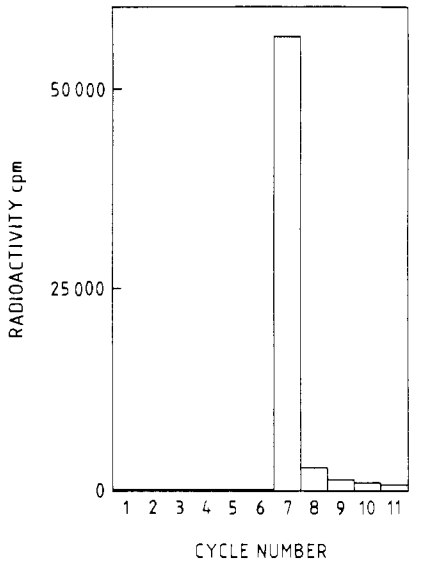

FIGURE 3: Edman degradation of the labeled tryptic peptide from bakers' yeast glucose-6-phosphate dehydrogenase established a sequence of 11 residues (Table II). The residue cleaved in cycle 7 contained the radioactive label.

Environment of Reactive Lysine Residues. Reactive lysine residues have been characterized in several dehydrogenases, and the structures around such residues are shown in Table III. Acetylsalicylic acid, the reagent used in this study, has long been known to modify some proteins [e.g., Pinckard et al. (1968)] but has seldom been used as a labeling reagent. However, the lysine residue that it specifically acetylates in human serum albumin has been characterized and is included in Table III. Interestingly, acetylsalicylic acid specifically acetylates and inactivates prostaglandin endoperoxide synthetase, but for this enzyme, the modification has been reported to be O-acetylation of a serine residue not N-acetylation of a lysine residue (van der Ouderaa et al., 1980).

Pyridoxal 5′-phosphate labels reactive lysine residues in several proteins, and a frequent occurrence of serine, threonine, lysine, and arginine has been noticed (Minchiotti et al., 1981) at positions three and four residues distant from the labeled residue (corresponding to positions −4, −3, 3, and 4 in Table III). The glyceraldehyde-3-phosphate dehydrogenase reactive lysine residue that is shown in sequence 18 of Table III is acetylated not directly by the reagent (*p*-nitrophenyl acetate) but via an active site cysteine residue (they are Lys-183 and Cys-149 in the protein sequence) (Harris & Waters, 1976). Rearrangements of label in the other enzymes may also be possible.

Reactions of lysine residues with acetylsalicylic acid, pyridoxal 5′-phosphate, trinitrobenzenesulfonic acid, and *p*-nitrophenyl acetate, summarized in Table III, involve different reagent structures and reactions. Nevertheless, the reagents have some features in common (aromatic ring and, in the first three, negative charge); furthermore, the reactions all involve nucleophilic attack by the lysine ϵ-amino group at an electron-deficient carbon (Figure 4). Comparison of all the sequences around the reactive lysine residues in Table III shows that glycine or alanine residues often occupy positions −3, −2, −1, 1, 2, or 3. Sequence similarities involve proteins likely to be related, such as the glutamate dehydrogenases [sequences 5, 6, and 7; cf. Austen et al. (1977)], but also to some extent some 6-phosphogluconate (sequence 3, Table III) and glyceraldehyde-3-phosphate dehydrogenases (sequences 17 and

Table III: Summary of Reported Structures in Dehydrogenases and in Serum Albumin around Lysine Residues Reactive with Different Reagents[a]

no.	protein	reagent	sequence −6	−4	−2	0	2	4	ref
1	glucose-6-phosphate dehydrogenase (bakers' yeast)	A	I D H	Y L G	K E L	V K			this study
2	glucose-6-phosphate dehydrogenase (*L. mesenteroides*)	P		F L L	K S P	S Y			Haghighi et al., 1982
3	6-phosphogluconate dehydrogenase (*C. utilis*)	P	L B Z	A G G	K G Z	T K			Minchiotti et al., 1981
4	6-phosphogluconate dehydrogenase (*C. utilis*)	P		T V S	K V D	H F			Minchiotti et al., 1981
5	glutamate dehydrogenase (NADP) (*N. crassa*)	P	L S M	G G G	K G G	A D			Wootton et al., 1974
6	glutamate dehydrogenase (bovine liver)	P	V P F	G G A	K A G	V K			Talbot et al., 1977
7	glutamate dehydrogenase (NAD) (*N. crassa*)	P	I P E	G G S	K G V	I L			Austen et al., 1977
8	glutamate dehydrogenase (bovine liver)	T	L E R	K F G	K H G	G T			Coffee et al., 1971
9	glutamate dehydrogenase (bovine liver)	T	Q E S	L E R	K F G	K A			Coffee et al., 1971
10	glutamate dehydrogenase (bovine liver)	P	S E K	Q L T	K S N	A P			Talbot et al., 1977
11	glutamate dehydrogenase (NAD) (*N. crassa*)	P	E L R	R L A	K A R	A M			Austen et al., 1977
12	glutamate dehydrogenase (NAD) (*N. crassa*)	P	A S T	Q Q R	K N K	D I			Austen et al., 1977
13	glutamate dehydrogenase (NAD) (*N. crassa*)	P	I V K	S R S	K E A	Y Q			Austen et al., 1977
14	glutamate dehydrogenase (NAD) (*N. crassa*)	P		K A T	K N T	K			Austen et al., 1977
15	glutamate dehydrogenase (NAD) (*N. crassa*)	P		L E	K K				Austen et al., 1977
16	glyceraldehyde-3-phosphate dehydrogenase (rabbit muscle)	P	V D G	P S G	K L W	R D			Forcina et al., 1971
17	glyceraldehyde-3-phosphate dehydrogenase (rabbit muscle)	P	A S T	G A A	K A V	G K			Forcina et al., 1971
18	glyceraldehyde-3-phosphate dehydrogenase (pig muscle)	N	A I T	A T Q	K T V	D G			Harris & Waters, 1976
19	glyceraldehyde-3-phosphate dehydrogenase (bakers' yeast)	N	S S T	G A A	K A V	G K			Jones & Harris, 1972
20	glyceraldehyde-3-phosphate dehydrogenase (bakers' yeast)	N	A A K	A V G	K V L	P E			Jones & Harris, 1972
21	glyceraldehyde-3-phosphate dehydrogenase (bakers' yeast)	N	K A A	A E G	K L K	G V			Jones & Harris, 1972
22	malate dehydrogenase (pig heart mitochondria)	P			K P G	M T			Wimmer & Harrison, 1975
23	alcohol dehydrogenase (horse liver)	P	V D I	N K D	K F A	K A			Sogin & Plapp, 1975
24	albumin (human serum)	A	S A K	Q R L	K C A	S L			Walker, 1976

[a] Reagent designations are as follows: A, *O*-acetylsalicylic acid; P, pyridoxal 5′-phosphate; T, 2,4,6-trinitrobenzenesulfonic acid; N, *p*-nitrophenyl acetate. Amino acid residues are represented by one-letter symbols, and sequences are centered around the reactive lysine residue placed in position 0.

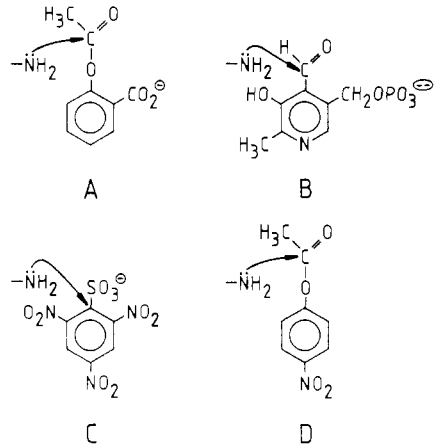

FIGURE 4: Labeling of a protein by modification of the ϵ-amino group ($-NH_2$) of a lysine residue with acetylsalicylic acid (A), pyridoxal phosphate (B), trinitrobenzenesulfonate (C), or nitrophenyl acetate (D) involves in each case nucleophilic attack by the amino group at an electron-deficient carbon. These reagents are not closely similar in structure, but all contain an aromatic ring, and three of the reagents (A–C) are negatively charged. As shown, the reacting carbon atoms also have different positions relative to the rings.

19, as shown in Table IV). These similarities involve proteins not only from widely different organisms but also with distinct substrates and reactions (decarboxylation, deamination, or phosphorylation).

Relationships between Widely Different Glucose-6-phosphate Dehydrogenases: Likely Position of Reactive Lysine Residue. Human erythrocyte glucose-6-phosphate dehydrogenase has been labeled on a reactive lysine residue (Camardella et al., 1981) and initial studies (but not the amino acid sequence) of a labeled fragment have been reported (Camardella et al., 1982). Interestingly, the tentative primary structure for this human enzyme (Beutler, 1983) contains a segment clearly homologous to the presently determined

Table IV: Comparison of Sequences around Lysine Residues in 6-Phosphogluconate Dehydrogenase (3), Glutamate Dehydrogenases (5–7), and Glyceraldehyde-3-phosphate Dehydrogenases (17 and 19)[a]

amino acid sequence	sequence no. in Table III
Leu Asx **Glx** Ala **Gly Gly Lys Gly** Glx Thr **Lys**	3
Leu Ser Met **Gly Gly Gly Lys Gly Gly** Ala Asp	5
Ile **Pro Glu Gly Gly** Ser **Lys Gly Val** Ile Leu	7
Val **Pro** Phe **Gly Gly Ala Lys Ala Gly** Val **Lys**	6
Ala **Ser Thr Gly Ala Ala Lys Ala Val Gly Lys**	17
Ser **Ser Thr Gly Ala Ala Lys Ala Val Gly Lys**	19

[a] Where positions (relative to the reactive lysine) are occupied by identical residues in two or more sequences, these residues are shown in bold type.

```
                    93  94  95  96  97  98  99 100 101 102 103
Human erythrocyte   Ile-Asp-His-Tyr-Leu-Gly-Lys-Glu-Asp-Glu-Ile

Bakers' yeast       Ile-Asp-His-Tyr-Leu-Gly-Lys*-Glu-Leu-Val-Lys
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FIGURE 5: Comparison of the presently determined structure around the reactive lysine residue (shown by an asterisk) of bakers' yeast glucose-6-phosphate dehydrogenase (lower line) and the amino acid sequence reported to occur (Beutler, 1983) in human erythrocyte glucose-6-phosphate dehydrogenase (upper line) at the positions indicated by the top numbers. It is not known whether lysine-99 in the human enzyme is reactive, but somewhere, the erythrocyte protein does contain a reactive lysine residue (Camardella et al., 1981).

structure with the reactive lysine residue in the yeast enzyme (eight residue identities, including actual lysine position; cf. Figure 5). It therefore appears likely that the yeast and human erythrocyte enzymes have related structures and that the reactive lysine residue occupies similar positions in both enzymes.

However, the amino acid sequence containing the reactive lysine residue of *Leuconostoc* glucose-6-phosphate de-

hydrogenases shows differences more obvious than similarities in relation to the yeast enzyme (sequences 1 and 2, Table III). Comparing the eight residues known for both sequences, it is seen that, apart from the lysine residues placed at position 0, the only identity is the leucine residues at position −2. A structural relationship with the *Leuconostoc* enzyme is therefore not yet established (the dissimilarities may indicate that another region is labeled or that the enzymes are very different), whereas the comparisons show that the evolutionarily far-separated yeast and human enzymes are clearly related. The results also position a labeled residue and couple it to enzyme inactivation. It appears possible that further investigation of these enzymes will facilitate assignments of structure–function relationships for glucose-6-phosphate dehydrogenases, as well as possibly evolutionary and metabolic relationships, in the same way as earlier work also starting with enzyme inactivations and labelings (Jeffery et al., 1981) did in the case of polyol and alcohol dehydrogenases (Jörnvall et al., 1981; Jeffery & Jörnvall, 1983).

Registry No. Glucose-6-phosphate dehydrogenase, 9001-40-5; lysine, 56-87-1; acetylsalicylic acid, 50-78-2.

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