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Sequence identity between a lysine-containing peptide from *Leuconostoc mesenteroides* glucose-6-phosphate dehydrogenase and an active site peptide from human erythrocyte glucose-6-phosphate dehydrogenase

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Peptides recently isolated and sequenced from a bacterial (*Leuconostoc mesenteroides*) glucose-6-phosphate dehydrogenase are remarkably homologous to an active site region of the human erythrocyte enzyme, although the enzymes differ in their overall amino acid composition and kinetic properties. The computer program ALIGN, used to determine the best alignment between the two enzyme sequences, gives match-scores which are statistically highly significant.

Glucose-6-phosphate dehydrogenase; Sequence homology; (Human erythrocyte, *Leuconostoc mesenteroides*)

1. INTRODUCTION

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:$NADP^+$ 1-oxidoreductase, EC 1.1.1.49) from *Leuconostoc mesenteroides* catalyzes the oxidation of glucose 6-phosphate by either $NADP^+$ or NAD^+, both in vitro [1] and in vivo [2]. This enzyme serves two distinct metabolic functions, depending on whether it utilizes $NADP^+$ or NAD^+ [2]. Mechanisms whereby the choice of coenzyme utilization are regulated have been proposed [3,4] and depend on differences between the steady-state kinetic mechanisms of the NADP- and NAD-linked reactions [5]. Experiments have been reported that document differences between the conformations of the *L. mesenteroides* G6PD-$NADP^+$ and NAD^+ complexes [6,7].

The dual nucleotide coenzyme specificity of *L. mesenteroides* G6PD contrasts sharply with the $NADP^+$ specificity of most other G6PDs under physiological conditions [8]. These NADP-preferring G6PDs catalyze the first reaction of the hexose monophosphate shunt pathway which generates pentoses required for the biosynthesis of nucleic acids and several coenzymes, and produces NADPH required for a variety of biosynthetic and detoxication reactions. Human erythrocyte G6PD is of special interest because of the very large number of variants found in the human population and their correlation with clinical problems such as various hemolytic abnormalities [9]. Despite the importance of the human G6PD, few structural studies have been reported on this enzyme [8,10,11].

As an extension of our previous investigations of

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Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; HPLC, high performance liquid chromatography; PTH, phenylthiohydantoin

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the *L. mesenteroides* G6PD and as part of our interest in understanding the structural basis of its dual nucleotide specificity, we are determining the enzyme's complete amino acid sequence and its three-dimensional structure using X-ray crystallographic techniques [12]; preliminary X-ray studies on the rat liver enzyme have also been reported [13].

Two recent reports of the complete amino acid sequence of human G6PD [14,15] are in agreement for 479 residues from the C-terminus with only 3 exceptions, but the sequence of Takizawa et al. [15] contains 52 additional residues N-terminal to the first residue of the sequence of Persico et al. [14]. A reactive lysine residue has been labelled on G6PD from *L. mesenteroides* [16], baker's yeast [17] and human erythrocytes [18,19], and fragments containing this lysine have been sequenced for all but the human enzyme. The tentative amino acid sequence of the human enzyme proposed by Beutler [11] contains a fragment extensively homologous to the yeast reactive lysine peptide [17] and the homology is now confirmed in the complete sequence of the human enzyme. The amino acid sequence of the *L. mesenteroides* G6PD peptide containing the reactive lysine showed more differences from, than similarities to, either of the other peptides and it was therefore concluded that the *L. mesenteroides* enzyme is very different from the human enzyme. On the basis of the recent amino acid sequencing results, we wish to point out that this is not so and that remarkable similarity exists between the *L. mesenteroides* and the human G6PDs in the active site region of the human enzyme.

2. MATERIALS AND METHODS

L. mesenteroides G6PD was obtained from Cooper Biomedical, Inc. Cyanogen bromide cleavage [20] yielded 4–5 broad peaks when the peptides were subjected to HPLC using TSK250 and TSK125 gel filtration columns in tandem. These were resolved into individual peptides using a Waters μBondapak C_{18} column. During the overall sequencing strategy, which will be described elsewhere, several peptides were obtained from native *L. mesenteroides* G6PD by cleavage with various proteases, one of which was thermolysin. The sequence of one of the thermolytic peptides, T-10, overlapped with that of CNBr peptide C-3b. Isolated peptides were sequenced by Edman degradation using an Applied Bio-Systems gas-phase model 470A protein/peptide sequencer [21]. PTH-amino acids were identified by a minor modification of the method of Johnson et al. [22].

We have used a computer program ALIGN [23] based on the algorithm of Needleman and Wunsch [24] to find the best alignment between two amino acid sequences and its statistical significance. An alignment score is calculated by taking the difference between the maximum match score and the maximum match score for average random permutations of the two sequences and dividing by the standard deviation of the random scores [24]. Insertions and deletions may be accommodated by allowing a break in either sequence. However, with each break in the sequence, the match score is reduced by an amount (break penalty parameter) specified by the user. If the alignment score is more than 3 standard deviations, with or without allowing breaks, the sequence homology can be considered statistically significant.

3. RESULTS AND DISCUSSION

The sequence of *L. mesenteroides* G6PD peptides C-3b and T-10 revealed that there was significant overlap between the C-terminal end of the former and the N-terminus of the latter, thus extending the sequence of C-3b to give a 42-residue peptide. The alignment of this sequence to that of the human erythrocyte enzyme [14], as shown in fig.1, gave the alignment score shown in table 1. A maximum alignment score of 15.2 standard deviations was found for this peptide without necessitating any breaks in the sequence. This clearly shows a high degree of homology between the *L. mesenteroides* and human G6PDs. As is seen from fig.1, the 42 residues of the *L. mesenteroides* enzyme align best with residues 133–174 of the human enzyme. Five consecutive residues starting from 134 are identical, only 4 non-consecutive residues match between 139 and 161, a region in which there are several replacements but no insertions or deletions. Finally and most interestingly, the last 13 residues of this peptide match entirely with residues 162–174 of the human enzyme, the region containing the reactive lysine (residue 169) in this enzyme. The

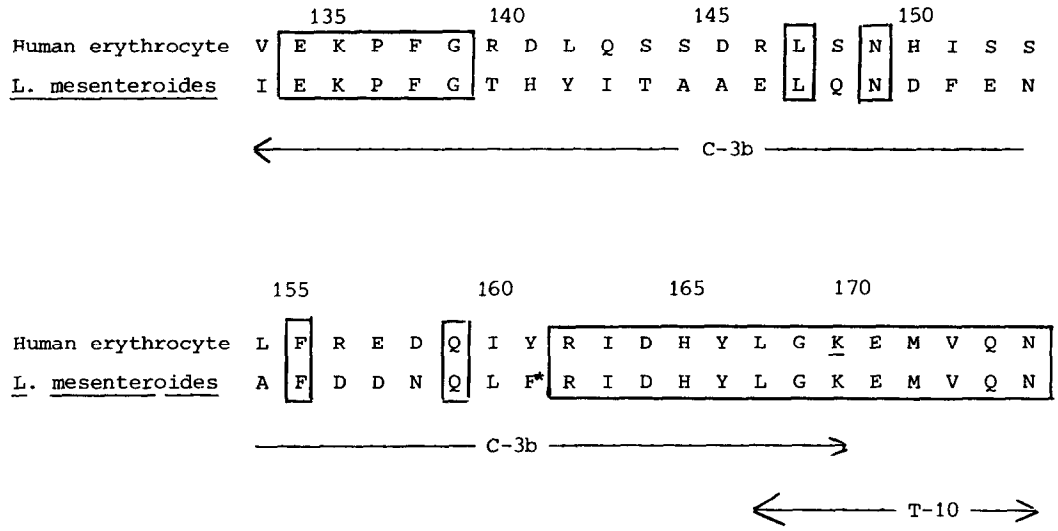

Fig.1. *L. mesenteroides* G6PD peptides. C-3b and T-10 aligned with human erythrocyte G6PD sequence. Reactive Lys-169 is underlined. * Sequence contains either L or F at this position. Both give very similar alignment scores.

8-residue peptide thought to contain the essential lysine in *L. mesenteroides* G6PD [16] gives alignment scores close to zero with both the human sequences [14,15] suggesting that this region which might be important in the activity of the *L. mesenteroides* enzyme is not conserved in the human enzyme. The 11 residues around the reactive lysine of the human and yeast enzymes, together with the homologous peptide fragment of *L. mesenteroides* G6PD are shown in fig.2. These 11 residues are entirely identical in the *L. mesenteroides* and human enzymes and almost identical in the yeast enzyme. A sequence identical for these 11 residues with that of the human and *L. mesenteroides* enzymes has also been reported for *Drosophila melanogaster* (Manning quoted in [14]).

Table 1

Alignment scores for the *L. mesenteroides* G6PD peptides

Peptide	No. of residues	Alignment score (in SD units)
C-3b	37	11.6
T-10	8	8.5
C-3b and T-10	42	15.2

The *L. mesenteroides* G6PD differs from the human enzyme in its amino acid composition and kinetic properties. The *L. mesenteroides* enzyme can utilize both $NADP^+$ and NAD^+ for its catalytic reaction, whereas the human enzyme is highly specific for $NADP^+$; the *L. mesenteroides* enzyme contains no cysteines whereas the human enzyme contains 18 of these residues and there are large differences in the contents of several other amino acids; unlike the human enzyme, *L. mesenteroides* G6PD is not inhibited by steroids [8]. Although these two enzymes are distant in

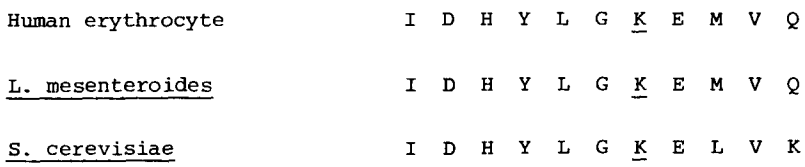

Fig.2. Peptides containing the reactive lysine (underlined) of various G6PDs.

evolutionary terms, the sequence comparisons reported here strongly suggest that the two enzymes are structurally related.

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REFERENCES

[1] De Moss, R.D., Gunsalus, I.C. and Bard, R.C. (1953) J. Bacteriol. 66, 10–16.
[2] Kemp, R.G. and Rose, I.A. (1964) J. Biol. Chem. 239, 2998–3006.
[3] Levy, H.R. and Daouk, G.H. (1979) J. Biol. Chem. 254, 4843–4847.
[4] Levy, H.R., Daouk, G.H. and Katopes, M.A. (1979) Arch. Biochem. Biophys. 198, 406–413.
[5] Levy, H.R., Christoff, M., Ingulli, J. and Ho, E.M.L. (1983) Arch. Biochem. Biophys. 222, 473–488.
[6] Grove, T.H., Ishaque, A. and Levy, H.R. (1976) Arch. Biochem. Biophys. 177, 307–316.
[7] Haghighi, B. and Levy, H.R. (1982) Biochemistry 21, 6421–6428.
[8] Levy, H.R. (1979) Adv. Enzymol. Relat. Areas Mol. Biol. 48, 97–192.
[9] Luzzatto, L. and Battistuzzi, G. (1985) Adv. Human Genet. 14, 217–329.
[10] Luzzatto, L. and Testa, U. (1978) Curr. Top. Haematol. 1, 11–70.
[11] Beutler, E. (1983) in: Metabolic Basis of Inherited Diseases (Stanbury, J.B. et al. eds) 5th edn, pp.1629–1653, McGraw-Hill, New York.
[12] Adams, M.J., Levy, H.R. and Moffat, K. (1983) J. Biol. Chem. 258, 5867–5868.
[13] Ammon, H.L., Murphy, K.C., Bhattacharjee, S.K., Szepesi, B. and Hansen, R.J. (1983) J. Mol. Biol. 171, 233–236.
[14] Persico, M.G., Viglietto, G., Martini, G., Toniolo, D., Paonessa, G., Moscatelli, C., Dono, R., Vulliamy, T., Luzzatto, L. and D'Urso, M. (1986) Nucleic Acids Res. 14, 2511–2522.
[15] Takizawa, T., Hunag, I.-Y., Ikuta, T. and Yoshida, A. (1986) Proc. Natl. Acad. Sci. USA 83, 4157–4161.
[16] Haghighi, B., Flynn, T.G. and Levy, H.R. (1982) Biochemistry 21, 6415–6420.
[17] Jeffery, J., Hobbs, L. and Jornvall, H. (1985) Biochemistry 24, 666–671.
[18] Camardella, L., Romano, M., Di Prisco, G. and Descalzi-Cancedda, F. (1981) Biochem. Biophys. Res. Commun. 103, 1384–1389.
[19] Camardella, L., Romano, M., Di Prisco, G. and Descalzi-Cancedda, F. (1982) Abstr. Intr. Congr. Biochem. 12, 308.
[20] Gross, E. (1967) Methods Enzymol. 11, 238–255.
[21] Hewick, R., Hunkapiller, M., Hood, L. and Dryer, W. (1981) J. Biol. Chem. 256, 7990–7997.
[22] Johnson, N.D., Hunkapiller, M. and Hood, L. (1978) Anal. Biochem. 100, 335–338.
[23] Orcutt, B.C., Dayhoff, M.O., George, D.G. and Baker, W.C. (1984) ALIGN, Alignment Score Program of the Protein Identification Resource, National Biomedical Research Foundation, Georgetown University Medical Center, Washington, DC, USA.
[24] Needleman, S.B. and Wunsch, C.D. (1979) J. Mol. Biol. 48, 443–453.