# SEQUENCE HOMOLOGIES AMONG PYRIDINE NUCLEOTIDE-LINKED DEHYDROGENASES: POSSIBLE PARTIAL GENE DUPLICATIONS IN GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

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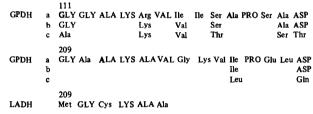
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# 1. Introduction

Homologous amino acid sequences neighbouring the essential cysteine residues of several dehydrogenases including glyceraldehyde-3-phosphate dehydrogenase (GPDH) suggest a possible common evolutionary ancestry [1-3], although, for such relatively short peptide homologies, the possibility of convergent evolution cannot be discounted. Although glutamate dehydrogenase (GDH) has no essential cysteine residues, the sequences surrounding the essential lysine 126 in GDH and lysine 212 in GPDH exhibit significant homology [4, 5]. Both these lysines react readily with pyridoxal-5'-phosphate.

GDH possesses within a single subunit both a catalytic site and one or more regulator sites specific for the catalytic coenzymes and their structural analogues ADP and GTP. Engel [6] suggested that the evolution of such regulator sites, perhaps better described as "homosteric" than "allosteric", might be most easily explained by partial gene duplication. The apparent duplication in GDH of an extensive region containing lysine 126 was adduced in support of this hypothesis.

The recent report [7] that GPDH also possesses a separate regulator site for ATP, ADP and NAD therefore prompted a re-examination of the amino acid sequence of this enzyme. The present paper reports evidence of a partial gene duplication in GPDH analogous to that found in GDH.



Register shift = 98 residues.

Fig. 1. Internal comparisons of 2 sections of the amino acid sequence of GPDH. The sequences [8, 9] of pig (a), lobster (b) and yeast (c) GPDH are shown on separate lines where they differ. Capital letters are used to emphasise the internal homologies where they occur. A small segment of the sequence of LADH [10] is also shown for comparison.

# 2. Results

Fig. 1. shows an alignment of 2 sections of the GPDH sequences published by Harris and his colleagues [8, 9]. One of these, containing lys 212, has been shown previously [4-6] to be homologous with the duplicated sequence in GDH. In these two highly-conserved pieces of sequence 6 positions out of 14 are occupied by identical residues.

The same figure also shows part of the sequence of horse liver alcohol dehydrogenase [10]. Jörnvall detected significant in-register homology between GPDH and LADH from residues 1 to 60 but not beyond. In view of the homologies between GDH and GPDH it is perhaps significant that LADH also

```
Homology 2A
   VAL VAL ASP LEU Thr Cys Arg Leu
                                       Glu LYS
                         VAL Arg
                                       Gly
                         VAL Lys
   VAL VAL ASP LEU Met VAL His Met Ala Ser
ь
       He
                     Leu Lys
                                  Met Gln LYS
       VAL
                     Val Glu
                                 Val/Ile Ala LYS
           Register shift = 82 residues.
                                                           Homology 2B
   PRO ALA SER Thr Gly Ala Ala LYS ALA VAL Gly LYS Val Ile Pro GLU
       Ser
                                                           Len
   249
  PRO ALA Lys Tyr Asp Asp lie LYS Lys VAL Val LYS Gin Ala Ser GLU
                                                               Ser
  Glu Cys SER
                        Asp
                                     ALA Ala Met
                                                       Thr
                                     Lys VAL Val
       Thr Thr
                         Glu
                                                       Ala
                                                               Ala
                       Register shift = 44 residues.
                Homology 2C
   109
   LEU Lys Gly Gly ALA LYS
h
   Phe Lys Gly
С
  Ile
       Asp Ala
   LEU Ala Pro Leu ALA LYS
b
               Val
               Leu
  Register shift = 45 residues.
                                                Homology 2D
   VAL ASP Gly Phe GLY Arg Ile Gly Arg LEU Val THR Arg -
ь
   II.E
С
   HE
                                                     Ala Gly
   VAL Glu Ser Thr GLY Val Phe Thr Thr Met Glu Lys
                                Thr Thr Ile Glu Lys
ь
   VAL Glu
                                Lys Glu LEU ASP THR
                                                         Gln
   ILE ASP
c
   18
   ALA Ala PHE Asn Ser GLY Lys Val Asp Ile
а
           LEU Ser Cys GLY - Ala Gln VAL
                                                         Val
b
   ALA
            LEU Ser Arg Pro Asx Val Glx VAL
                                                      SER/THR
С
   Ile
   109
                                                         SER
   ALA His LEU Lys Gly GLY Ala Lys Arg VAL Ile
                                                  He
                                              VAL
                                                         SER
ь
   ALA.
           PHE Lvs Glv
                                     Lys
                                     Lys
            He
               Asp Ala
                                              VAL
                                                         THR
c
   Lys
   Asn, Asp PRO Phe Ile
                       ASP Leu His Tyr Met VAL
                                Glu
                                        Met VAL
                       Ala
С
   Asx Asx PRO
                       Asx
                               Asp
                                        Ala Ala
   120
       Ala PRO Ser Ala ASP Ala Pro Met Phe VAL
                    Ala ASF
                    Ser Thr
           Register shift = 88-89 residues
                              Homology 2E
 Leu GLY TYR Thr Glu Asp Gln VAL Val Ser
                          Asp
 315
 Phe GLY TYR Ser Asn Arg Val VAL Asp Leu
                  Gln
                              [le
                              VAL
                  Thr
          Register shift = 44 residues.
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Fig. 2. Further internal comparisons of the amino acid sequence of GPDH displayed as in fig. 1.

has lysine at position 212. LADH has no lysine at position 114, but there is lysine at position 113 (cf. fig. 1).

Whilst the partial gene duplication suggested by the evidence in fig. 1 may be related to the presence of regulator sites, attention must also be drawn to several other fragments of apparent sequence homology shown in fig. 2. In most of the positions not showing identity the alterations can be accounted for in terms of single base changes or by a simple chain of such mutations in those positions where the pig, lobster and yeast enzymes differ.

A further homology between beef liver GDH and pig liver GPDH has also been found and is shown in fig. 3. Five of the ten residues compared are identica The sequence of the 'B' peptide from *Neurospora* NADP-linked GDH [11] bears out this homology, adding an identity in a sixth position. With the exception of the change of threonine to valine, which requires two nucleotide base changes, all replacements can be accounted for by single base changes in the gen ome. It is interesting that the beef liver GDH fragment shows greater similarity to the fragment from GPDH than to the Neurospora GDH fragment.

### 3. Discussion

The homologies presented above contribute furthe evidence that the pyridine nucleotide-linked dehydrogenase share common ancestry. Without further information from chemical modification studies and 3-dimensional structure determinations one cannot be confident that the amino acid duplications now documented in both GDH and GPDH represent the duplication of functional binding sites. On the evidence to date, however, it is attractive to speculate that duplication of a lysine-containing sequence in an ancestral protein is reflected in the present structures of GDH, GPDH and LADH, and that, while

Fig. 3. Comparison of the amino acid sequences of GDH from beef liver [12] and *Neurospora* [11] with those of GPDH [8, 9 displayed as in fig. 1 and 2.

the duplicated sequence is perhaps non-functional in LADH, it has evolved a regulatory function in GDH and GPDH.

The different register shifts shown in figs. 1 and 2 may be an indication that the observed homologies have arisen by chance or through convergent evolution. On the other hand they may reflect repeated partial gene duplication in the evolution of these proteins. Homologous regions of DNA in the gene, once established, would facilitate the recurrence of such duplications. In this connection it may be noted that:

- i) sequences 111... and 209... are homologous
- each of these sequences is homologous with a sequence nearer the carboxyl end, the register shift being in one case 44, and, in the other, 45, residues.
- iii) homology 2D, which again involves residues 111-124, requires a register shift of 88, exactly twice 44.
- iv) homology 2E, between 2 sequences near the carboxyl terminal, again requires a register shift of 44 residues.

These relationships may reflect random coincidence. Nevertheless it seems clear that the available dehydrogenase sequences should be subjected to a close and systematic scrutiny by the methods developed by Fitch [13]. Such a study should provide good

evidence for or against the hypothesis of repeated duplication and is now in progress.

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