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The relationships between transketolase, yeast pyruvate decarboxylase and pyruvate dehydrogenase of the pyruvate dehydrogenase complex

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The amino acid sequences of four thiamine pyrophosphate-requiring enzymes were aligned with the published amino acid sequence of the transketolase of *Hansenula polymorpha*. Sequences of the combined α and β subunits of the E_1 enzyme of the pyruvate dehydrogenase complexes of *Homo sapiens* and *Bacillus stearothermophilus* aligned well with the transketolase while the E_1 of the pyruvate dehydrogenase complex of *Escherichia coli* aligned easily provided a non-aligning segment of 77 amino acids was omitted. The non-acetylating pyruvate decarboxylase of *Saccharomyces cerevisiae* could only be aligned if the sequence was cut in two with the C-terminus corresponding to the N-terminus of the other TPP-dependent enzymes. Using the published 2.5 Å resolution of the X-ray crystal structure of *Saccharomyces cerevisiae* transketolase as a template we show that a hydrophobic region of the β-subunit of the PDH $E_1\alpha\beta$ enzymes likely contains a binding site for the thiazolium ring of TPP and key motifs are retained in common by all the TPP-dependent enzymes considered, which are essential for catalysis

Pyruvate dehydrogenase, Transketolase, Thiamine pyrophosphate; Thiazolium binding

1. INTRODUCTION

The investigation of the structure and function of thiamine pyrophosphate (TPP)-binding enzymes was assisted by the recent publication of the X-ray crystal structure at 2.5 Å resolution of the yeast, *Saccharomyces cerevisiae*, transketolase [1]. This showed how the structural binding site motif $GDGX^{26/27}NN$, previously identified by sequence homology studies of TPP-binding enzymes [2], is actually concerned primarily with binding the pyrophosphate end of the TPP molecule using an incorporated calcium ion. The thiazolium ring structure, on the other hand, is held in a hydrophobic pocket at the other end of the transketolase monomer such that each dimeric functional transketolase molecule binds two molecules of TPP in a head-to-tail fashion [1].

While this provides a working model that may encompass all TPP-dependent enzymes, two remaining problems have to be answered with respect to the pyruvate decarboxylating enzymes that are TPP-dependent. What is the function of the β subunit in the $\alpha_2\beta_2$ tetrameric pyruvate dehydrogenase (PDH) E_1 present in the pyruvate dehydrogenase complex of yeast, bacteria and higher animals and plants, and how does this relate to the structure of the family of TPP-requiring decarboxylase enzymes recently compared by Green [3], which seem to be a different evolutionary group.

Here we use the published *Hansenula polymorpha* transketolase amino acid sequence as a template on which to align important functional motifs in a variety of pyruvate decarboxylating enzymes. This shows that the β subunit of the pyruvate dehydrogenase enzymes is involved in TPP binding and that the non-acetylating pyruvate decarboxylase group of enzymes has the same functional groups arranged in an orientation which suggests a re-arrangement of the ancestral gene for this family.

2. EXPERIMENTAL

Since the amino acid sequence of *S. cerevisiae* transketolase was not published with the X-ray structure, we used the sequence of *H. polymorpha* [4] as a template. We compared its sequence with four other sequences. The first one was a combined human pyruvate dehydrogenase $E_1\alpha\beta$ protein constructed by removing the leader sequences from the $E_1\alpha$ [5,6] and $E_1\beta$ [7,8] human subunits and joining them N-terminus (β) to C-terminus (α). Homology searches using Pearson–Lipmann algorithms [9] consistently misaligned these proteins with respect to their functional motifs, or aligned them with significant homology only for a short stretch of 30–40 amino acids. On the other hand, a very simplistic alignment of the proteins by eye, starting at residue number 1 in each case, gave an alignment with widely spaced, but consistent homology, 68 residues being identical (Fig. 1). The second sequence aligned was the $E_1\alpha\beta$ subunits of the pyruvate dehydrogenase of *Bacillus stearothermophilus*, another $\alpha_2\beta_2$ tetramer [10] again organized as a combined single $E_1\alpha\beta$ protein sequence. Not surprisingly these two proteins had 98 residues in common. The third sequence was the non-acetylating pyruvate decarboxylase of *S. cerevisiae* [11]. In this case alignment was achieved again by eye, the initial alignment being made by the juxtaposition of the consensus sequence

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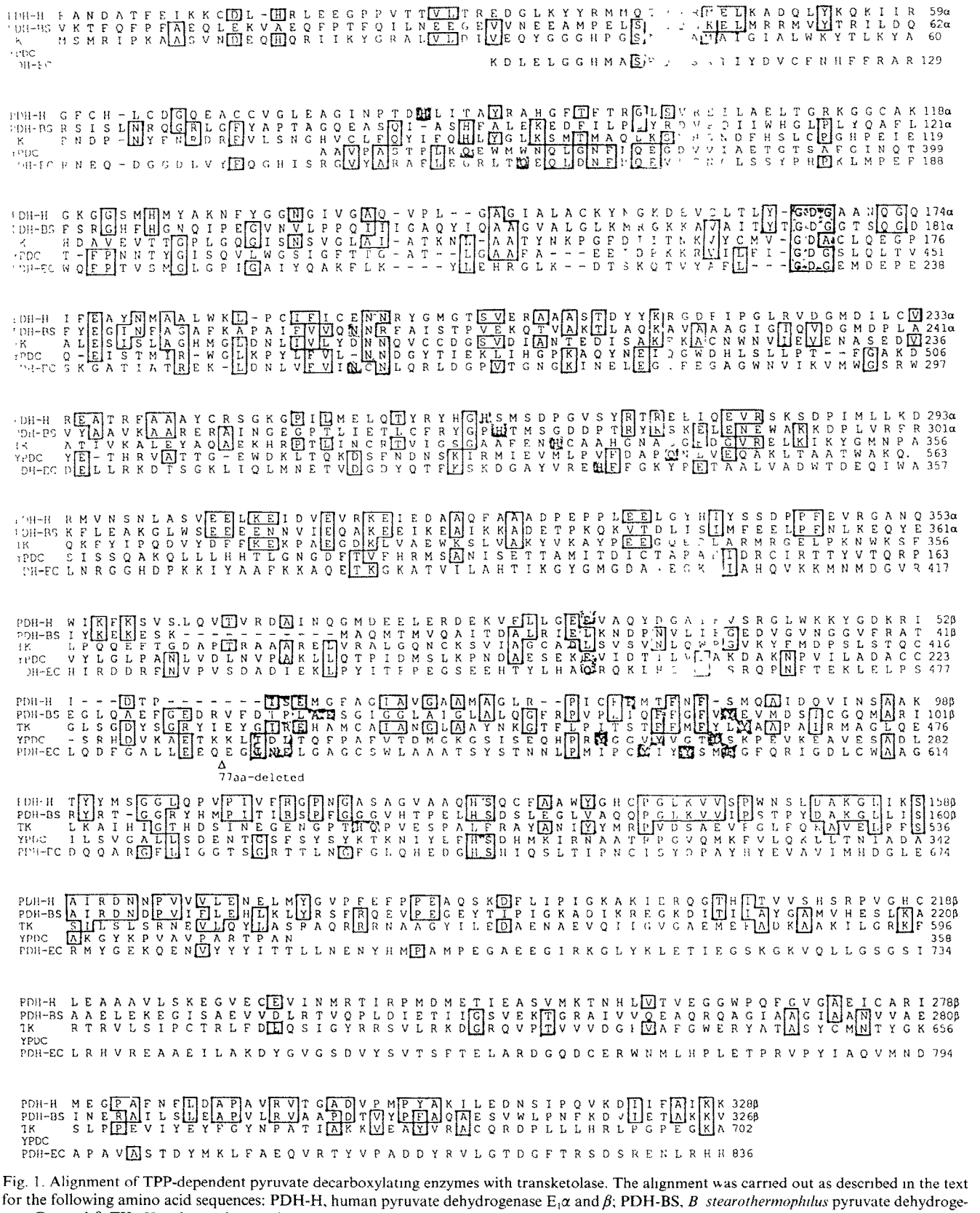

Fig. 1. Alignment of TPP-dependent pyruvate decarboxylating enzymes with transketolase. The alignment was carried out as described in the text for the following amino acid sequences: PDH-H, human pyruvate dehydrogenase $E_1\alpha$ and β; PDH-BS, *B stearothermophilus* pyruvate dehydrogenase $E_1\alpha$ and β; TK, *H polymorpha* transketolase; YPDC, *S cerevisiae* pyruvate decarboxylase (in two segments 103–357 and 358–563), PDH-EC, *E coli* pyruvate dehydrogenase E_1. A 77-amino acid segment 478–555 which does not align is omitted as indicated, as are the 50 C-terminal residues 837–886; identical residues are boxed. Residues thought to be involved either in important binding or in catalytic roles within the active site are shaded.

for TPP binding. When this was done, it became obvious that the yeast pyruvate decarboxylase sequence had to be broken such that the second half of the molecule aligned approximately with the $E_1\alpha$ sequence, while the first half of the decarboxylase, starting at residue 1, aligned with the human $E_1\beta$ sequence. This gave an alignment which was also close to linear, such that 31 residues were held in common with transketolase, 43 residues in common with human PDH $E_1\alpha\beta$ and 33 in common with the *B. stearothermophilus* enzyme. Finally the E_1 of the *Escherichia coli* PDH complex [12] was aligned and this gave 44 identical residues to human E_1, 40 identical residues to *B. stearothermophilus* E_1, 48 with transketolase and 43 with *S. cerevisiae* pyruvate decarboxylase. A short 77-amino acid non-homologous region was deleted from the *E. coli* sequence as indicated and the final C-terminal 50 amino acid residues are not shown for the *E. coli* sequence. Computerized alignment by a variety of protein alignment algorithms consistently failed to align functional domains as identified below.

3. FUNCTIONAL DOMAINS PREDICTED BY ALIGNMENT WITH THE TRANSKETOLASE STRUCTURE

The region showing the most conservation amongst these sequences was, as expected, the section concerned with the binding of the pyrophosphate end of the cofactor TPP, the consensus being GDG/A X^{24-26}N/CN.

Since in transketolase these residues are concerned with the co-ordination of a calcium ion with the pyrophosphate structure, it is reasonable to infer that calcium, magnesium or a similar divalent cation is similarly involved in TPP binding in the pyruvate decarboxylating enzymes. Two further histidine residues required for pyrophosphate co-ordination through hydrogen bonding in *S. cerevisiae* transketolase [1] are probably represented by H84 and H263 for human PDH $E_1\alpha$, H90 and H272 for *B. stearothermophilus* PDH $E_1\alpha$ and by Q161 and H335 for *E. coli* PDH. The most likely residues for *S. cerevisiae* pyruvate decarboxylase are Q368 and Q547 since there is no histidine in the vicintiy and an amide can give a satisfactory hydrogen bond with the phosphate oxygen residues. The serine residue next to H263 in human PDH $E_1\alpha$ is one of the residues which undergoes phosphorylation/dephosphorylation in the vertebrate PDH complex, while the corresponding histidine–threonine combination in *B. stearothermophilus* does not undergo phosphorylation [13]. A child with severe lactic acidosis due to PDH E_1 deficiency was shown by us to have an H263L mutation with only 2.5% residual activity in the PDH complex [14]. This mutation could have affected the pyrophosphate binding or the serine phosphorylation site at S264. The aspartate residue D382 in *S. cerevisiae* transketolase is also prominent in the active site and has its equivalent at E29β of the human sequence, E17β of the *B. stearothermophilus* sequence, E198 of the decarboxylase and possibly Q454 in *E. coli* PDH E_1.

Of particular interest is the thiazolium binding area of transketolase and possible homologous regions of the pyruvate decarboxylating enzymes that might serve a similar function. In *H. polymorpha* transketolase this is made up of F458, F461, Y464 preceeded by E433 which co-ordinates the nitrogen atom of the pyridine ring [4]. This is almost identical spacing to the E418, F442, F445 and Y448 of the *S. cerevisiae* transketolase enzyme. The glutamate residue is retained in human and *B. stearothermophilus* PDH $E_1\alpha\beta$ at E59β and E60β, respectively, and the same function is probably served by D237 in *S. cerevisiae* pyruvate decarboxylase (which is highly conserved in this group of enzymes) [4]. The equivalent thiazolium binding pocket for $E_1\alpha\beta$ is most likely to be made up of F80β, F83β and F85β for human, and F83β, F86β and Y88β for *B. stearothermophilus*. In the yeast decarboxylase it is probably made up of Y260β, Y264β and L268β, and in *E. coli* of Y345, Y348 and F351. The branched-chain keto acid dehydrogenase $E_1\beta$ subunit also has a similar motif of FXXYI/FF/Y, starting at about 90β in a wide variety of organisms [14]. A strategically placed histidine, 33 amino acids C-terminal to the end of the thiazolium ring binding pocket, is thought

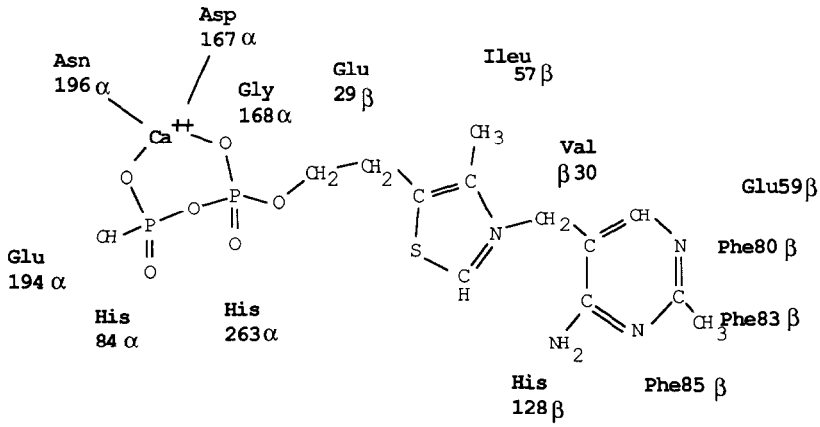

Fig. 2. Model of the thiamine pyrophosphate-binding site of human pyruvate dehydrogenase E_1. Based on the homologies seen in Fig. 1 and the published X-ray crystal structure of transketolase [1], a parallel model of the thiamine pyrophosphate-binding site in human pyruvate dehydrogenase E_1 was constructed. Residues belonging to the PDH-$E_1\alpha$ subunit are designated α, while residues belonging to the $E_1\beta$ subunit are designated β.

to be very important in the catalytic mechanism of the transketolase [1]. This residue, which is probably instrumental in creating a reactive thiazole carbanion for receipt of the acetyl function, occurs at 481 in *S. cerevisiae* transketolase and at 503 in *Hansenula polymorpha* transketolase. For all the pyruvate and branched-chain keto acid decarboxylating enzymes an HS combination occurs about 40 amino acids downstream of the thiazolium binding site [15], and this compelling homology suggests that this histidine is the catalytic residue which is held in common. No other histidine residues are common to all sequences in this vicinity.

A possible model for the active site of human pyruvate dehydrogenase E_1 is shown in Fig. 2, incorporating the key amino acids mentioned above and exhibiting marked similarity to the transketolase active site [1]. This model clearly shows that the function of the β subunit of the $\alpha_2\beta_2$ tetrameric enzymes is to bind the thiazolium ring of TPP and to provide a key catalytic histidine residue for the active site. It also shows that the fundamental catalytic residues are present in similar configurations in all of the enzymes considered, although it is obvious that a splitting and rejoining of the ancestral gene has taken place to create two enzyme types, those with the TPP-binding site at the end of the protein and those with this site at the beginning. Which is the most primitive arrangement is impossible to say at this point. Parsimony analysis programmes give different answers to this question depending on the parameters chosen for the analysis. However, simple consideration of the homology between sequences as presented here suggests a close relationship between human and *B. stearothermophilus* PDH E_1 enzymes with transketolase, while the relationship of these entities to *E. coli* PDH E_1 and *S. cerevisiae* pyruvate decarboxylase are more distant. The validity of this model will eventually be tested when resolution of the PDH E_1 structure is revealed by X-ray crystallography and the importance of key residues revealed either by site-directed mutagenesis as has been shown for both *E. coli* PDH E_1 [16] and for *Zymomonas mobilis* pyruvate decarboxylase [17] around the $GDGX^{24-26}$ NN thiamine pyrophosphate-binding motif, or by examination of naturally occurring mutations in the human population [18].

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