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A common structural motif in thiamin pyrophosphate-binding enzymes

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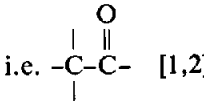
The amino acid sequences of a wide range of enzymes that utilize thiamin pyrophosphate (TPP) as cofactor have been compared. A common sequence motif approximately 30 residues in length was detected, beginning with the highly conserved sequence -GDG- and concluding with the highly conserved sequence -NN-. Secondary structure predictions suggest that the motif may adopt a $\beta\alpha\beta$ fold. The same motif was recognised in the primary structure of a protein deduced from the DNA sequence of a hitherto unassigned open reading frame of *Rhodobacter capsulata*. This putative protein exhibits additional homology with some but not all of the TPP-binding enzymes.

Thiamin pyrophosphate; Structural motif; Sequence homology; Pyruvate dehydrogenase complex; Pyruvate decarboxylase; Transketolase

1. INTRODUCTION

Thiamin pyrophosphate (TPP, vitamin B_1) is widely employed as a cofactor by enzymes that catalyse reactions involving the rupture of the carbon-carbon bond adjacent to an oxo function,



i.e. -C(-)-C(=O)- [1,2].

Prominent among them are pyryvate decarboxylase, the 2-oxo acid decarboxylase (E1) components of the 2-oxo acid dehydrogenase multienzyme complexes, pyruvate oxidases, acetolactate synthases and the transketolases. For all these enzymes, which cover a wide range of metabolic pathways, one or more representative structural genes have been cloned and sequenced in recent years, as summarized in table 1.

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Abbreviation: TPP, thiamin pyrophosphate

Surprisingly, despite the identical nature of the reactions they catalyse, no amino acid sequence homology could be detected between the E1p and E1o polypeptide chains of the pyruvate and 2-oxoglutarate dehydrogenase complexes, respectively, of *Escherichia coli* [3]. Moreover, as pointed out elsewhere [4], neither chain shows any homology with the E1α or E1α chains of the human pyruvate dehydrogenase [5,6] or *Pseudomonas putida* branched chain 2-oxo acid dehydrogenase [4] complexes. These latter are representative of those 2-oxo acid dehydrogenase complexes in which the E1 component is a tetramer composed of E1α and E1β chains (reviewed in [7,8]). On the other hand, systematic comparisons of the published gene sequences [9,10] have recently indicated that there is considerable homology between the amino acid sequences inferred for the pyruvate decarboxylase, pyruvate oxidase (cytochrome) and acetolactate synthase polypeptide chains. However, no sequence homology could be detected between this pyruvate decarboxylase-like group of enzymes and the E1p polypeptide chain of the pyruvate dehydrogenase complex from *E. coli*. This was taken to indicate that the pyruvate decarboxylase group may share a common ancestor dif-

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Table 1
Enzymes utilizing TPP as a cofactor

Enzyme name (EC number)	Reaction catalysed	Source	Cloned sequence	Ref.
Pyruvate decarboxylase (EC 4.1.1.1)	pyruvate = acetaldehyde + CO_2	*Zymomonas mobilis*	Zmpdc	[31]
		Saccharomyces cerevisiae	Scpdc 1	[32]
Acetolactate synthase (EC 4.1.3.18)	2 pyruvate = acetolactate + CO_2	*Nicotiana tabacum*	Ntsura	[33]
		Saccharomyces cerevisiae	Scilv2	[34]
		Escherichia coli	EcilvI	[35]
Pyruvate oxidase (cytochrome) (EC 1.2.2.2)	pyruvate + H_2O + ferricytochrome *b*1 = acetate + CO_2 + ferrocytochrome *b*1	*Escherichia coli*	EcpoxB	[36]
Pyruvate dehydrogenase complex, E1 component (EC 1.2.4.1)	pyruvate + lipoamide = *S*-acetyldihydrolipoamide + CO_2	Human	HuE1αp	[5]
		Human	HuE1βp	[6]
		Bacillus stearothermophilus	BsE1α	[37]
		Escherichia coli	EcaceE	[17]
2-Oxoglutarate dehydrogenase complex, E1 component (EC 1.2.4.2)	2-oxoglutarate + lipoamide = *S*-succinyldihydrolipoamide + CO_2	*Saccharomyces cerevisiae*	Sckgd1	[18]
		Escherichia coli	EcsucA	[3]
Branched-chain 2-oxo acid dehydrogenase complex, E1 component (EC 1.2.4.4)	3-methyl-2-oxobutanoate + lipoamide = *S*(2-methylpropanoyl) dihydrolipoamide + CO_2	Human	HuE1αb	[16]
		Ox	OxEqαb	[38]
		Rat	RaE1α	[39]
		Pseudomonas putida	PpbkdA1	[4]
Formaldehyde transketolase (Dihydroxyacetone synthase) (EC 2.2.1.3)	D-xylulose 5′-phosphate + formaldehyde = glycerone + glyceraldehyde 3-phosphate	*Hansenula polymorpha*	Ysmdas	[11]

ferent from that of *E. coli* E1p [10] and, by inference, that of *E. coli* E1o.

What has been strikingly absent from the sequence comparisons thus far has been any indication of a sequence motif that might be related to be need of all these proteins to bind the essential cofactor, TPP. This has prompted us to re-examine the sequences and led us to uncover a structural motif found in the sequences of all these enzymes, and indeed in that of a hitherto unconsidered, but mechanistically related enzyme, dihydroxyacetone synthase [11]. This latter enzyme is exemplary of the transketolases. By searching the sequence databases, we have been able further to identify the same motif in the primary structure of a protein deduced from the DNA sequence of an unassigned open reading frame of *Rhodobacter capsulata* [12]. We show also that the potential product of this open reading frame exhibits sequence homology with the dihydroxyacetone synthase (formaldehyde transketolase) and the E1α and E1β subunits of the 2-oxo acid dehydrogenase complexes, pointing the way to a possible biochemical function for this putative protein.

2. MATERIALS AND METHODS

Sequence homologies were initially explored using the program BESTFIT [13]. Protein sequence databases (PIR, SWISSPROT and DOOLITTLE) were searched using the program FASTP [14]. The program FASTA was used to search the DNA sequence databases (GENBANK and EMBL) for matches with all possible translation products [15]. Sequence alignments were ultimately ordered using the editing program LINEUP and its associated program PRETTY [13].

3. RESULTS AND DISCUSSION

3.1. *Identification of a common sequence motif*

Careful visual inspection of the amino acid sequences of the pyruvate decarboxylase group of enzymes aligned by Green [10] and of the E1α chains of the human pyruvate dehydrogenase complex [4]

and the branched chain 2-oxo acid dehydrogenase complexes of humans [16] and *P. putida* [4] enabled us tentatively to identify a sequence motif that is common to all these proteins (fig.1). We were similarly able to identify the same motif in the sequences of the *E. coli* E1p [17] and E1o [3] chains and in the sequence of the E1o component of the 2-oxoglutarate dehydrogenase complex of yeast [18]. The relevant regions of all these proteins are aligned in fig.1. The relative positions of this structural motif in the sequences of the various TPP-utilizing enzymes are shown in fig.2.

Further scrutiny of the databases revealed two other proteins that contain the same sequence motif: these are the formaldehyde transketolase of *Hansenula polymorpha* [11] and the potential product (Rcrcfp) of a hitherto unidentified open reading frame that lies immediately downstream from the gene cluster encoding the photosynthetic reaction centre proteins in *R. capsulata* [12]. The relevant regions of these proteins too are aligned in fig. 1 and fig. 2.

As shown in fig. 1, the common sequence motif begins with the highly conserved sequence -GDG-, and concludes with the highly conserved sequence -NN-. In between there are approximately 30 residues whose sequence is much less conserved but which exhibit several common features. For example, about 10 residues to the C-terminal side of the -GDG- sequence there is usually a negatively-charged residue (E or D), followed about 5 residues and 11 residues further on by a generally conserved alanine and proline residue, respectively. Immediately preceding the -NN- sequence is a cluster of 6 or 7 largely hydrophobic side-chains.

3.2. *A possible role for the sequence motif*

Given that the only shared property of the enzymes listed in fig. 1 is the requirement for TPP as a cofactor, it is tempting to speculate that the conserved sequence motif may be part at least of a common TPP-binding site. Its occurrence in the E1α subunits of the 2-oxo acid dehydrogenase complexes is consistent with this possibility, since the binding site for TPP is thought to reside in the E1α and not the E1β subunits of the ox kidney and heart pyruvate dehydrogenase complexes [19].

A prediction of the secondary structure [20] associated with the sequence motif shown in fig. 1 suggests that the -GDG- sequence might form a turn that separates a preceding β-strand from a succeeding α-helical segment of approximately 20 residues. The cluster of hydrophobic residues immediately before the -NN- sequence is predicted to form another β-strand, whereas the sequence immediately following the -NN- dipeptide is likely to be α-helical.

This predicted structure bears a striking resemblance to a region in the active sites of a large number of dinucleotide-binding enzymes. In such enzymes the binding of the dinucleotide occurs in a typical β-turn-$\alpha\beta$ fold in which a conserved sequence pattern -G-X-G-X-X-G- forms the tight turn that permits a favourable dipole-charge in-

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Zmpdc     APERRNILMVGDGS.FQLTAQEVAQMVR.LKL.PVIIFLINNY.GYTIEVM
Scpdc1    DPKKRVILFIGDGSL.QLTVQEISTMIRWGLK.PY.LFVLNND.GYTIEKL
Ntsura    RPDEVVVDIDGDGS.FIMNVQELATIKVENL..PVKIMLLNNQ.HLGMVVQ
Scilv2    KPESLVIDIDGDAS.FNMTLTELSS.AVQAGT.PVKILILNNEE.QGMVTQ
Ecilvi    LPEETVVCVTGDGSI.QMNIQELST.ALQYEL.PVLVVNLNNR.YLGMVKQ
Ecpoxb    EPERQVVAMCGDGG.FSMLMGDFLS.VVQMKL.PVKIVVFNNS.VLGFVAM
HuE1αp    GKDEVCLTLYGDGAANQGQIFEAYNMAALWKL.PCIFICENNY.GMGTSVE
BsE1α     GKKAVAITYTGDGGTSQGDFYEGINFAGAFKA.PAIFVVQNNR..FAISTP
EcaceE    TSKQTVYAFLGDGEMDEPESKGAITIATREKL.DNLVFVINCNLQR.LDGP
Sckgd1    LLHGDA.AFAGQGVV.YETM.GFLTL.PEYSTGGTIHVITNNQIGFT.TDP
EcsucA    TIHGDA.AVTGQGVV.QETL.NMS.KARGYEVGGTVRIVINNQVGFTTSNP
HuE1αb    NANRVVICYFGEGAASEGDAHDGFNFAATLEC.PIIFFCRNN..GYAISTP
OxE1αb    NANRVVICYFGEGAASEGDAHAGFNFAATLEC.PIIFFCRNN..GYAISTP
RaE1αb    NANQIVICYFGEGAASEGDAHAGFNFAATLEC.PIIFFCRNN..GYAISTP
PpbkdA1   GDTKIASAWIGDGATAESDFHTALTFAHVYRA.PVILNVVNNQ..WAISTF
Ysmdas    IITNKVYCMVGDACLQEGPALESISLAGHMGLDNLIVLYDNNQVCCDGSVD

Rcrcfp    QPVGDTIAIIGDGSITAGMAYEALNHAGHLK..SRMFVILNDND.MSIAPP
           ----β----t----------α----------t--β---t----α----
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Fig. 1. Putative TPP-binding motif underlined by the secondary structure prediction of Chou and Fasman [20].

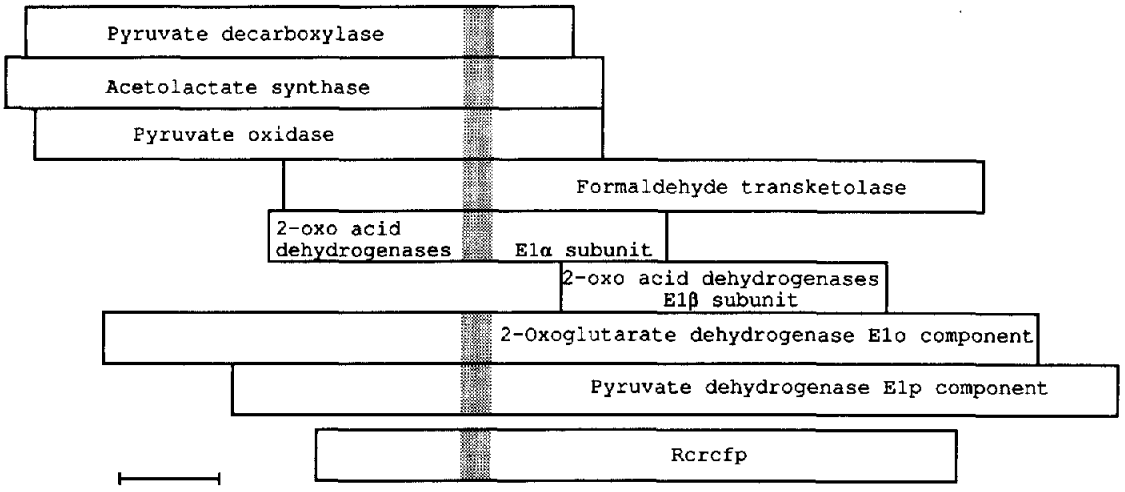

Fig. 2. The various TPP-utilizing enzymes aligned on the proposed TPP-binding motif (shaded box) together with the unidentified *R. capsulata* protein, Rcrcfp. Scale bar, 100 residues.

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          1                                                                  90
Ysmdas                                                    MSMRIPKAASVNDEQHQRIIKYGRALVLDIVEQY
HuE1αp    MRKMLAAVSRVLSGASQKPASRVLVASRNFANDATFEIKKCDLHRLEEGPPVTTVLTREDGLKYYRMMQTVRRMELKADQLYKQKIIRGF
Rcrcfp                                                    MSATPSRTPHLDRVTGPADLKAMSIADLTALASE..VRREIVEVV

          91                                                                 180
Ysmdas    G..GGHPGSAMGAMAIGIALWKYTLKYAPNDPNYFNRDRFVLSNGHVCLFQYIFQHLYGLKSMTMAQLKSYHSNDFHSLCPGHPEIEHDA
HuE1αp    CHLCDGQEACCVGLEAGINPTDHL....ITAYRAHGFTFTRGLSVREILAELTGRKGGCAKGKGGS...MHMYAKNFYG..........
Rcrcfp    SQTGGHLGSSLGVVELTVALHAVF....NSPGDKLIWDVGHQCYPHKILTGRRSRMLTLRQAGGISGFPKRSESPHDAF..........

          181                                                                270
Ysmdas    VEVTTGPLGQGISNSVGLAIATKNLAATYNKPGFDIITNKVYCMVGDACLQEGPALESISLAGHMGLDNLIVLYDNNQVCCDGSVDI.AN
HuE1αp    ...GNGIVGAQVPLGAGIALA..........CKYNGKDEVCLTLYGDGAANQGQIFEAYNMAALWKL.PCIFICENNY.GMGTSVER...
Rcrcfp    ...GAGHSSTSISAALGFAVG..........RELGQPVGDTIAIIGDGSITAGMAYEALNHAGHLK..SRMFVILNDND.MSIAPPVGAL
                                                 ----β---t----------α----------t---β---t----α----
          271                                                                360
Ysmdas    TEDISAKFKACNWNVIEVENASEDVATIVKALEYAQAEKHRPTLI...NCRTVIGSGAAFENHCAAHGNALGE..DGVRELKIKYGMNPA
HuE1αp    ........AAASTDYYKRGDFIPGLRVDGMDILCVREATRFAAAYCRSGKGPILMELQTYRYHGHSMS.DPGVSYRTREEIQEVRSKSDP
Rcrcfp    QHYLNTIARQAPFAALKAAAEGIEMHLPGPVRDGARRARQMVTAM...PGGATLFEELGFDYIGPVDGHDMAELVETLRVTRARASGPVL

          361                                                                450
Ysmdas    QKFYIPQDVYDFFKEKPAEGDKLVAEWKSLVAKYVKAYPEEGQEFLARMRGELPKNWKSFLPQQEFTGDAPTRAAARELVRALGQNCKSV
HuE1αp    IMLLKDRMVNSNLASVEELKEIDVEVRKEIEDPAQFAAADPEPPLEELGYHIYSSDPPFEVRGANQWIKFKSVS
HuE1βp                                                    VQVTVRDAINQGMDEELERDEKVFLLGEEVAQYDGAYKVSRGLWKKYG
Rcrcfp    IHVCTTKGKGYAPAEGAEDKLHGVSKFDIETGKQKKSIP......NAPNYTAVFGERLTEEAARDQAIVAVTAAMPTGTGLDIMQ..KRF

          451                                                                540
Ysmdas    IAGCADLSVSVNLQWPGVKYFMDPSLSTQCGLSGDYSGRYIEYGIREHAMCAIANGLAAYNKGTFLPITSTFFMFYLYAAPAIRMAGLQE
HuE1βp    DKRIIDTPISEMGFAGIAVGAAMAGLRPICEFMTFNFSMQAIDQVINSAAKTYYMSGGLQPVPIVFRGPNGASAGVAAQHSQCFAAWYGH
Rcrcfp    PRRVFDVGIAEQHAVTFAAGMAAAGLKPFLALYSSFVQRGCYDQLVHDVALQNLPVRLMIDRAGLVGQDGATHAGAFDVSMLANLPNFTV

          541                                                                630
Ysmdas    LKAIHIGTHDSINEGENGPTHQPVESPALFRAYANIYYMRPVDSAEVFGLFQKAVELPFSSILSLSRNEVLQYLASRAQRRRNAAGYILE
HuE1βp    CPGLKVVSPWNSEDAKGLIKSAIRDNNPVVVLENELMYGVPFEFLRKLSQKILLIPIGKAKIERQGTHITVVSHSRPVGHCLEAAAVLSK
Rcrcfp    MAAADEAELCHMVVTAAAHDSGPIALRYPRGEG.....RGVEMPERGEVLEIGKGRVMTEGTEVAILSFGAHLAQALKAAEMLEA

          631                                                                720
Ysmdas    DAENAEVQIIGVGAEMEFADKAAKILG.RKFRTRVLSIPCTRLFDEQSIGYRRSVLRKDGRQVPTVVVDGHVAFGWERYATASYCMNTYG
HuE1βp    EGVECEVINMRTIRPMDMETIEASVMKTNHLVTVEGGWPQFGVGAEICARINGSPAFNFLDAPAVRVTGADVPMPYAKILEDNSIPQVKD
Rcrcfp    EGVSTTVADARFCRPLDTDLIDRLIEGHAALITLEQG.AMGGFGAMVLHYLARTGQLEKGRA.IRTMTLPDCYIDHGSPEEMYAWAGLTA

          721
Ysmdas    KSLPPEVIYEYFGYNPATIAKKVEAYVRACQRDPLLLHRLPGPEGKA
HuE1βp    IIFAIKKTLNI
Rcrcfp    NDIRDTALAAARPSKSVRIVHSA
```

Fig. 3. Alignment of the E1α and E1β components of the human pyruvate dehydrogenase complex with the formaldehyde transketolase (Ysmdas) and the unidentified protein from *R. capsulata* (Rcrcfp). The proposed TPP-binding motif is marked with the prediction [20] for secondary structure. Residues with a score ≥2 on the Dayhoff similarity matrix [40] are in bold typeface.

teraction between the N-terminal end of the α-helix and the pyrophosphate moiety of the dinucleotide [21]. The highly conserved -GDG- sequence in fig. 1 might have the same function in permitting a β-turn-αβ structural motif.

There is no comparable clue to a possible function for the highly conserved -NN- sequence at the C-terminal end of the sequence motif in fig. 1. However, asparagine side-chains participate readily in hydrogen bonds and it is easy to envisage, for example, one or more hydrogen bonds being formed with nitrogen atoms of the thiamin moiety of TPP. Thus, it is known that absence of the 1′-nitrogen, but not the 3′-nitrogen, from the pyrimidine ring prevents binding of TPP to yeast pyruvate decarboxylase [22].

3.3. *An unidentified open reading frame in* Rhodobacter capsulata

The lack of homology between the primary structures of *E. coli* E1p and the various E1α and E1β subunits of the 2-oxo acid dehydrogenase complexes has been commented on before [4]. However, as shown in fig. 3, significant homology can be detected between the sequences of the formaldehyde transketolase, the unidentified open reading frame in *R. capsulata* and both the E1α and E1β subunits of the human pyruvate dehydrogenase complex. The sequence homology with the E1β subunits is of interest in that the putative TPP-binding site described above is found in the E1α subunits of these complexes. The sequence homology between the potential product of the *R. capsulata* open reading frame and these other enzymes adds weight to the inference that this protein is an enzyme utilizing TPP as a cofactor. On the evidence before us we would speculate that it is a transketolase (note its alignment in fig. 2) or a component of a 2-oxo acid dehydrogenase complex.

3.4. *Conclusions*

Despite numerous attempts to probe the active sites of TPP-dependent enzymes by means of chemical modification [22–26], no amino acid sequences have yet been identified, although it is widely accepted that the TPP-binding sites must be hydrophobic in character [27–30]. Our identification of a common sequence motif in TPP-dependent enzymes catalysing a wide variety of chemical reactions now opens the way to a systematic study of this motif by means of site-directed mutagenesis and protein engineering.

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