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# Determination of the DNA and Amino Acid Sequences of the Lactate Dehydrogenase Gene from *Plasmodium falciparum* Strains K1 and PF FCBR: A Route to the Design of New Antimalarials

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**Abstract :** This paper describes cloning of the gene coding for the lactate dehydrogenase (LDH) gene from the two different strains of *Plasmodium falciparum*, K1 and PF FCBR. The DNA sequences of LDH genes of these two strains were found to be identical. Amino acid sequence alignment of LDH from *P. falciparum* strains K1 and PF FCBR (*PfLDH*) to some other known LDH sequences showed that *PfLDH* has 29% residue identities with *Bacillus stearothermophilus* (*BsLDH*) and 29%, 31%, 33% with dogfish, man-M<sub>4</sub>, and pig-M<sub>4</sub> LDHs, respectively. It was also shown that *PfLDH* has insertions and single amino acid deletions. Two deletions are glutamate-48 and glycine-217. It has a single amino acid insertion, a tyrosine, between residues 73 and 74, and most remarkably a five residue insertion in the catalytic loop compared to other LDH sequences. This five residue insertion could be exploited in drug design.

**Key Words:** lactate dehydrogenase / antimalarials / DNA sequencing / gene cloning / catalytic loop

## *Plasmodium falciparum* soyları K1 and PF FCBR'ın Laktat Dehidrogenaz Geninin DNA ve Aminoasit Dizilerinin Belirlenmesi: Yeni Antimalariallerin Tasarımına Bir Yol

**Özet :** Bu makalede laktat dehidrogenaz (LDH) geninin *Plasmodium falciparum*'un iki farklı soyu olan K1 ve PF FCBR'dan klonlanması tanımlanmaktadır. Bu iki soyun nükleotid dizilerinin tümüyle birbirinin aynı olduğu bulunmuştur. *P. falciparum* soyları K1 ve PF FCBR'ın LDH'lerinin (*PfLDH*) amino asit dizilerinin, amino asit dizileri bilinen diğer bazı LDH dizileri ile karşılaştırılması *PfLDH*'in; *Bacillus stearothermophilus* LDH'i (*BsLDH*) ile % 29, köpek balığı LDH'i ile % 29, insan-M<sub>4</sub> LDH'i ile % 31 ve domuz-M<sub>4</sub> LDH'i ile de % 33 oranında aynı aminoasitleri içerdiği gösterilmiştir. *PfLDH* 'in diğer LDH'lere göre amino asit ilaveleri ve tek aminoasit eksiklikleri olduğunda gösterilmiştir. Glutamat-48 ve glisin-217 eksik olan iki aminoasittir. *PfLDH*, diğer LDH'ler ile karşılaştırıldığı zaman 73. ve 74. rezidüler arasında tek bir amino asit ve en önemlisi katalitik halkada ilave 5 amino asit bulunmaktadır. Tespit ettiğimiz bu 5 rezidü ilavesinin proteine kazandırdığı yapısal ve kinetik özelliklerin araştırılmasının, sıtmaya karşı etkin olacak yeni ilaç tasarımları çalışmalarına katkı sağlayacağı kanısındayız.

**Anahtar Sözcükler:** Laktat dehidrogenaz / antimalarial / DNA dizisi / gen klonlaması / katalitik halka

## Introduction

The emergence of the malaria parasite strains which are resistant to conventional drug therapy has stimulated the search for antimalarials with novel modes of action. This necessitates new approaches for the development of drugs. It will be possible to understand the structure and function of the *Plasmodium falciparum* gene products by cloning them into the *E. coli* cells. This approach could be particularly helpful in studying parasite enzymes because it could provide ways to distinguish them from the host enzymes.

In this study, *Plasmodium falciparum* lactate dehydrogenase (*PfLDH*) was chosen as a target with the aim of developing a new antimalarial. The selective inhibition of specific parasite enzymes leaving any corresponding human enzymes unaffected is a therapeutic strategy ideally suited to *PfLDH* because the plasmodial form of this protein has significantly different properties from the enzymes catalyzing similar biochemical reactions in the human (1,2). LDH is essential to the life cycle of the plasmodium. The human malaria parasites in the erythrocyte lack a functional tricarboxylic acid cycle (3); therefore the asexual stage of the parasite, residing in the mature red blood cells, depends mainly on glycolysis for its ATP requirements. Compounds that inhibit the enzyme also kill the plasmodium (1). It is hoped that this major metabolic pathway of the parasite will be a target for enzyme inhibition.

The first attempt to clone the LDH gene was by Simmons et al. (4) from *P. falciparum* strain K1. They used a polyclonal antibody against *PfLDH*, and several *PfLDH* cDNA clones were isolated by screening a  $\lambda$ gt11Tn5 expression library. DNA sequence analysis of one of these clones revealed a single open reading frame which showed a degree of homology to the N-terminal domain between residues 17 and 45 in the published LDH sequences. The complete gene coding for *PfLDH* strain Honduras I was isolated by Bzik et al. (5).

*P. falciparum* occurs as many different strains. In this study, the same gene was isolated from two different strains of *P. falciparum*, K1 and PF FCBR, to determine whether the amino acid sequence was going to be sufficiently similar that an antimalarial designed against one *PfLDH* strain would work against all other strains.

To this end, the *PfLDH* gene was isolated from genomic DNA by PCR and cloned into the vector pUC18, and the whole gene was sequenced in both directions.

## Materials and Methods

### Bacterial strains and growth media

The host bacterial strain used to prepare DNA for mutagenesis and sequencing in pUC-18 (Pharmacia Biotech, Uppsala, Sweden) was *Escherichia coli* TG2 {*supE hsdΔ5 thiΔ(lac-proAB) Δ(srl-recA) (306::Tn 10) (tetr) F'[traD36 proAB<sup>+</sup> lacIq lacZΔM15 d]*}. The *E. coli* TG2 cells were cultured in double strength (2X) YT broth. Where necessary, ampicillin (100 mg/ml) was used

in media for the selection and growth of transformants. T4 DNA Ligase and restriction enzymes (*Eco* RI and *Pst* I) were purchased from Boehringer Corp. *Taq* DNA Polymerase was obtained from Pharmacia Biotech, Uppsala, Sweden.

#### Polymerase Chain Reaction (PCR)

Two oligonucleotide primers used to amplify *P. falciparum* genomic DNA, 5'ATGGCTCCA AAAGCAAAAATCG3' (*Eco* RI site) and 5'GAGAATGAAGGCATTAGCTTAA 3' (*Pst* I site), were complementary to the forward-reverse strands of *P. falciparum* strains of K1 and PF FCBR LDHs. The PCR was carried out in the presence of 5  $\mu$ l *Taq* buffer (supplied with enzyme), 5  $\mu$ l (10ml of each 100 mM dNTPs and 10 ml of H<sub>2</sub>O) stock dNTPs, 2.5  $\mu$ l (at 20 pmoles) N-term. primer, 2.5  $\mu$ l (at 20 pmoles) C-term. primer, 1  $\mu$ l (of 0.5  $\mu$ g/ $\mu$ l) genomic DNA, 2.5 units *Taq* DNA polymerase and 33.5 ml H<sub>2</sub>O to the final volume of 50 $\mu$ l. A layer of mineral oil was placed over the reaction mixture to prevent evaporation. The PCR was carried out at 94°C for 1.5 min, 55°C for 2 min, and 72°C for 2 min for 20 cycles.

#### Resolubilizing DNA from Agarose Gel

Agarose gel electrophoresis was performed in a horizontal gel apparatus according to Sambrook et al. (6) following PCR. After the electrophoresis; 200  $\mu$ l TE buffer (10 mM Tris HCl, 1 mM EDTA) was added to the gel slices containing the DNA, and they were incubated in a waterbath at 68°C for 10 min. Phenol was equilibrated by mixing 4 ml of redistilled phenol with the same volume of 0.5 M Tris. HCl pH 8.0. The bottom layer was phenol.

When the agar slice had melted, 200  $\mu$ l of phenol was added. It was vortexed vigorously and spun for 5 min in a microcentrifuge in order to separate the phases. The aqueous layer which contained the DNA was removed into a clean tube spun for another 5 min and then the DNA was ethanol precipitated. Ethanol precipitation was as follows: the DNA was mixed with 1/10 of its volume of 4 M NaCl and then 2.5 volumes of cold 100% ethanol and left at -20°C for at least 30 min. The DNA was spun down for 15 min, the supernatant discarded and 500 ml cold 70% ethanol was added to the pellet which was spun down for a further 10 min. The supernatant was discarded and the pellet was dissolved in 200  $\mu$ l sterile H<sub>2</sub>O for the second ethanol precipitation. After this the DNA was dissolved in an appropriate volume of sterile H<sub>2</sub>O.

#### Restriction of DNA

The DNA coding for wild type LDH from *P. falciparum* and the chosen vectors were digested with the restriction enzymes *Eco* RI and *Pst* I under the conditions recommended by the suppliers of each enzyme. The reaction was as follows: 20  $\mu$ l DNA (from phenol extraction), 3  $\mu$ l 10 x buffer (supplied with enzymes), 2  $\mu$ l (of 26 U/ $\mu$ l) *Eco* RI, 2  $\mu$ l (of 10 U/ $\mu$ l) *Pst* I, 2  $\mu$ l RNase and 1  $\mu$ l H<sub>2</sub>O. Restriction digests were performed in a waterbath, at 37°C, for 1 h. After the digest they were run out on an LMP gel and cleaned by phenol extraction, and their concentrations were estimated on 1% agarose gel before ligation.

### Ligation and Transformation

The *Eco* RI/*Pst* I digested *Pf*LDH gene was ligated into similarly digested cloning vector pUC18. The reaction mixture contained 100 ng DNA (insert), 3:1 Insert:vector ratio (molar), 2  $\mu$ l 10 x Ligase buffer, 2  $\mu$ l (1 U/ $\mu$ l) T4 DNA ligase and X  $\mu$ l H<sub>2</sub>O to final volume of 20  $\mu$ l. Ligations were set up at 16°C for 16 h in a Perkin Elmer thermal cycler.

Transformation of *E. coli* cells was carried out according to Sambrook et al. (6) after the ligation.

### Small Scale DNA Preparation (Miniprep)

A single bacterial colony was put into 5 ml 2 x YT broth containing 100  $\mu$ g/ml ampicillin. It was grown overnight at 37°C with shaking. After that DNA preparation was performed using QIAprep Spin Miniprep Kit (50), Qiagen.

### DNA Sequencing

The DNA was sequenced either manually by Sanger's dideoxy-mediated chain termination method (7) using a Sequenase Version 2.0 kit from US Biochemicals, Cleveland, OH, or automatically on a Du Pont Genesis 2000 automated DNA sequencer in the Department of Biochemistry, Bristol, UK. The plasmid primers used to sequence two ends of the *Pf*LDH were as follows:

5'-AGCGGATAACAATTTTCACACAGGA-3' (M13/pUC Reverse)

5'-GTAAAACGACGGCCAGT-3' (M13/pUC Forward)

5'-CGACATCATAACGGTTCTGG-3' (M13/pKK223-3 N-terminus)

5'-GTATCAGGCTGAAAATCTTC-3' (M13/pKK233-3 C-terminus).

The other primers used to sequence the LDH gene from *P. falciparum* strains K1 and PF FCBR are given in the Table.

## Results and Discussion

### Nucleotide Sequence of the *Pf*LDH Gene from Strains K1 and PF FCBR

Once the correct insert was detected, a single colony carrying the insert was tooth-picked into 2 x YT broth containing ampicillin for each strain and grown overnight at 37°C in a shaker, and was prepared for sequencing. The DNA was manually sequenced from both directions by the method of Sambrook et al. (6) using the sequencing primers given in Materials and Methods.

The *Pf*LDH gene consist of an open reading frame (ORF) of 951 nucleotides (316 amino acids) initiated with an ATG start codon and ending with a TTA codon. As was shown by Bzik et al. (5), the *P. falciparum* LDH gene contains no introns. The sequencing of the whole K1 and

Table The sequencing primers of the LDH gene from *P. falciparum* strains K1 and *PF* FCBR. *Pf*LDH sequencing primer's numbers are given in parentheses and their positions are shown in Figure 1 in bold.

<i>Pf</i> LDH 5'→3' sequencing primers	<i>Pf</i> LDH 3'→5' sequencing primers
5' -CCACATGGAAAAGCTTTAGA-3' (1)	5' -GTGTCCATATTGTCCTTCTA-3' (7)
5' -AGGCCCCAGGAAAAGAGTGAC-3' (2)	5' -TAAAGGGATACCACCTACAG-3' (8)
5' -TCAACATTTCAGGTGTTTCCTA-3' (3)	5' -GATGTATCTAATAACACCACC-3' (9)
5' -TAAATGCACACATTGTAGGT-3' (4)	5' -GTACCATAACATCTACTGGG-3' (10)
5' -TTACATGCATCACCATATGT-3' (5)	5' -CAAATCGTCATAAGTGTGTTG-3' (11)
5' -TAGAAGGACAATATGGACAC-3' (6)	5' -TTCCATGTGGCATGTTCTTT-3' (12)

*PF* FCBR genes showed that there is no variation between the LDH amino acid and the LDH gene DNA sequences of these two strains, but there is no guarantee that all the strains are going to be the same. The complete DNA and amino acid sequences from these two strains are presented in Figure 1.

### Sequence Comparisons

Sequences are known for 41 LDH genes from a large number of species. Figure 2 presents an alignment of six LDH sequences made using the Genetics Computer Group program PILEUP and consideration of the crystal structures of *Bs*LDH (8) and pig  $M_4$ -LDH (9).

In the present study, *Pf*LDH was cloned from strains K1 and *PF* FCBR and the gene sequence was found to be identical with the published sequence from the *P. falciparum* strain Honduras 1 (5). *Pf*LDH has the key residues involved in catalysis (shown in bold italics in Figure 2): arginine-171 (binds pyruvate), aspartate-168 and histidine-195 (the proton donor couple), and arginine-109 (polarizes the pyruvate carbonyl group). The presence of so many characteristic LDH residues leaves no doubt about the cloned *P. falciparum* sequence codes for an LDH. This conclusion is supported by the observation that the amino terminal amino acid sequence of *P. knowlesi* LDH is essentially identical (19 out of 21 amino acids are identical) to that of *Pf*LDH (10). Although many characteristic residues are conserved, *Pf*LDH has 18 positions (shown underlined in Figure 2) where residues previously conserved in 40 gene-derived sequences are different. For example, in all known LDHs, residue 163 is a serine except *Pf*LDH, whereas residue 163 is a leucine, a threonine residue at position 246 is a proline. When

```

ATGGCTCCAAAAGCAAAAATCGTTTTAGTTGGCTCAGGTATGATTGGAGGAGTAATGGCT
1 -----+-----+-----+-----+-----+-----+ 60
TACCGAGGTTTTCGTTTTTAGCAAAATCAACCGAGTCCATACTAACCTCCTCATTACCGT
a: MetAlaProLysAlaLysIleValLeuValGlySerGlyMetIleGlyGlyValMetAla

ACCTTAATTGTTTCAGAAAAATTTAGGAGATGTAGTTTTGTTTCGATATTGTAAAGAACATG
61 -----+-----+-----+-----+-----+-----+ 120
TGGAAATTAACAAGTCTTTTTAAATCCTCTACATCAAAACAAGCTATAACATTTCTTTGTAC
a: ThrLeuIleValGlnLysAsnLeuGlyAspValValLeuPheAspIleValLysAsnMet

(1) →
CCACATGGAAAAGCTTTAGATACATCTCATACTAATGTTATGGCATATTCAAATTGCAAA
121 -----+-----+-----+-----+-----+-----+ 180
GGTGTACCTTTTCGAAATCTATGTAGAGTATGATTACAATACCGTATAAGITTAACGTTT
a: ProHisGlyLysAlaLeuAspThrSerHisThrAsnValMetAlaTyrSerAsnCysLys
← (12)
GTAAGTGGTTCAAACACTTATGACGATTTGGCTGGAGCAGATGTAGTAATAGTAACAGCT
181 -----+-----+-----+-----+-----+-----+ 240
CATTCCACCAAGTTTGTGAATACTGCTAAACCGACCTCGTCTACATCATTATCATTGTCGA
a: ValSerGlySerAsnThrTyrAspAspLeuAlaGlyAlaAspValValIleValThrAla
(2) → ← (11)
GGATTTACCAAGGCCCCAGGAAAGAGTGACAAAGAATGGAATAGAGATGATTTATTACCA
241 -----+-----+-----+-----+-----+-----+ 300
CCTAAATGGTTCGGGGTCCCTTTCTCACTGTTTCTTACCTTATCTCTACTAAATAATGGT
a: GlyPheThrLysAlaProGlyLysSerAspLysGluTrpAsnArgAspAspLeuLeuPro

TTAACAACAAGATTATGATTGAAATGGTGGTCATATTAAGAAGAATTGTCCAAATGCT
301 -----+-----+-----+-----+-----+-----+ 360
AATTTGTGTCTAATACTAACTTTAACCACCAGTATAATTCCTCTAACAGTTTACGA
a: LeuAsnAsnLysIleMetIleGluIleGlyGlyHisIleLysLysAsnCysProAsnAla
(3) →
TTTATTATTGTTGTAACAAACCCAGTAGATGTTATGGTACAATTATTACMTCAACATTCA
361 -----+-----+-----+-----+-----+-----+ 420
AAATAATAACAACATTGTTTGGGTCATCTACAATACCATGTTAATAATGTAGTTGTAAGT
a: PheIleIleValValThrAsnProValAspValMetValGlnLeuLeuHisGlnHisSer
← (10)
GGTGTTCCTAAAAACAAGATTATTGGTTTAGGTGGTATTAGATACATCAAGATTGAAG
421 -----+-----+-----+-----+-----+-----+ 480
CCACAAGGATTTTGTCTAATAACCAAATCCACCACATAATCTATGTAGTTCTAACTTC
a: GlyValProLysAsnLysIleIleGlyLeuGlyGlyValLeuAspThrSerArgLeuLys
← (9)

```

Figure 1. Complete DNA and amino acid sequences of *P. falciparum* strains K1 and PF FCBR LDH.

(4)→

TATTACATATCTCAGAAATTAATGTATGCCCAAGAGAT**TTAAATGCACACATTGTAGGT**  
 481 -----+-----+-----+-----+-----+-----+ 540  
 ATAATGTATAGAGTCTTTAATTTACATACGGGTCTCTACATTTACGTGTGTAACATCCA  
 a: TyrTyrIleSerGlnLysLeuAsnValCysProArgAspValAsnAlaHisIleValGly

GCTCATGGAAATAAAATGGTCTTTTAAAAAGATACATTACTGTAGTGGTATCCCTTTA  
 541 -----+-----+-----+-----+-----+-----+ 600  
 CGAGTACCTTTATTTACCAAGAAAATTTTCTATGTAAT**GACATCCACCATAGGGAAAT**  
 a: AlaHisGlyAsnLysMetValLeuLeuLysArgTyrIleThrValGlyGlyIleProLeu

← (8)

CAAGAATTTATTAATAACAAGTTAATTTCTGATGCTGAATTAGAAGCTATATTTGATAGA  
 601 -----+-----+-----+-----+-----+-----+ 660  
 GTTCTTAAATAATTATTGTTCAATTAAGACTACGACTTAATCTTCGATATAAACTATCT  
 a: GlnGluPheIleAsnAsnLysLeuIleSerAspAlaGluLeuGluAlaIlePheAspArg

(5)→

ACTGTTAATACTGCATTAGAAAATTGTAAAC**TTACATGCATCACCATATGT**TGCACCAGCT  
 661 -----+-----+-----+-----+-----+-----+ 720  
 TGACAATTATGACGTAATCTTTAACATTTGAATGTACGTAGTGGTATACAACGTGGTCGA  
 a: ThrValAsnThrAlaLeuGluIleValAsnLeuHisAlaSerProTyrValAlaProAla

GCTGTATTATCGAAATGGCTGAATCCTACTTAAAAGATTGAAAAAGTATTAATTTGC  
 721 -----+-----+-----+-----+-----+-----+ 780  
 CGAGATAATAGCTTTACCGACTTAGGATGAATTTTCTAAACTTTTTCATAATTAACG  
 a: AlaAlaIleIleGluMetAlaGluSerTyrLeuLysAspLeuLysLysValLeuIleCys

(6)→

TCAACCTTG**TTAGAAGGACAATATGGACAC**TCCGATATATTCGGTGGTACACCTGTGT  
 781 -----+-----+-----+-----+-----+-----+ 840  
 AGTTGGAACA**ATCTTCCTGTTATACCTGTG**AGGCTATATAAGCCACCATGTGGACAACAA  
 a: SerThrLeuLeuGluGlyGlnTyrGlyHisSerAspIlePheGlyGlyThrProValVal

← (7)

TTAGGTGCTAATGGTGTGAACAAGTTATCGAATTACAATTAATAAGTGAGGAAAAAGCT  
 841 -----+-----+-----+-----+-----+-----+ 900  
 AATCCACGATTACCACAACCTTGTTCAATAGCTTAATGTTAATTTATCACTCCTTTTTCGA  
 a: LeuGlyAlaAsnGlyValGluGlnValIleGluLeuGlnLeuAsnSerGluGluLysAla

AAATTTGATGAAGCCATAGCTGAAACTAAGAGAATGAAGGCATTAGCTTAA  
 901 -----+-----+-----+-----+-----+-----+ 951  
 TTTAAACTACTTCGGTATCGACTTTGATTCTCTTACTTCCGTAATCGAATT  
 a: LysPheAspGluAlaIleAlaGluThrLysArgMetLysAlaLeuAlaEnd

Figure 2. Sequence alignment of *PfLDH* to some other LDHs. Residues conserved in nearly all LDHs, including *PfLDH*, are in bold type, key catalytic residues are in bold italics, and residues conserved in nearly all LDHs but not in *PfLDH* are underlined.

		27	29	32	48	53	61	65	72	76	85
1	man-m	ATLKDQLIYNL	.LKEEQTPQNKITVVG	VGAVGMACAISILMKDLAD	EALVVDVIEDKLGEMMDLQHGSL	EEL	.RTPKIVSGKDYNV	TANSKILVI			
	pig-m	ATLKDQLIHNL	.LKEEHPHNKITVVG	VGAVGMACAISILMKELAD	EALVVDVIEDKLGEMMDLQHGSL	EEL	.RTPKIVSGKDYNV	TANSRLVV			
	dogf-m	ATLKDKLIGHLATSQ	EPRSYNKITVVG	VGAVGMACAISILMKDLAD	EALVVDVIEDKLGEMMDLQHGSL	EEL	.HTAKIVSGKDY	SVSAGSKLIV			
	man-h	ATLKEKLIAPVAEEEA	TPNNKITVVG	VGQVGMACAISILGKSLAD	EALVVDVIEDKLGEMMDLQHGSL	EEL	.QTPKIVADKDY	SVTANSKIIV			
	b-stearo	.....	MKNNGGARVVVIG	AGFVGAASYFALMQG	IAD <del>E</del> IVLIDANESKAI	GDAMD	FNHGKVEA	.PKPVDIWHGDYD	DCRDADLIV		
	malaria	.....	MAPKAKIVLVG	SGMIGVGMATLIV	QKNLGD.VVLEFIVK	NMPHGKALD	TSHNTVMAY	SNCKVSGSNTY	DDLADAGDVVI		
	man-m	98	109	110	116	143	156	163	168	171	173
	pig-m	ITAGARQOEGES	.....	RNLVQRNVNIFKFI	IPNVKYSNCKLLIV	SNPVDILITVAVW	KLISGFFKNRVIG	SGCNLD	SARFYL	MGERLGVHP	
	dogf-m	ITAGARQOEGES	.....	RNLVQRNVNIFKFI	IPNIKYSNCKLLIV	SNPVDILITVAVW	KLISGFFKNRVIG	SGCNLD	SARFYL	MGERLGVHP	
	man-h	ITAGARQOEGES	.....	RNLVQRNVNIFKFI	IPNIKYSNCKLLIV	SNPVDILITVAVW	KLISGFFKNRVIG	SGCNLD	SARFYL	MGERLGVHP	
	b-stearo	VTAGVRQOEGES	.....	RNLVQRNVNIFKFI	IPNIKYSNCKLLIV	SNPVDILITVAVW	KLISGFFKNRVIG	SGCNLD	SARFYL	MGERLGVHP	
	malaria	ICAGANQKPGET	.....	RDLVDKNI	ALFRSIVESVMASGF	QGLFLVATNPVDILITVAVW	KLISGFFKNRVIG	SGCNLD	SARFYL	MGERLGVHP	
	man-m	188	195	204	237	243	246	250	270		
	pig-m	LSCHGWVIG	<u>E</u> HG <u>D</u> SSVPVW	SGNVAGVSLKTLHPD	LITD	KDKEQWKEVHKQ	IVESAZEV	IKLGYT	SWAIGLSVAD	LAESIMKNLRRVHPVSTM	
	dogf-m	CSCHGWVIG	<u>E</u> HG <u>D</u> SSVPVW	SGNVAGVSLKTLHPD	LITD	KDKEQWKEVHKQ	IVESAZEV	IKLGYT	SWAIGLSVAD	LAESIMKNLRRVHPVSTM	
	man-h	SSCHGWVIG	<u>E</u> HG <u>D</u> SSVPVW	SGNVAGVSLKTLHPD	LITD	KDKEQWKEVHKQ	IVESAZEV	IKLGYT	SWAIGLSVAD	LAESIMKNLRRVHPVSTM	
	b-stearo	QNVHAYVIG	<u>E</u> HG <u>D</u> TEL	PVW	SOAYIGVMP	IRKLVESK	GEEAOKD	.LE	IFVNRDAA	QIIEKKGAT	YVGIAMGLARVTRAILHNENA
	malaria	RDVNAHIVG	ARGNKWLLKRYIT	VGGIPLQEF	INNK	.LISDAE	LEAIF	DRVT	NTALEIV	NLHASP	VVAPAAAIIEMAESY
	man-m	280	290	311	318	322					
	pig-m	IKGLYGIKDDVFLSVP	CILGQNGISDLV	KVTLTSEEA	RLLKKSADTL	WGIQKELQF					
	dogf-m	IKGLYGIKENVFLSVP	CILGQNGISDVV	KVTLTPEEA	HLKKSADTL	WGIQKELQF					
	man-h	VKDFYGIKDNVFLSIP	CVLNDHGISNIV	KMLKPD	EEQQLQK	SATLWDIQDKLKF					
	b-stearo	VKGMVGIENEVFLSIP	CVLNDHGISNIV	KMLKPD	EEQQLQK	SATLWDIQDKLKF					
	malaria	LDGLYGER	.DVIYIGVAVIN	RNGIREVIEI	ELNDDEK	NRFFHS	SAATL	KS	VLARAFTR		

*PfLDH* is compared to *BsLDH* and mammalian LDHs (by PILEUP), it has 29% residue identities with *BsLDH* and 29%, 31% and 33% with dogfish, human-M<sub>4</sub>, and pig-M<sub>4</sub> LDHs, respectively, but lacks the amino terminal extension observed in mammalian LDHs. All this suggests that *PfLDH* is very different from bacterial and mammalian LDHs.

*P. falciparum* LDH contains several single amino acid deletions and insertions compared to other LDHs (see Figure 2). *PfLDH* has two single residue deletions; the first deletion is a glutamate residue at position 48 and the second one is a glycine residue at position 217 with respect to *BsLDH*. The deletion of glycine-217 occurs in the highly variable antigenic loop region of the protein.

A single residue insertion occurs between the residues alanine-73 and serine-74, which is a tyrosine, but the most remarkable feature of *PfLDH* is a five residue insertion (D<sub>108A</sub> K<sub>108B</sub> E<sub>108C</sub> W<sub>108D</sub> N<sub>108E</sub>) between positions serine-108 and arginine-109 in the catalytic loop. The insertion of amino acid residues itself is not unique to *PfLDH*. For example, the active site loop of the broad specificity LDH from the bacterium *Lactobacillus confusus* carries an extra four amino acids (11) but the region in *L. confusus* is disordered in the X-ray structure (12). The presence of the enlarged loop in *L. confusus* has not been adequately explained.

## Conclusion

As described in this paper, the DNA sequences of *PfLDH* strains that have been cloned are the same. The most remarkable feature of *PfLDH* is the insertion of five amino acid residues (DKEWN) adjacent to the active site, which is likely to provide a good target for the rational design of antimalarials. After the DNA was sequenced from both directions, the *PfLDH* protein was overproduced from the *P. falciparum* strain K1 (13) and the enzyme's ternary structure was determined (14). The reported *PfLDH* structure showed that *PfLDH* has a distinctive surface cleft, caused by the insertion of the five residues, which does not occur in mammalian LDHs. This comparison immediately suggests that the site is an attractive target for the development of inhibitors specific for the malarial enzyme. In addition to the structure study, mutagenic testing of the *PfLDH* with and without this unique insertion may provide more information on distinctive kinetic, biochemical and electrophoretic properties of *PfLDH*. We believe that exploitation of these unique differences will open a route to the design of new antimalarial agents.

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