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 (*Pf*LDH) to some other known LDH sequences showed that *Pf*LDH has 29% residue identities with B*acillus stearothermophilus* (*Bs*LDH) and 29%, 31%, 33% with dogfish, man-M$_4$, and pig-M$_4$ LDHs, respectively. It was also shown that *Pf*LDH 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 Antimalariallerın 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 biribirinin aynı olduğu bulunmuştur. *P. falciparum* soyları K1 ve *PF* FCBR'ın LDH'lerinin (*Pf*LDH) amino asit dizilerinin, amino asit dizileri bilinen diğer bazı LDH dizileri ile karşılaştırılması *Pf*LDH'in; *Bacillus stearothermophilus* LDH'i (*Bs*LDH) ile % 29, köpek balığı LDH'i ile % 29, insan-M$_4$ LDH'i ile % 31 ve domuz-M$_4$ LDH'i ile de % 33 oranında aynı aminoasitleri içerdiği gösterilmiştir. *Pf*LDH 'in diğer LDH'lere göre amino asit ilaveleri ve tek aminoasit eksiklikleri olduğuda gösterilmiştir. Glutamat-48 ve glisin-217 eksik olan iki aminoasittir. *Pf*LDH, 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 (*Pf*LDH) 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 *Pf*LDH 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 *Pf*LDH, and several *Pf*LDH cDNA clones were isolated by screening a λgt11Tn5 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 *Pf*LDH 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 *Pf*LDH strain would work against all other strains.

To this end, the *Pf*LDH 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⁺ lacIq lacZ*ΔM15 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 µl *Taq* buffer (supplied with enzyme), 5 µl (10ml of each 100 mM dNTPs and 10 ml of H_2O) stock dNTPs, 2.5 µl (at 20 pmoles) N-term. primer, 2.5 µl (at 20 pmoles) C-term. primer, 1 µl (of 0.5 µg/µl) genomic DNA, 2.5 units *Taq* DNA polymerase and 33.5 ml H_2O to the final volume of 50µl. A layer of mineral oil was placed over the reaction mixture to prevent evaporation. The PCR was carried out at 94ºC for 1.5 min, 55ºC for 2 min, and 72ºC 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 µl 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 68ºC 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 µl 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 -20ºC 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 µl sterile H_2O for the second ethanol precipitation. After this the DNA was dissolved in an appropriate volume of sterile H_2O.

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 µl DNA (from phenol extraction), 3 µl10 x buffer (supplied with enzymes), 2 µl (of 26 U/µl) *Eco* RI, 2 µl (of 10 U/µl) *Pst* I, 2 µl RNAse and 1 µl H_2O. Restriction digests were performed in a waterbath, at 37ºC, 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 μl 10 x Ligase buffer, 2 μl (1 U/μl) T4 DNA ligase and X μl H$_2$O to final volume of 20 ml. Ligations were set up at 16ºC 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 μg/ml ampicillin. It was grown overnight at 37ºC 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'-AGCGGATAACAATTTCACACAGGA-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 37ºC 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

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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.

*Pf*LDH 5'→3' sequencing primers	*Pf*LDH 3'→5' sequencing primers
5'-CCACATGGAAAAGCTTTAGA-3'(1)	5'-GTGTCCATATTGTCCTTCTA-3'(7)
5'-AGGCCCCAGGAAAGAGTGAC-3'(2)	5'-TAAAGGGATACCACCTACAG-3'(8)
5'-TCAACATTCAGGTGTTCCTA-3'(3)	5'-GATGTATCTAATACACCACC-3'(9)
5'-TAAATGCACACATTGTAGGT-3'(4)	5'-GTACCATAACATCTACTGGG-3'(10)
5'-TTACATGCATCACCATATGT-3'(5)	5'-CAAATCGTCATAAGTGTTTG-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
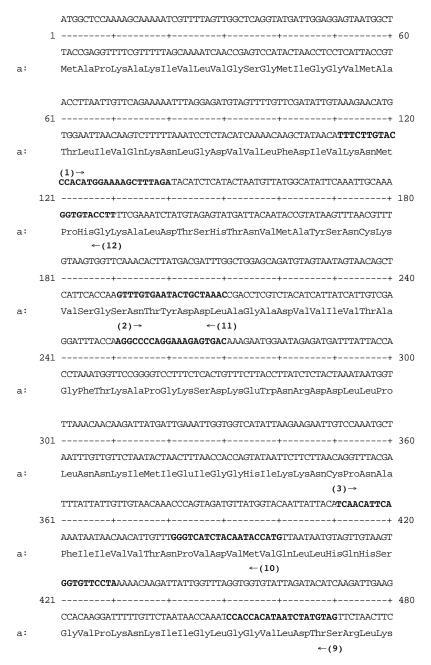
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      ATGGCTCCAAAAGCAAAAATCGTTTTTAGTTGGCTCAGGTATGATTGGAGGAGTAATGGCT
   1  ---------+---------+---------+---------+---------+---------+ 60
      TACCGAGGTTTTCGTTTTTAGCAAAATCAACCGAGTCCATACTAACCTCCTCATTACCGT
a:    MetAlaProLysAlaLysIleValLeuValGlySerGlyMetIleGlyGlyValMetAla


      ACCTTAATTGTTCAGAAAAATTTAGGAGATGTAGTTTTGTTCGATATTGTAAAGAACATG
  61  ---------+---------+---------+---------+---------+---------+ 120
      TGGAATTAACAAGTCTTTTTAAATCCTCTACATCAAAACAAGCTATAACATTTCTTGTAC
a:    ThrLeuIleValGlnLysAsnLeuGlyAspValValLeuPheAspIleValLysAsnMet

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


      TTAAACAACAAGATTATGATTGAAATTGGTGGTCATATTAAGAAGAATTGTCCAAATGCT
 301  ---------+---------+---------+---------+---------+---------+ 360
      AATTTGTTGTTCTAATACTAACTTTAACCACCAGTATAATTCTTCTTAACAGGTTTACGA
a:    LeuAsnAsnLysIleMetIleGluIleGlyGlyHisIleLysLysAsnCysProAsnAla
                                                     (3)→
      TTTATTATTGTTGTAACAAACCCAGTAGATGTTATGGTACAATTATTACATCAACATTCA
 361  ---------+---------+---------+---------+---------+---------+ 420
      AAATAATAACAACATTGTTTGGGTCATCTACAATACCATGTTAATAATGTAGTTGTAAGT
a:    PheIleIleValValThrAsnProValAspValMetValGlnLeuLeuHisGlnHisSer
                                            ←(10)
      GGTGTTCCTAAAAACAAGATTATTGGTTTAGGTGGTGTATTAGATACATCAAGATTGAAG
 421  ---------+---------+---------+---------+---------+---------+ 480
      CCACAAGGATTTTTGTTCTAATAACCAAATCCACCACATAATCTATGTAGTTCTAACTTC
a:    GlyValProLysAsnLysIleIleGlyLeuGlyGlyValLeuAspThrSerArgLeuLys
                                            ←(9)
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Figure 1.   Complete DNA and amino acid sequences of *P. falciparum* strains K1 and *PF* FCBR LDH.

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                                              (4)→
        TATTACATATCTCAGAAATTAAATGTATGCCCAAGAGATGTAAATGCACACATTGTAGGT
    481 ---------+---------+---------+---------+---------+---------+ 540
        ATAATGTATAGAGTCTTTAATTTACATACGGGTTCTCTACATTTACGTGTGTAACATCCA
  a:    TyrTyrIleSerGlnLysLeuAsnValCysProArgAspValAsnAlaHisIleValGly


        GCTCATGGAAATAAAATGGTTCTTTTAAAAAGATACATTACTGTAGGTGGTATCCCTTTA
    541 ---------+---------+---------+---------+---------+---------+ 600
        CGAGTACCTTTATTTTACCAAGAAAATTTTTCTATGTAATGACATCCACCATAGGGAAAT
  a:    AlaHisGlyAsnLysMetValLeuLeuLysArgTyrIleThrValGlyGlyIleProLeu
                                                            ←(8)


        CAAGAATTTATTAATAACAAGTTAATTTCTGATGCTGAATTAGAAGCTATATTTGATAGA
    601 ---------+---------+---------+---------+---------+---------+ 660
        GTTCTTAAATAATTATTGTTCAATTAAAGACTACGACTTAATCTTCGATATAAACTATCT
  a:    GlnGluPheIleAsnAsnLysLeuIleSerAspAlaGluLeuGluAlaIlePheAspArg
                                              (5)→
        ACTGTTAATACTGCATTAGAAATTGTAAACTTACATGCATCACCATATGTTGCACCAGCT
    661 ---------+---------+---------+---------+---------+---------+ 720
        TGACAATTATGACGTAATCTTTAACATTTGAATGTACGTAGTGGTATACAACGTGGTCGA
  a:    ThrValAsnThrAlaLeuGluIleValAsnLeuHisAlaSerProTyrValAlaProAla


        GCTGCTATTATCGAAATGGCTGAATCCTACTTAAAAGATTTGAAAAAAGTATTAATTTGC
    721 ---------+---------+---------+---------+---------+---------+ 780
        CGACGATAATAGCTTTACCGACTTAGGATGAATTTTCTAAACTTTTTTCATAATTAAACG
  a:    AlaAlaIleIleGluMetAlaGluSerTyrLeuLysAspLeuLysLysValLeuIleCys
                (6)→
        TCAACCTTGTTAGAAGGACAATATGGACACTCCGATATATTCGGTGGTACACCTGTTGTT
    781 ---------+---------+---------+---------+---------+---------+ 840
        AGTTGGAACAATCTTCCTGTTATACCTGTGAGGCTATATAAGCCACCATGTGGACAACAA
  a:    SerThrLeuLeuGluGlyGlnTyrGlyHisSerAspIlePheGlyGlyThrProValVal
                                  ←(7)
        TTAGGTGCTAATGGTGTTGAACAAGTTATCGAATTACAATTAAATAGTGAGGAAAAAGCT
    841 ---------+---------+---------+---------+---------+---------+ 900
        AATCCACGATTACCACAACTTGTTCAATAGCTTAATGTTAATTTATCACTCCTTTTTCGA
  a:    LeuGlyAlaAsnGlyValGluGlnValIleGluLeuGlnLeuAsnSerGluGluLysAla


        AAATTTGATGAAGCCATAGCTGAAACTAAGAGAATGAAGGCATTAGCTTAA
    901 ---------+---------+---------+---------+---------+- 951
        TTTAAACTACTTCGGTATCGACTTTGATTCTCTTACTTCCGTAATCGAATT
  a:    LysPheAspGluAlaIleAlaGluThrLysArgMetLysAlaLeuAlaEnd
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Figure 2. Sequence alignment of *Pf*LDH to some other LDHs. Residues conserved in nearly all LDHs, including *Pf*LDH, are in bold type, key catalytic residues are in bold italics, and residues conserved in nearly all LDHs but not in *Pf*LDH are underlined.

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              1                 27 29 32         48      53      61  65      72    76        85
man-m     ATLKDQLIYNL.LKEEQTPQNKITVVGVGAVGMACAISILMKDLADELALVDVIEDKLKGEMMDLQHGSLFL.RTPKIVSGKDYNVTANSKLVI
pig-m     ATLKDQLIHNL.LKEEHVPHNKITVVGVGAVGMACAISILMKELADEIALVDVMEDKLKGEMMDLQHGSLFL.RTPKIVSGKDYNVTANSRLVV
dogf-m    ATLKDKLIGHLATSQEPRSYNKITVVGVGAVGMACAISILMKDLADEVALVDVMEDKLKGEMMDLQHGSLFL.HTAKIVSGKDYSVSAGSKLVV
man-h     ATLKEKLIAPVAEEEATVPNNKITVVGVGQVGMACAISILGKSLADELALVDVLEDKLKGEMMDLQHGSLFL.QTPKIVADKDYSVTANSKIVV
b-stearo  ............MKNNGGARVVVIGAGFVGASYVFALMNQGIADEIVLIDANESKAIGDAMDFNHGKVEA.PKPVDIWHGDYDDCRDADLVV
malaria   .............MAPKAKIVLVGSGMIGGVMATLIVQKNLGD.VVLFDIVKNMPHGKALDTSHTNVMAYSNCKVSGSNTYDDLAGADVVI

              98      109 110  116           140 143        156     163  168 171 173
man-m     ITAGARQQEGES.....RLNLVQRNVNIFKFIIPNVVKYSPNCKLLIVSNPVDILTYVAWKISGFPKNRVIGSGCNLDSARFRYLMGERLGVHP
pig-m     ITAGARQQEGES.....RLNLVQRNVNIFKFIIPNIVKYSPNCKLLVVSNPVDILTYVAWKISGFPKNRVIGSGCNLDSARFRYLMGERLGVHP
dogf-m    ITAGARQQEGES.....RLNLVQRNVNIFKFIIPNIVKHSPDCIILVVSNPVDVLTYVAWKLSGLPMHRIIGSGCNLDSARFRYLMGERLGVHS
man-h     VTAGVRQQEGES.....RLNLVQRNVNVFKFIIPQIVKYSPDCIIIVVSNPVDILTYVTMKLSGLPKHRVIGSGCNLDSARFRYLMAEKLGIHP
b-stearo  ICAGANQKPGET.....RLDLVDKNIAIFRSIVESVMASGFQGLFLVATNPVDILTYATWKFSGLPHERVIGSGTILDTARFRFLLGEYFSVAP
malaria   VTAGFTKAPGKSDKEWNRDDLLPLNNKIMIEIGGHIKKNCPNAFIIVVTNPVDVMVQLLHQHSGVPKNKIIGLGGVLDTSRLKYYISQKLNVCP

              188     195       204                    237   243 246 250            270
man-m     LSCHGWVLGEHGDSSVPVWSGMNVAGVSLKTLHPDLGTDKDKEQWKEVHKQVVESAYEVIKLKGYTSWAIGLSVADLAESIMKNLRRVHPVSTM
pig-m     LSCHGWIIGEHGDSSVPVWSGVNVAGVSLKNLHPELGTDADKEHWKAVHKEVVDSAYEVIKLKGYTSWAIGLSVADLAESIMKNLRRVHPISTM
dogf-m    CSCHGWVIGEHGDSVPSVWSGMWNA...LKELHPELGTNKDKQDWKKLHKDYVDSAYEVIKLKGYTSWAIGLSVADLAETIMKNLCRVHPVSTM
man-h     SSCHGWIIGEHGDSSVAVWSGVNVAGVSLQELNPEMGTDNDSENWKEVHKMVESAYEVIKLKGYTNWAIGLSVADLIESMLKNLSRIHPVSTM
b-stearo  QNVHAYIIGEHGDTELPVWSQAYIGVMPIRKLVESKGEEAQKD.LERIFVNVRDAAYQIIEKKGATYYGIAMGLARVTRAILHNENAILTVSAY
malaria   RDVNAHIVGAHGNKMVLLKRYITVGGIPLQEFINNK.LISDAELEAIFDRTVNTALEIVNLHASPYVAPAAAIIEMAESYLKDLKKVLICSTL

              280     290         311         318 322
man-m     IKGLYGIKDDVFLSVPCILGQNGISDLVKVTLTSEEEARLKKSADTLWGIQKELQF.
pig-m     IKGLYGIKENVFLSVPCILGQNGISDVVKVTLTPEEEAHLKKSADTLWGIQKELQF.
dogf-m    VKDFYGIKDNVFLSLPCVLNDHGISNIVKMKLKPDEEQQLQKSATTLWDIQKDLKF.
man-h     VKGMYGIENEVFLSLPCILNARGLTSVINQKLKDDEVAQLKKSADTLWDIQKDLKDL
b-stearo  LDGLYGER.DVYIGVPAVINRNGIREVIEIELNDDEKNRFHHSAATLKSVLARAFTR.
malaria   LEGQYGHSDIFGGTPVVLGANGVEQVIELQLNSEEKAKFDEAIAETKRMKALA*....
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*Pf*LDH is compared to *Bs*LDH and mammalian LDHs (by PILEUP), it has 29% residue identities with *Bs*LDH and 29%, 31% and 33% with dogfish, human-M_4, and pig-M_4 LDHs, respectively, but lacks the amino terminal extension observed in mammalian LDHs. All this suggests that *Pf*LDH 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). *Pf*LDH 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 *Bs*LDH. 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 *Pf*LDH is a five residue insertion (D_{108A} K_{108B} E_{108C} W_{108D} N_{108E}) between positions serine-108 and arginine-109 in the catalytic loop. The insertion of amino acid residues itself is not unique to *Pf*LDH. 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 *Pf*LDH strains that have been cloned are the same. The most remarkable feature of *Pf*LDH 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 *Pf*LDH protein was overproduced from the *P. falciparum* strain K1 (13) and the enzyme's ternary structure was determined (14). The reported *Pf*LDH structure showed that *Pf*LDH 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 *Pf*LDH with and without this unique insertion may provide more information on distinctive kinetic, biochemical and electrophoretic properties of *Pf*LDH. We believe that exploitation of these unique differences will open a route to the design of new antimalarial agents.

Acknowledgements

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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