Sequence and expression of the *Mycobacterium leprae* *dnaJ* gene

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Study of *Mycobacterium leprae*, the causative agent of leprosy, has been advanced by the isolation of genes encoding mycobacterial proteins including *dnaK* encoding the *M. leprae* 70 kDa heat shock protein. The sequence downstream from *dnaK* revealed a second open reading frame coding for a protein of 389 amino acids with a calculated molecular mass of 41·2 kDa. Sequence analysis demonstrated significant DNA homology with the *dnaJ* gene of other organisms. High amino acid sequence identity was obtained between the DnaJ protein of *M. leprae* and *M. tuberculosis* (89%) with significant divergence between the two occurring only at the C-terminal end. The expressed recombinant DnaJ protein had a molecular mass of 42 kDa.

Introduction

Mycobacterium leprae, which is a significant human pathogen, cannot be cultivated outside an animal host. This has provided difficulties for the biochemical and immunological study of *M. leprae* proteins. The molecular cloning of *M. leprae* DNA has offered an alternative method for the characterization and production of *M. leprae* proteins (Young *et al.*, 1985).

The gene encoding a significant *M. leprae* antigen, the 70 kDa heat shock protein and DnaK homologue (p70), was cloned by identification of the gene product with a mycobacterial specific mouse monoclonal antibody L7 (Britton *et al.*, 1985; McKenzie *et al.*, 1991). In *Escherichia coli*, the *dnaK* heat shock promoter controls an operon containing the *dnaK* gene and a second heatshock gene, *dnaJ* (Cowing *et al.*, 1985). Initial sequencing downstream of the *M. leprae* p70 gene revealed an open reading frame, the translational product of which demonstrates some homology at the amino acid level with the *dnaJ* gene of *E. coli* (Garsia, 1989). Alignment of the *M. leprae* and *E. coli* sequences indicated that the *M. leprae* *dnaJ* gene begins 700 bp 3' to the *dnaK* TGA stop codon. Recent analysis of the *dnaK*–*grpE*–*dnaJ* gene cluster of *M. tuberculosis* demon-

strated that the *M. tuberculosis* *dnaJ* gene begins 792 bp downstream of the *dnaK* (Young *et al.*, 1988; Lathigra *et al.*, 1988; D. Young, personal communication). We have now isolated the complete *M. leprae* *dnaJ* gene and in this report present its DNA sequence and the expression of the recombinant *M. leprae* DnaJ protein.

Methods

DNA sequence analysis. A 3·6 kb *Eco*RI fragment of the cosmid clone ML231 (McKenzie *et al.*, 1991) was subcloned into the plasmid vector pBluescript SK and designated pSKdnaJ. The 3·6 kb fragment contained the 3' part of the *M. leprae* *dnaK* gene, previously reported as the fragment JKL2 (Garsia *et al.*, 1989; McKenzie *et al.*, 1991), and an additional 1·5 kb of *M. leprae* DNA. pSKdnaJ was denatured (Zhang *et al.*, 1988) prior to sequencing with the Sequenase version 2·0 DNA sequencing kit (United States Biochemical Corp.) according to the manufacturer's instructions. Synthetic oligonucleotides designed using the DNA sequence downstream of *M. leprae* *dnaK* (Garsia, 1989) served as primers. Both strands of DNA were sequenced and the amino acid sequence of the open reading frame deduced. Homology searches used the MATCHFASTA programs of Pearson & Lipman (1988) and sequence comparisons were made using the GCG package (Genetics Computer Group Inc., Madison, WI, USA). The databases were accessed via ANGIS (Australian National Genomic Information Service, University of Sydney).

Expression of recombinant *M. leprae* *dnaJ*. The *M. leprae* *dnaJ* gene was ligated to the 3' end of the coding sequence for the C-terminal portion of the glutathione *S*-transferase (GST) of *Schistosoma japonicum*, under the control of the *lacZ* promoter, in the expression vector pGEX-2T (Smith & Johnson, 1988). A 1·5 kb fragment of DNA was amplified from plasmid pSKdnaJ by polymerase chain reaction (PCR) (Higuchi *et al.*, 1988). An oligonucleotide complementary to the 5' end of the *M. leprae* *dnaJ* gene and the pBluescript reverse sequencing primer were used as primers in the PCR reaction. The PCR DNA product, containing a unique 3' *Eco*RI site derived from pBluescript, was digested with *Eco*RI and ligated between the *Sma*I

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Abbreviation: GST, glutathione *S*-transferase.

The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession number M95576.

TCACCGAAGGCAATCCACACGAAACAGGTGACGGTCACCGACAAACGACGGATCGATCCCGATACTGGCGAAGTACGATCT	80
* ATACCTCTCGCGACACGCCGGGAGGTAGCGAGCCGGCTGGTATGACCGAAGTGCCAAACAGTACCGACACAATGGTCCGAC	160
AAAGTTGCTGAGCTGACTAGCGATCTGCAGCGGGTGCAGGCGCGATTTTGCCAACTACCGTAAGCGAGCGTTGCGCGACCA	240
GCAGACGGCTTCGGATCGAGCCAAGGCGAGTGTATCAGCCAATTGTTGGGTGTGCTTGACGATTTTCGATCGTGCCCGCG	320
AGCACGGCGATCTGGACTCGGGTCCGCTGAAGTCGGTTCGCCGACAAGCTAAGAGCGCATTCACCGGGCTCGGGCTGGTGG	400
CGTTTGGCGTCGAGGGAGAAGACTTTCGACCCGGTGTCTGCACGAAGCCGTGCAGCATGAGGGCGATGGTGGGGAAGGCTCC	480
AAGCCGGTGATCGGTGACGTCCTCCGGCACGGCTACAAATTGGGCGATCAAGTGCTGCGGCACGCCTTGGTCGGGGTGGT	560
TGACACGATCGCAGGGCGATGGTGGCGAAACGGTTGCCATAGTCGCACCTGTGACAGCACAGCCAAAACCGAGCAAGGTG	640
AACTGGGCGATAAGGTGACACCGCACAAAGGAGGACGGTGGTAGGGGGTGCACGCGAT ATG GCC CAG CAT GAA TGG	714
met ala gln arg glu trp	6
GTC GAA AAG GAC TTC TAC AAG GAG CTA GGC GTT TCC TCT GAC GCC AGT CCC GAA GAG ATT	774
val glu lys asp phe tyr lys glu leu gly val ser ser asp ala ser pro glu glu ile	26
AAC CGT GCC TAT CGG AAG TTG GCA CGC TAC CTT CAT CCT GAC GCT AAT CCC GAC AAC TCC	834
asn arg ala tyr arg lys leu ala arg tyr leu his pro asp ala asn pro ala asn ser	46
TCT GGT GAA CGG TTT AAG GTG GTG TCT GAG GCG CAC AAT GTG TTG TCG GAT CCG GTC AAG	894
ser gly glu arg phe lys val val ser glu ala his asn val leu ser asp pro val lys	66
CGC AAA GAG TAC GAT GAA ACT CGC CGG CTG TTT GCC GGC GGC TGC TTA GGC GGT CGT CGG	954
arg lys glu tyr asp glu thr arg arg leu phe ala gly gly cys leu gly gly arg arg	86
TTC GAC AGT AGT TTT GGT GAC TTT GGA ATG GGC GGC GAC GGT GCT GAG TTC AAC CTC AAC	1014
phe asp ser ser phe gly asp phe gly met gly gly asp gly ala glu phe asn leu asn	106
GAC TTG TTC GAT GCT GCC AGT CGA ACC GGG GGC ACC AAC ATC GGT GAC TTG TTC GGT GGC	1074
asp leu phe asp ala ala ser arg thr gly gly thr asn ile gly asp leu phe gly gly	126
TTG TTT GGA CGC GGC GCC AGT GTC CGC CCC AGT AGC CGA CGC GGC AAC GAC CTG GAG ACC	1134
leu phe gly arg gly ala ser val arg pro ser ser arg arg gly asn asp leu glu thr	146
GAG GCA GAT TTG GAT TTT GTC GAA GCG GCC AAA GGT GTA GCG ATG CCG CTG CGG CTG ACC	1194
glu ala asp leu asp phe val glu ala ala lys gly val ala met pro leu arg leu thr	166
AGC CCA GCG CCG TGC ACC AAT TGC CAT GGC AGC GGG GCA CCT CCG GGA ATG AGT CCG AAA	1254
ser pro ala pro cys thr asn cys his gly ser gly ala arg pro gly ile ser pro lys	186
GTG TGT TCC ACT TGC AAC GGT TCC GGC GTG ATC AAC CGC AAT CAG GGT GCC TTT GGT TTC	1314
val cys ser thr cys asn gly ser gly val ile asn arg asn gln gly ala phe gly phe	206
TCT GAG CCT TGT ACC GAA TGT CGG GGT AGC GGG TCG ATC ATC GAG CAC CTC CGC GAA GAG	1374
ser glu pro cys thr glu cys arg gly ser gly ser ile ile glu his leu arg glu glu	226
TGC AAA GGC ACC GGG GTT ACC ACC CGG ACC CGA ACC ATC AAT GTG CGG ATC CCG TCC GGT	1434
cys lys gly thr gly val thr thr arg thr arg thr ile asn val arg ile pro ser gly	246
GTC GAA GAT GGA CAA CGG ATT CGG CTG GCA GGG CAG GGC GAG GCG GGG TTG CGT GGC GCT	1494
val glu asp gly gln arg ile arg leu ala gly gln gly glu ala glu leu arg gly ala	266
CCG TCG GGT GAT CTG TAT GTG ACG GTC CAT GTG CGG CCT GAC AAG ATC TTT GGT CGA AAT	1554
pro ser gly asp leu tyr val thr val his val arg pro asp lys ile phe gly arg asn	286
GGC GAC GAC CTC ACC GTG ACC ATT CCG GTT AGC TTC ACT GAA TTA GCT TTG GGC TCA ACC	1614
gly asp asp leu thr val thr ile pro val ser phe thr glu leu ala leu gly ser thr	306

CTT TCA GTG CCC ACG CTG GAC GGA ACA GTC GGT GTC CGG GTG CCA AAA GGT ACC TCA GAT	1674
leu ser val pro thr leu asp gly thr val gly val arg val pro lys gly thr ser asp	326
GGC CGT ATC CTG CGG GTA CGA GGG CGT GGT GTA CCC AAA CGC AGT GGT GGC CAT GGC GAC	1734
gly arg ile leu arg val arg gly arg gly val pro lys arg ser gly gly his gly asp	346
TTG CTT GCT ACA GTC AAG GTG GCA GTT CCG CTG AAC TTG GAA GGT GCC GCG CAA AAG GCA	1794
leu leu ala thr val lys val ala val pro leu asn leu glu gly ala ala gln lys ala	366
CTG GAG GCC TAT+GCC GCC GCC GAG CGT CAA GTG GTT TCA ACC CTC GGG CGG GAT GGG GCA	1854
leu glu ala tyr ala ala ala glu arg gln val val ser thr leu gly arg asp gly ala	386
GGT AAT CGG TGATGACTAGAACTCTAGAGACCCAGGCAACAAGAATCCCGGACGTTTTTGTCTCGGCGGCTGC	1930
gly asn arg * *	389

Fig. 1. Nucleotide and deduced amino acid sequence of the *M. leprae* *dnaJ* gene. Potential promotor sequences are boxed. A Shine–Dalgarno (S–D) sequence is underlined; * represents a stop codon. The numbering of nucleotides begins with the stop codon of the *dnaK* open reading frame and numbering of amino acids is from the start of the coding region. Amino acids are represented by their three letter abbreviations.

and *EcoRI* sites of the expression vector pGEX-2T. The presence, in frame, of the *M. leprae* *dnaJ* gene was confirmed by sequencing from the 3' end of the GST coding sequence within the new construct. Recombinant *M. leprae* DnaJ protein fused to GST (DnaJf) was cleaved with thrombin to yield soluble DnaJ (DnaJc) (Smith & Johnson, 1988; McKenzie *et al.*, 1991).

Electrophoresis. The recombinant proteins were separated by 12% SDS-PAGE and transferred to nitrocellulose paper as described (McKenzie *et al.*, 1991). The immunoblots were probed with antisera from Balb/c mice immunized with recombinant cleaved DnaJ in incomplete Freund's adjuvant.

Results and Discussion

DNA sequence of the *M. leprae* *dnaJ* gene

A total of 1930 bp of DNA sequence was determined downstream of the *M. leprae* *dnaK* gene. The largest open reading frame within this region begins 700 nucleotides downstream of the *dnaK* gene and encodes a protein of 389 amino acids with a calculated molecular mass of 41.2 kDa (Fig. 1). Sequence homology of this open reading frame with the sequences of *dnaJ* from other organisms strongly supported the notion that the sequence encoded a DnaJ equivalent protein in *M. leprae*. A Shine–Dalgarno sequence was identified immediately upstream of the DnaJ coding region, in addition to several possible promoters. Expression of *dnaJ* may therefore be under the control of its own promoter in *M. leprae* rather than using the same promoter as the *dnaK* gene, as has been suggested for other bacteria, including *M. tuberculosis* (Dale & Patki, 1990) and *E. coli* (Cowing *et al.*, 1985).

Expression of the recombinant *M. leprae* DnaJ protein

The *M. leprae* *dnaJ* gene was cloned into the expression vector pGEX-2T resulting in the production of recombinant *M. leprae* DnaJ protein fused to GST (DnaJf),

with a molecular mass of 66 kDa (Fig. 2a). The fusion protein reacted strongly with polyclonal rabbit anti-GST antisera (data not shown) and murine anti *M. leprae* DnaJc (Fig. 2b). Cleavage of this fusion protein with thrombin resulted in the release of recombinant *M. leprae* DnaJ protein (DnaJc) with a molecular mass of 42 kDa (Fig. 2). The DnaJc protein was partially degraded by thrombin resulting in additional immunoreactive bands of 32 kDa and less than 15 kDa in the DnaJc sample (Fig. 2).

Comparison of the DnaJ protein sequences

The amino acid sequence of the *M. leprae* DnaJ protein was compared with those in the GenBank and EMBL data bases. A comparison of the amino acid sequence of *M. leprae* DnaJ with those from the prokaryotes *M. tuberculosis* and *E. coli* and the eukaryotic yeast *Saccharomyces cerevisiae* is shown in Fig. 3. The maximum amino acid sequence identity (89%) was obtained with the DnaJ protein of the closely related *M. tuberculosis*. The *M. leprae* DnaJ protein is 33 amino acids (9%) longer than that of *M. tuberculosis* (356 amino acids) and considerable sequence divergence was observed at the C-terminal end, where 80% of the 15 C-terminal residues of *M. tuberculosis* varied from the *M. leprae* sequence. Conservation is highest in the internal two-thirds of DnaJ with 94% identity occurring between amino acids 97–335 in the proteins of *M. leprae* and *M. tuberculosis*.

The lower degree of conservation (24–42% amino acid identity) shown between the DnaJ proteins of mycobacteria and those of other organisms (including *E. coli*, *Bacillus subtilis*, *Caulobacter crescentus*, *S. cerevisiae* and *Homo sapiens*) contrasts with the 45–55% sequence identity observed between the prokaryotic and eukaryotic 65 and 70 kDa heat shock proteins (McKenzie *et al.*,

Fig. 2. SDS-PAGE and immunoblot of recombinant *M. leprae* DnaJ. The separated proteins (10 µg per track) were stained with Coomassie Brilliant Blue (a). The proteins (2 µg per track) were transferred to nitrocellulose and probed with murine anti-DnaJ antiserum (b). Tracks: 1, mol. mass markers; 2, GST-DnaJ fusion protein (DnaJf); 3, DnaJ cleaved protein (DnaJc); 4, GST. The position of the cleaved DnaJc protein is indicated by an arrowhead.

1991; Young *et al.*, 1988). In the case of the *E. coli* and mycobacterial DnaJ proteins, there are two regions of greater conservation: residues 10–40 (50% identity) and 238–277 (68% identity). In contrast, there is a gap in the *E. coli* protein corresponding to positions 100–121 in the *M. leprae* product. The four tandem repeats of a motif consisting of Cys-X-X-Cys-X-Gly-X-Gly identified in *M. tuberculosis* and *E. coli* DnaJ proteins (Lathigra *et al.*, 1988) are also conserved in the *M. leprae* DnaJ protein (Fig. 3), except that the fourth copy lacks the initial Cys residue. The conserved regions may be significant in the proposed function of DnaJ in binding to subunits during the assembly of multimeric proteins (Langer *et al.*, 1992). The highly conserved N-terminal region of the amino acid sequence of the related 70 kDa heat shock protein is necessary for the ATPase activity of the molecule (Peake *et al.*, 1991). In contrast, the C-terminal region of Hsp70 varies between species, with the terminal 70 residues of *M. leprae* being completely divergent from those of the *M. tuberculosis* protein (McKenzie *et al.*, 1991). Divergence of the mycobacterial DnaJ protein from *E. coli* and eukaryotic DnaJ proteins could be explained if the mycobacterial gene products are no longer utilized.

However, this is unlikely as the expression of the *M. tuberculosis* DnaJ protein is increased at higher temperatures (Lathigra *et al.*, 1991). The high level of conservation between the *M. leprae* and *M. tuberculosis* DnaJ proteins would suggest that *M. leprae* DnaJ is also expressed. However, it is not possible to test this experimentally at present.

In *E. coli*, the GrpE heat shock protein has been shown to collaborate with the DnaK and DnaJ proteins in the folding of some proteins (Langer *et al.*, 1992). DnaK recognizes extending polypeptide chains and cooperates with DnaJ in stabilizing this intermediate conformation. The GrpE protein and ATP hydrolysis are required for both the transfer of the protein to GroEL (hsp60) and subsequent GroEL-mediated folding of the native protein. The *grpE* gene of *M. tuberculosis* resides between the *dnaK* and *dnaJ* genes. The corresponding region in *M. leprae* shows 78% nucleotide identity with that of *M. tuberculosis*. However, alignment of the two mycobacterial sequences revealed two gaps in the *M. leprae* sequence of 46 and 40 nucleotides corresponding to regions of the *M. tuberculosis* sequence containing the first 22 nucleotides and the last 34

Mlep	MAQREWVEKD	FYKELGVSSD	ASPEEINRAY	RKLARYLHPD	ANPDN-SSGE	49
Mtub		.Q.....	K.....	D.....	...G.PAA...	50
Ecol	-MAKQ.	Y.EI...KT	.EER..RK..	KR..MKY...	R.QGDKEAEA	45
Scer	---MVK.TK	..DI...PVT	.TDV..KK..	..C.LKY...	K..SE-EAA.	45
Mlep	RFKVVSSEAHN	VLSDPVKRKE	YDE-TRRIFA	GGCLGGRRFD	S----SFGDF	94
Mtub	..A.....	..A.....		..GF.....	.GFGGG..G.	99
Ecol	K..EIK..YE	.T.SQ..AA	.QYGHAA.E	Q.GM.....	-----G.	84
Scer	K..EA.A.YE	..E..DI	..QFGEDGLS	.AGGA-----	---GFPG.G.	88
Mlep	GMGGDGAEFN	LNDLFDAASR	TGGTNIGDLF	GGLFGRGASV	RPS-SRRGND	143
Mtub	.V.....	T.....	G.A	..RP.....		149
Ecol	.G.A-----	.FS.I-----	V.	.DI..-GRG	.Q-RAA..A.	116
Scer	.F-----	-----D.I.	SQF..A..AQ	..RGPQ..K.	115	
Mlep	LETEADLDFV	EAAKGVAMPL	RLTSPAPCTN	CHGSGARPGI	SPKVCSTCNG	193
Mtub	...TE....		T	P....	199	
Ecol	.RYNME.TLE	.VR..TKEI	.IPTLEE.DV	K..T	Q.QT.P..H.	166
Scer	IKH.ISASLE	.LY..RTAK.	A.NKQIL.KE	.E.R.GKK.-	AV.K.TS...	164
Mlep	SGV--INRNQ	GAPG--FSEP	CTECRGSGSI	IE--HLREEC	KGTCVTTRTR	237
Mtub			..D.....	PC...		243
Ecol	.Q--VQMR.	.F.A--VQQT	.PH.Q.R.TL	.K--DPCNK.	H.H.RVE.SK	210
Scer	Q.IKFVT.QM	.PMIQR.QTE	.DV.H.T.D.	.DPKDRCKS.	N.KK.ENERK	203
Mlep	TINVRIPSGV	EDGQRIRLAG	QGEAGLRGAP	SGDLYVTVHV	RPDKIFGRNG	287
Mtub	P....				A.....D.	293
Ecol	.LS.K..A..	.DT.D.....	E.....EH...	A.....Q.Q.	KQHP..E.E.	260
Scer	ILE.HVEP.M	K.....VFK.	EADQAPDVI.	GDVVFI--SE	..H.S.K.D.	252
Mlep	DDLTVTIPVS	FTLEALGSTL	SVPTLDG---	TVGVVRPKGT	SDGRILRVRG	334
Mtub	V....				A.....D.	340
Ecol	NN.YCEV.IN	.AMA...GEI	E.....	R.KLK..GE.	QT.KLF.M..	307
Scer	...VYEAID	LTAIA.GEF	ALEHVS.DWL	K..IVPGEVI	AP.MRKVIE.	302
Mlep	RGVPK-RSGG	HGDLATVKV	AVPLN--LEG	AAQKALEAYA	AAERQVVSTL	381
Mtub	.VC.S-AV.V	AATY.SP				356
Ecol	K..KSV.G.A	Q...CR.V.	ET.VG--NE	RQKQL.QELQ	ESFSGPTGEH	355
Scer	K.M.IPKY..	Y.N.IIKFTI	KF.E.HFTSE	ENL.K..EIL	PPRIVPAIPK	352
Mlep	GRDGAGNR					389
Mtub						
Ecol	NSPRSKSFFD	GVKKFFDDLT	R			376
Scer	KATVDECVLA	DFDPAKYNRT	RASRGGANYS	SDEERQGGEG	VQCASQ	398

Fig. 3. Alignment of the *M. leprae* DnaJ deduced amino acid sequence with DnaJ amino acid sequences from different species. Mlep, *M. leprae* DnaJ; Mtub, *M. tuberculosis* DnaJ (Lathigra *et al.*, 1988); Ecol, *E. coli* DnaJ (Bardwell *et al.*, 1986); and Scer, *S. cerevisiae* DnaJ (Caplan & Douglas, 1991). Amino acids are represented by their single letter abbreviations. Amino acids identical to those of *M. leprae* are represented as a full stop (.). Gaps in the sequence, where additional amino acids are present in the sequence of other species, are represented as a hyphen(-). The four tandem repeats of the conserved Cys-X-X-Cys-X-Gly-X-Gly motif (Lathigra *et al.*, 1988) are underlined in the *M. leprae* sequence.

nucleotides of the *grpE* gene. There are additional nucleotide differences between the two mycobacterial sequences. Further, there is no significant open reading frame in this region of the *M. leprae* genome. In *B. subtilis*, *grpE* is thought to be expressed as part of the *dnaK* operon (Wetzstein & Schumann, 1990), whereas in *E. coli* it forms part of a separate operon. The latter, together with the apparent ubiquity of the GrpE protein among prokaryotes suggests that an intact copy of the *grpE* gene is likely to be present elsewhere in the *M. leprae* genome.

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