

Nucleotide sequence of the *Klebsiella pneumoniae nifD* gene and predicted amino acid sequence of the α -subunit of nitrogenase MoFe protein

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The nucleotide sequence of the *Klebsiella pneumoniae nifD* gene is presented and together with the accompanying paper [Holland, Zilberstein, Zamir & Sussman (1987) *Biochem. J.* 247, 277–285] completes the sequence of the *nifHDK* genes encoding the nitrogenase polypeptides. The *K. pneumoniae nifD* gene encodes the 483-amino acid-residue nitrogenase α -subunit polypeptide of M_r 54 156. The α -subunit has five strongly conserved cysteine residues at positions 63, 89, 155, 184 and 275, some occurring in a region showing both primary sequence and potential structural homology to the *K. pneumoniae* nitrogenase β -subunit. A comparison with six other α -subunit amino acid sequences has been made, which indicates a number of potentially important domains within α -subunits.

INTRODUCTION

Biological nitrogen fixation is catalysed by nitrogenase (Gibson & Newton, 1981), an O_2 -sensitive metalloenzyme that is composed of two separate proteins (Lowe *et al.*, 1985). In *Klebsiella pneumoniae* and other genetically characterized diazotrophs the *nifH*, *nifD* and *nifK* genes encode the Fe protein and the α - and β -subunits of the MoFe protein respectively. The MoFe protein is a tetramer of the form $\alpha_2\beta_2$ and contains the site at which reducible substrates are bound (reviewed in Smith, 1977; Thorneley *et al.*, 1978). Prosthetic groups associated with *K. pneumoniae* MoFe protein include four [4Fe-4S] clusters, two FeMo cofactors and one further class of Fe atoms (Orme-Johnson & Munck, 1980; Smith, 1983; Burgess, 1984). Analysis of *K. pneumoniae nifV* mutants suggests that FeMo cofactor is important in substrate specificity and reduction (Hawkes *et al.*, 1984).

Recombinant DNA methods have permitted the identification and isolation of *nifH*, *nifD* and *nifK* genes from a number of diazotrophs, facilitated by the strong interspecies homology that exists between individual *nifH*, *nifD* and *nifK* genes and the availability of *nif* gene probes derived from the *K. pneumoniae nif* gene cluster (Mazur *et al.*, 1980; Hennecke, 1981; Quiviger *et al.*, 1982; Rice *et al.*, 1982; Ruvkun *et al.*, 1982). The present paper reports the completed *K. pneumoniae nifD* sequence.

MATERIALS AND METHODS

Restriction enzymes were obtained from Boehringer Mannheim or Northumbria Biologicals, and T4 DNA ligase was from Bethesda Research Laboratories. Digestions were performed in 33 mM-Tris/acetate buffer, pH 7.9, containing 66 mM-potassium acetate, 10 mM-magnesium acetate, 4 mM-spermidine and 5 μ g of pan-

creatic ribonuclease/ml. Ligations were performed in 66 mM-Tris/HCl buffer, pH 7.5, 6.6 mM-MgCl₂, 10 mM-dithiothreitol and 1 mM-rATP. DNA sequencing used [α -³⁵S]thio]dATP (Biggin *et al.*, 1983) as the label (Amersham International) with deoxynucleoside and dideoxynucleoside triphosphates from P & L Laboratories. The 17 bp primer was purchased from Celltech.

The *nif* DNA to be sequenced was excised from pWF23 (Filler *et al.*, 1986) as a 6.4 kb *EcoRI* fragment (Cannon *et al.*, 1979), which contains the *K. pneumoniae nifHDKY* operon. Primary subclones of this *EcoRI* fragment were made in M13 mp18 and mp19 by restriction with *Bam*HI and *Hind*III (Fig. 1). The *Bam*HI site delineates the 3' end of the partial *nifD* sequence previously determined (Scott *et al.*, 1981), and the *Hind*III site lies in *nifK* (see also Holland *et al.*, 1987). Subclones of the *Bam*HI–*Hind*III fragment for sequencing were made in M13 mp8, mp9, mp18 and mp19 (Messing, 1983) (see Fig. 1). DNA sequencing was carried out by using the chain termination method (Sanger *et al.*, 1977). Secondary-structure predictions were made (Chou & Fasman, 1978) by using the Wisconsin PEPLOT software package.

RESULTS AND DISCUSSION

Nucleotide sequence of the *nifD* gene

The nucleotide sequence of the *nifD* gene is shown in Fig. 2. Sequence was determined on both strands (Fig. 1), which with the data of Scott *et al.* (1981) (nucleotides 1–630 in Fig. 2) completes the 1449 bases of the *nifD* sequence and predicts a 483-amino acid-residue polypeptide of M_r 54 156. Codon usage is typical of the *nifH* and *nifK* genes, with a preference for G or C in the third position (Scott *et al.*, 1981; Holland *et al.*, 1987). The *nifD* reading frame terminates with a single stop codon UGA, followed 55 bp downstream by the initiation codon for

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These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00316.

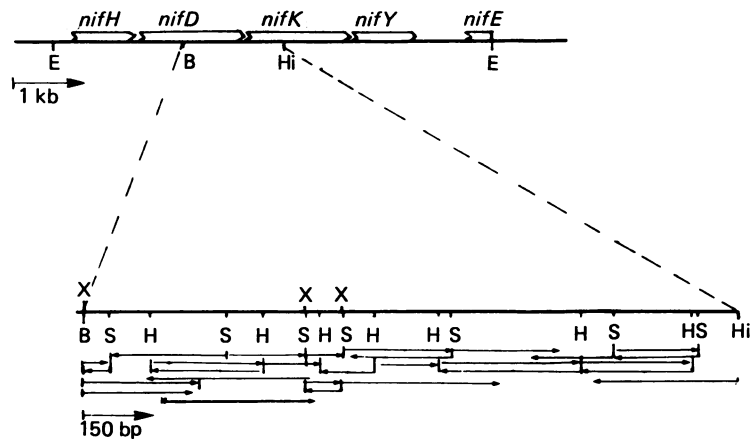


Fig. 1. Physical and genetic map of the *K. pneumoniae* *nifD* gene

Positions of restriction sites are given, and arrows indicate the direction and extent of each sequence obtained from subcloning into M13. A 23 bp synthetic oligonucleotide, complementary to nucleotides 827–849, was used to prime the sequencing indicated by the heavy arrow. The *Bam*HI site in the *nifD* gene was overlapped by sequencing from the *Nru*I site (13 bp downstream of the *Bam*HI site) into the previously determined *nifD* gene sequence reported by Scott *et al.* (1981). Sequence through the *nifK* gene to the *Hind*III site was identical with that reported by Holland *et al.* (1987). B, *Bam*HI; S, *Sau*3A; H, *Hpa*II; Hi, *Hind*III; X, *Xho*II.

the *nifK* gene. The *nifD*–*nifK* intercistronic region shows homology to sequences upstream of the *nifH* gene translation start extending beyond the Shine & Dalgarno region (see Fig. 2). These sequences may have a role in ribosome binding and translation initiation during expression of the *nifH* and *nifK* genes (see, e.g., Stanssen *et al.*, 1986), but apparently not that of the *nifD* gene.

Amino acid sequence of *nifD*-gene product

Sequences for the *nifD*-gene products from *Anabaena* (Lammers & Haselkorn, 1983), *Bradyrhizobium japonicum* (Kaluza & Hennecke, 1984), *Rhizobium* sp. *Parasponia* (Weinman *et al.*, 1984), *Azotobacter vinelandii* (Brigle *et al.*, 1985), *Rhizobium* sp. cowpea (strain IRc78) (Yun & Szalay, 1984), *Clostridium pasteurianum* (Chen *et al.*, 1986) and *K. pneumoniae* are aligned in Fig. 3. The C- and N-termini are least well conserved, and account for the size variation amongst α -subunits. Homology of the *K. pneumoniae* α -subunit with other α -subunits is high, the greatest being with *A. vinelandii* at 72%, followed by *Rhizobium* sp. *Parasponium*, *Rhizobium* sp. cowpea (strain IRc78) and *B. japonicum* each at 70%, and then *Anabaena* at 67%. Least homology (44%) is found with *C. pasteurianum*.

Runs of two or more identical residues are marked with vertical lines and include up to eight identical amino acid residues (e.g. residues 229 through to 236; Fig. 3). Regions showing least homology are from residues 1–50, 210–218 and 390–396. These could be sites at which deletion and insertion events have occurred, as we predict in our alignment. As variation within proteins that have diverged from a common evolutionary origin is likely to occur on the protein surface, internal changes being constrained by the dense molecular packing of globular protein interiors, the rather divergent regions 210–220 and 390–396 of the α -subunits may be situated within looping-out strands of the overall tertiary protein structure.

Cysteine residues

Up to nine cysteine residues are found in α -subunits; six cysteine residues are present in the *K. pneumoniae* α -subunit and five (Cys-63, Cys-89, Cys-155, Cys-184 and Cys-275) are found in highly homologous regions of those α -subunits compared (boxed in Fig. 3). Cysteine residues may be ligands to the prosthetic groups of *K. pneumoniae* MoFe protein. Conserved Cys-63 is found in a hydrophobic region adjacent to amino acid residues (Gly-62 and Ala-64) with small side chains that are unlikely to hinder the binding of prosthetic groups should the peptide conformation at position 63 exist as a turn rather than an extended form. Secondary-structure predictions suggest that Cys-63, Cys-155 and Cys-184 occur at turns (indicated by T in Fig. 3). Cys-89 is situated in a hydrophobic region, and residues in its proximity are usually small and conserved (Gly-88, often Val-87, except for Ile-87 in *C. pasteurianum* and Ala-87 in *K. pneumoniae*, and Gly-90). In the *C. pasteurianum* α -subunit Ser-90 is present (rather than Gly-90), which has ligand potential. Prosthetic groups associated with Cys-89 in *C. pasteurianum* may therefore have different properties to those in, for example, *K. pneumoniae*. Cys-184 is conserved and found within a hydrophobic region flanked by the rather small residues Arg-183, Val-182 and Gly-186, which may favour prosthetic-group binding. Cys-275 is flanked by bulky residues (His-274 and Tyr-276), which could hinder [4Fe-4S]-group binding and create a different environment to, for example, Cys-63. It is possible that His–Cys-275–Tyr ligates FeMo cofactor. Cys-324 of *A. vinelandii* and Cys-298 and Cys-353 of *C. pasteurianum* are not conserved and are substituted by alanine, methionine, threonine, tyrosine or phenylalanine residues in other α -subunits. With the possible exception of tyrosine, these residues have a weaker potential than cysteine as ligands.

Amino acid sequences surrounding conserved cysteine residues show little conservation among themselves, in contrast with the cysteine residues of ferredoxins (Yas-

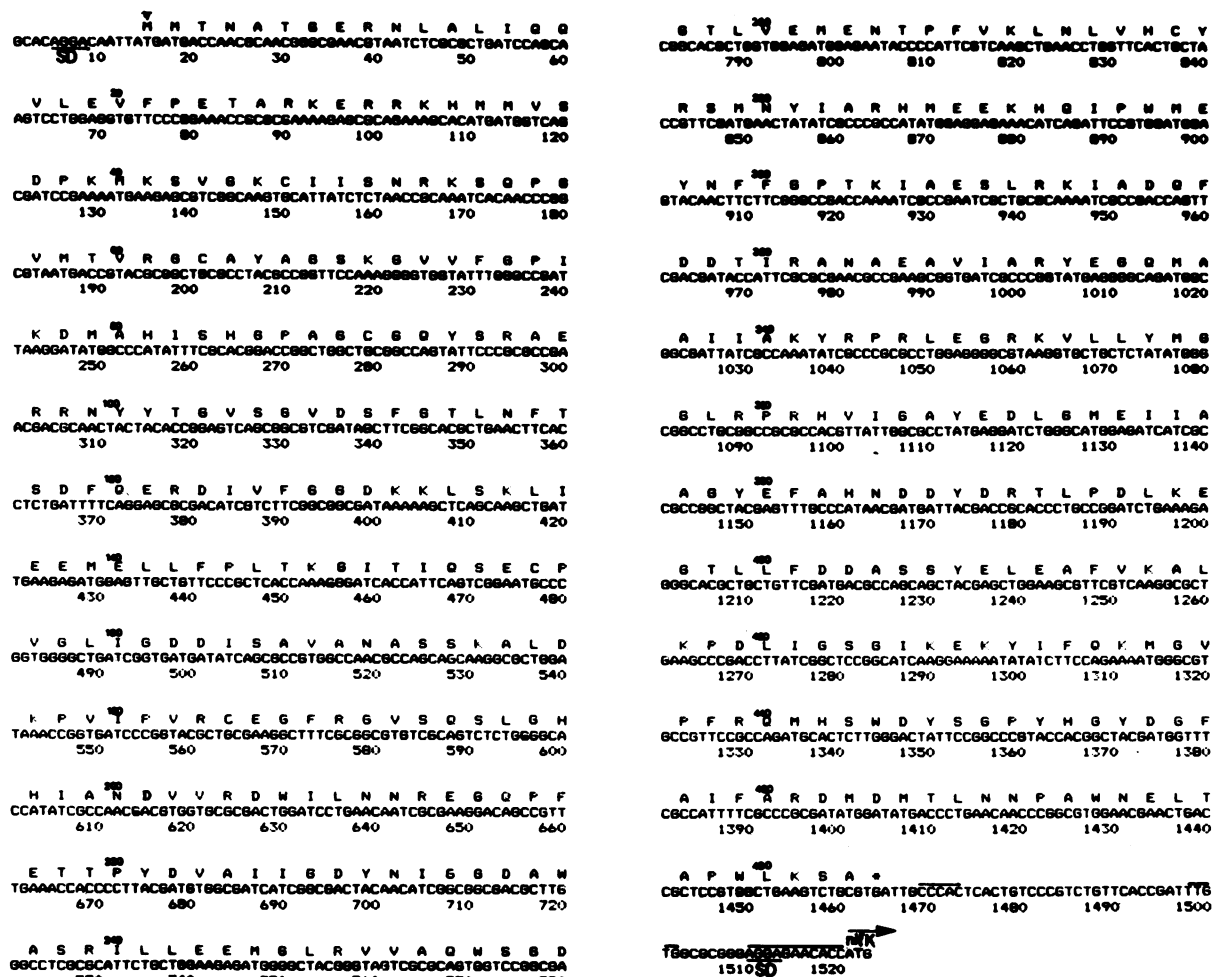


Fig. 2. Complete nucleotide sequence of the *nifD* gene of *K. pneumoniae* and its predicted translation product

Sequence up to the *Bam*HI site (nucleotides 1–630) was determined previously (Scott *et al.*, 1981). The single stop codon of the *nifD* gene is marked with an asterisk. The overlined sequences upstream of the *nifK*-gene translation start are homologous to sequences upstream of the *nifH*-gene translation start. These are illustrated in the following sequence alignment:

nifH (upstream sequences): --CCCAC---TTGTG---AGGAGAAGT CACC
nifK (upstream sequences): --CCCAC---TTGTG---AGGAGAA--CACC

unobu & Tanaka, 1973). Cys-155 is the sole example within the α -subunits that is associated with an amino acid sequence similar to the sequence Glu-Cys-Pro-Val-Gly-Xaa-Ile (Xaa is a variant residue) believed to be important for iron-sulphur-centre binding in bacterial ferredoxins. The ferredoxin cysteine residue found within this sequence is contained within a type I hydrogen-bonded β -turn (Adman *et al.*, 1975). Secondary-structure analysis for the *Anabaena* (Lammers & Haselkorn, 1983) and *B. japonicum* (Kaluza & Hennecke, 1984) α -subunits in the Cys-155 region predicts a β -sheet before the Cys-155 residue followed by a turn and an α -helix. The same holds true for the *K. pneumoniae* α -subunit, indicating that Cys-155 is a [4Fe-4S]-centre ligand candidate.

Secondary-structure predictions around other conserved cysteine residues of the *K. pneumoniae* α -subunit indicate little similarity in structure to the ferredoxins. These cysteine residues do not conform to the sequence Cys-Xaa-Xaa-Cys typical of the [4Fe-4S]-cluster-binding site of ferredoxins. This may explain why the redox and

spectral properties of the [4Fe-4S] clusters of the MoFe protein are different to those of ferredoxins if the conserved cysteine residues are ligands. Some cysteine residues (Cys-63, Cys-89, Cys-155 and Cys-184) are flanked by potential oxygen-donor residues (Tyr-65, Tyr- or Gln-93 or -92, Asp-162 or -163, and Gln- or Tyr-152 respectively), which might modify the cluster's properties. Cys-275 has residues with amide and carboxylate functions surrounding it (Asn-271, Asn-280 and Glu-287) and, on the need for solvents with an amide function for extraction of the FeMo cofactor (Shah & Brill, 1977), and the binding of only one thiol molecule per molecule of cofactor (Burgess *et al.*, 1980), may be a FeMo cofactor ligand (Brigle *et al.*, 1985).

Comparison of the *K. pneumoniae* α - and β -subunits

The *K. pneumoniae* *nifK*-gene-product sequence was kindly provided by A. Zamir (Holland *et al.*, 1987). The α - and β -polypeptides have similar α -helix and β -sheet content (23% β -sheet and 30% α -helix for the α -subunit,

1 10 20 30 40 T 50
 Ep: MH₂MTWATGERHW(L)ALIQOVLEVFPETAREERRENNMVDPENESVGR(C)I⁷-S⁸RRKS
 Av: MH₂MTRNSREVEESLIEVLEVYFPEKARDRNKHLAVNDPAVTS-KECI¹I-S²RRKS
 Rc: MH₂MSLASTQSIARIRARNEELIQEVLEVYFPEKARRAKHLVNDPAVTS-DC¹OVKSNIKS
 Rp: MH₂MSLATTQSIARIRARNEELIEVLEVYFPEKARRAKHLVNDPAVTS-DC¹OVKSNIKS
 Bj: MH₂MSLATTQSVARIRARNEELIEVLEVYFPEKARRAKHLVNDPAVTS-DC¹OVKSNIKS
 An: MH₂MTPFENRHLVDENEELIQEVLEKTPKRRERREKHLVNDPAVTS-DC¹OVKSNIKS
 Cp: MH₂SEELKDEILEKYI¹PKTKETESONIVIKTE-ETP²PH³I⁴VA-N⁵TRT

60 T 70 80 90 100 110
 Ep: QPGLM(TV)RGCAYAGSKE(V)YVFPF¹IKDMANIS(N)QFAG(C)QYSLAER(R)NYTGVSG(V)DSFGTLMF
 Av: QPGLMTIRGCAIYAGSKEQVYVWGF¹IKDMINISWOPVQGCQYSLAERINYYIGTTGVNAPVTMNF
 Rc: I¹PGVMTIRGCAIYAGSKEQVYVWGF¹IKDMVNSWOPVQGCQYSLWGRINYYVGTGIDSPVTLQF
 Rp: I¹PGVMTIRGCAIYAGSKEQVYVWGF¹IKDMVNSWOPVQGCQYSLWGRINYYVGTGIDSPVTLQF
 Bj: I¹PGVMTIRGCAIYAGSKEQVYVWGF¹IKDMVNSWOPVQGCQYSLWGRINYYVGTGIDSPVTLQF
 An: V¹PGVMTARGCAYIYAGSKEQVYVWGF¹IKDMIHISWOPVQGCQYSLWGRINYYVGTGIDSPVTLQF
 Cp: V¹PGIITARGCAYIYAGCKEQVYVWGF¹IKDRVNTMOPVQGCQYSLWGRINYSPTNGORISKPEDGTGLN-FNEYV¹F
 F K

120 130 140 150 B T 160 170
 Ep: TSDFO(E)RDI(V)PQDDEKLSKLIENEL(L)PFLTKGIIQSE(C)FVGL(I)QDD(S)AV(A)M(A)SS(E)ALD-
 Av: TSDFOEKD¹VFGDDKELAKLIDEVETL¹PFLNKGISVQSE(C)PIGLI¹QDD¹ESVSEKGAELS-
 Rc: TSDFOEKD¹VFGDDKELVKILDEIQEL¹PFLNKGITIQSE(C)PIGLI¹QDD¹EAVSRAKSKETQO
 Rp: TSDFOEKD¹VFGDDKELIKVLDIQEL¹PFLNKGITIQSE(C)PIGLI¹QDD¹EAVSRAKSKETQO
 Bj: TSDFOEKD¹VFGDDKELDKILDEIQEL¹PFLNKGITIQSE(C)PIGLI¹QDD¹EAVSRAKSKETQO
 An: TSDFOEKD¹VFGDDKELTELISELDVLP¹FLNKGISVQSE(C)PIGLI¹QDD¹EAVAKKTSKQIG-
 Cp: STDNQESD¹VFGVMELEDAINEAYENF-N¹FAAIGVYAT(L)FVGLI¹QDD¹LAVAATASKEIGI

180 190 200 210 T 220 230
 Ep: KPVIVPVR(C)RPGVVSQSLQ(N)ANDVVRDMLN¹NRGQF¹-----FETTFYDVAII¹GDY¹NI¹Q
 Av: KTIVPVR(C)RPGPROVSQSLQNNI¹ANDAVRDWVLD¹GRDADTT¹-----FASTPYDVAII¹GDY¹NI¹Q
 Rc: KTIVPVR(C)RPGPROVSQSLQNNI¹ANDAVRDWVLD¹QVADGKPEV¹-----EPTPYDVAII¹GDY¹NI¹Q
 Rp: KTIVPVR(C)RPGPROVSRSLQNNI¹ANDAVR-LIP¹DELPEGEPEF¹-----QTPPYDVAII¹GDY¹NI¹Q
 Bj: KTIVPVR(C)RPGPROVSQSLQNNI¹ANDAVRDWVIF¹GNIEAEGEPEF¹-----EPTPYDVAII¹GDY¹NI¹Q
 An: KPVVPLR(C)RPGPROVSQSLQNNI¹ANDAIRDWI¹PFYDKLKEETRLDFE¹PSPYDVALI¹GDY¹NI¹Q
 Cp: -FVNAFS(C)R¹TYKQVSQSAQNNI¹AMNTVMTDII¹QKGNKEEKE¹-----YSINVLGEY¹NI¹Q

240 250 260 A T 270 B 280 290
 Ep: GDAWASRIL(E)MGLRVVAQ(S)DGTLMENELT¹FPVKLNLVH(C)YRS¹MNYI¹ARHMEERKQI(P)¹
 Av: GDAWSSRILLEENGLRVAQWSODGYISQ¹IELTPEVKLNLVH(C)YRS¹MNYI¹SRHMEERKYGIF¹
 Rc: GDAWSSRILLEENGLRVIAQWSODGSLA¹LEAVVEGKLNILH(C)YRS¹MNYI¹SRHMEERKFOI¹
 Rp: GDAWSSRILLEENGLRVIAQWSODGSLA¹LEATPKAKLNILH(C)YRS¹MNYI¹SRHMEERKFOI¹
 Bj: GDAWSSRILLEENGLRVIAQWSODGSLA¹LEATPKAKLNILH(C)YRS¹MNYI¹SRHMEERKFOI¹
 An: GDAWASRILLEENGLRVVAQWSODGT¹LNELIQPAAKLVLIH(C)YRS¹MNYI¹CRSLEEQYGNP¹
 Cp: GDAWENDRVLEKIGYHV¹NATLTG¹DATYEK¹VQWADKADLMLVQ(C)HRS¹IN¹YI¹AEMMETKYGIF¹

300 310 320 330 340 350
 Ep: MEYN¹FPPTKIAESL¹RKIA¹DQ¹DD¹TIRAWAEAV¹ART(E)QMAAII¹AKYR¹PRLE(C)R(K)V¹LV(L)Y¹M
 Av: MEYN¹FPPTKIESL¹RAIAA¹EP¹DESIQE¹KEEV¹AKYEP¹WEAEAV¹VAKYR¹PRLEGRV¹MLY¹I
 Rc: CEYN¹FPPTKIAESL¹RIAGY¹DD¹EIKEGAER¹V¹EKY¹QPLVMAVIA¹KYR¹PRLEGRK¹TVMLY¹V
 Rp: CEYN¹FPPTKIAESL¹RIAGY¹DD¹EIKEGAER¹V¹EKY¹QPLVD¹AVIA¹KYR¹PRLEGRK¹TVMLY¹V
 Bj: CEYN¹FPPTKIAESL¹RIAGY¹DD¹EIKEGAER¹V¹ERY¹QPLVD¹AVIA¹KYR¹PRLEGRK¹TVMLY¹V
 An: MEFN¹FPPTKIAESL¹RIAA¹EP¹DESKIQE¹NAEK¹V¹AKYTP¹VMAVLD¹KYR¹PRLEGRK¹TVMLY¹V
 Cp: IKCN¹FIQVNGI¹VETL¹DMACK¹FD-EL¹TER¹EV¹AEEIAA¹IQDD¹LDY¹FKEK¹LOGK¹TAC¹LY¹V

360 370 380 390 T 400 410
 Ep: GGLR(E)R¹PHVIGAYEDL¹QHEIIAAGY¹FA¹NHDD¹YDRTL¹-PDL¹KEGTLL¹LP(D)ASSYELEAFV¹KAL
 Av: GGLRFRPHVIGAYEDL¹QHEVVG¹TOY¹FA¹NHDD¹YDRTM¹-KEM¹GDSTLL¹YDD¹VTOME¹PEP¹FVERI
 Rc: GGLRFRPHVIGAYEDL¹QHEVIG¹TOY¹FA¹GNHDD¹YQRTAQ¹HYV¹KGDTL¹I¹ND¹OVNGY¹EP¹RFVEKL
 Rp: GGLRFRPHVIGAYEDL¹QHEVVG¹TOY¹FA¹GNHDD¹YQRTAQ¹HYV¹KGDTL¹I¹YDD¹OVNGY¹EP¹RFVEKV
 Bj: GGLRFRPHVIGAYEDL¹QMDVIG¹TOY¹FA¹GNHDD¹YQRTAQ¹HYV¹KGDTL¹I¹YDD¹OVNGY¹EP¹RFVERL
 An: GGLRFRPHV¹PAFEDL¹QIKV¹VG¹TCY¹FA¹NHDD¹YKRTT¹-HY¹IDNATI¹IYDD¹VTAYE¹PEP¹FVEAK
 Cp: GGRSHTY¹M--L¹KSP¹FGV¹DSLVA¹QEP¹FA¹NHDD¹YEQ-M-KEM¹HDGT¹ILID¹MMNH¹MEV--VLEKL

420 430 440 450 460 470
 Ep: KFDLI(C)S¹Q¹KE(K)YI¹FO¹KMG(V)F¹RQMH¹SWDY¹SC¹PFYHGYDGP(A)I¹(F)A(R)DM¹DM¹HTL¹LN¹PAW¹HELT¹AP
 Av: KFDLIQSG¹Q¹KEK¹YI¹FO¹KMCI¹FFRQMH¹SWDY¹SC¹PFYHGF¹DGFA¹IFARD¹DM¹DM¹HTL¹LN¹PCW¹KK¹LQAF
 Rc: QFDLVGS¹Q¹KEKY¹VFO¹KMG¹QFFRQMH¹SWDY¹SC¹PFYHGYDGP(A)IFARD¹DM¹DM¹HA¹INS¹FW¹KEK¹TA¹P
 Rp: QFDLVGS¹Q¹KEKY¹VFO¹KMS¹VFFRQMH¹SWDY¹SC¹PFYHGYDGP(A)IFARD¹DM¹DM¹AV¹MS¹PI¹WEK¹TA¹P
 Bj: QFDLVGS¹Q¹KEKY¹VFO¹KMS¹VFFRQMH¹SWDY¹SC¹PFYHGYDGP(A)IFARD¹DM¹DM¹AV¹MS¹PI¹WEK¹TA¹P
 An: KFDLIASG¹Q¹KEKY¹VFO¹KMGL¹QFFRQMH¹SWDY¹SEL¹QDGV¹QMS¹DEV¹RF¹FC¹EG¹REK¹SFLA¹
 Cp: KFDN¹FFA¹Q¹IEL¹FPV¹IQ¹QCV¹LSK¹QL¹HSY¹DY¹NO¹PY¹AG¹FR¹QV¹VN¹PH¹EL¹VN¹CI¹Y¹TP¹AW¹K¹NI¹TP¹

480
 Ep: W(L)KSA*
 Av: WEASEGAEKVAASA*
 Rc: WKEASRAKLLAAE*
 Rp: WKEAAKPKLLAAE*
 Bj: WKDAERQDSRLQNNATRLALRESPOIFI*
 An: WKDAERQDSRLQNNATRLALRESPOIFI*
 Cp: WKEASSESEKVVVCGEA*

and 20% β -sheet and 35% α -helix for the β -subunit). Only in the *N*-terminal third of each subunit was structural similarity found. The first 45 amino acid residues are predicted to fold as an α -helix followed by β -sheeted regions through to position 125. Long β -sheeted areas ranging from position 176 through to position 230 were predicted for the α -subunit.

Homology between α - and β -subunits has been observed for *B. japonicum* (Thöny *et al.*, 1985) and *Anabaena* (Lammers & Haselkorn, 1983; see also Holland *et al.*, 1987). Structural homology is suggested by crystallographic studies of the *C. pasteurianum* α - and β -subunits, which indicate a 2-fold relationship between the α - and β -chains (Yamane *et al.*, 1982). Within the *N*-terminal of the *K. pneumoniae* β -subunit there exists sequence homology to the *K. pneumoniae* α -subunit. Residues with identity are marked with circles in Fig. 3. Little overall homology is observed except for two runs of four amino acid residues each within the third region described by Thöny *et al.* (1985). Some conserved cysteine residues are found in alike positions (Cys-63, Cys-89 and Cys-155). Those in the *N*-terminal regions (see above) may lie within a similar secondary structure. The significance of this remains to be shown.

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Fig. 3. Comparison of the *K. pneumoniae* (Kp) α -subunit sequence with those from different organisms

Conserved cysteine residues are boxed. Residues identical with amino acids present in the *K. pneumoniae* β -subunit and which occur in a similar position are circled. Runs of two or more identical residues between subunits are indicated with vertical lines. Regions of low homology referred to in the text are underlined below with a broken line. To maximize homologies, packing residues indicated by dashes were introduced. Secondary structures are indicated by: α , α -helix; β , β -sheet; T, turn. Sequences compared are from *A. vinelandii* (Av), *Rhizobium* sp. cowpea (strain IRc78) (RC), *Rhizobium* sp. *Parasponium* (RP), *B. japonicum* (Bj), *Anabaena* (An) and *C. pasteurianum* (Cp). The nucleotide sequence of the *nifD* gene from *C. pasteurianum* (Chen *et al.*, 1986) predicts asparagine and arginine at positions 103 and 52 respectively rather than aspartate and lysine as deduced from protein sequencing.