BIOCHEMICAL JOURNAL LETTERS

The PLEES proteins: a family of structurally related enzymes widely distributed from bacteria to humans

Aromatic compounds including biphenyls and polychlorinated-biphenyls (PCBs) are among the most serious and persistent environmental pollutants, owing to their chemical stability and widespread industrial use [1]. Over the last few years, a variety of bacterial strains with the ability to degrade these aromatic compounds have been isolated and used for the bioremediation of contaminated soil and water. These micro-organisms use different enzymes for the initial attack on the diverse aromatic substrates, but the catabolic pathways converge on just a few central intermediates such as catechol or substituted catechols. The conversion of catechols into tricarboxylic-acid-cycle intermediates is mediated in many cases by common enzymic steps called the *meta*-cleavage pathway. One of the key reactions in this route is catalysed by a serine hydrolase called BphD, which has the ability to perform the hydrolytic cleavage of carbon–carbon bonds, one of the rarest of all enzyme reaction types [2]. At present, eight bacterial hydrolases catalysing this type of reaction have been characterized at the amino-acid-sequence level. These hydrolases are involved in the degradation of biphenyls and their polychlorinated derivatives and show a high degree of sequence similarity. The recent isolation, from a breast carcinoma cDNA library, of a novel human serine hydrolase (Bph-rp) [3], with sequence similarity to the bacterial BphD enzymes involved in PCB degradation, led us to search for common motifs within these enzymes which might establish possible functional relationships between them.

A search against a nonredundant protein sequence database (SWISS-PROT Release 33.0, PIR Release 47.0, CDS translations from GenBank Release 95.0 and daily updates of these databases until 12 June 1996) with human Bph-rp using BLASTP [4] resulted in a series of statistically significant hits with other bacterial serine hydrolases involved in the degradation of a variety of aromatic compounds, including toluene, benzoate, phenol and xylenes [probability of matching by chance (P) $< 10^{-5}$]. In addition, significant similarities were also found with bacterial lipases, esterases, peroxidases, haloalkane dehalogenases and proline iminopeptidases, as well as with epoxide hydrolases, acylaminoacyl peptidases, dipeptidyl peptidases and prolyl oligopeptidases from different organisms, including yeast, plants and mammals [5–7]. A multiple sequence alignment was constructed using the sequence analysis program MACAW [8] (Figure 1). This analysis identified six regions displaying the highest degree of sequence similarities to Bph-rp, from 59–60 % with serine hydrolases, esterases and lipases, to 55–57 % with peroxidases, haloalkane dehalogenases, epoxide hydrolases and peptidases. Three of these blocks (III, IV and V) were conserved around the amino acids which would form the catalytic triad (Ser/Asp)-(Asp/Glu)-His, characteristic of all these proteins, and presumed to be essential for their enzymic activity. This catalytic triad is reminiscent of the archetypal one present in serine proteinases and originally described in chymotrypsin [10], but with opposite handedness.

The sequence similarity of the proteins aligned in Figure 1, together with the presence of a catalytic triad (Ser/Asp)-(Asp/Glu)-His with high conserved patterns around the residues forming the active centre, and the conservation of several residues which would play an important role in the conformation of these enzymes, led us to propose that these proteins may have very similar three-dimensional structures. In this regard, it is worthwhile mentioning that the three-dimensional structure of some of the proteins with similarity to human Bph-rp has been elucidated by X-ray crystallography [11,12], and in all cases they are organized into an α/β hydrolase fold, which is characterized by the presence of a central β-pleated sheet surrounded by α-helices [13]. Therefore it is likely that, in addition to their similarities in amino acid sequences and enzymic mechanism, the different proteins aligned in Figure 1 may also share a similar α/β folding.

Since virtually all these proteins related to human Bph-rp can be classified as **p**eptidases, **l**ipases, **e**sterases, **e**poxide-hydrolases, or **s**erine hydrolases, we propose to call them '**PLEES**' enzymes. These proteins could thus constitute a large family of structurally related enzymes which are able to metabolize a wide variety of substrates by using a common mechanism based on a catalytic triad of topologically conserved (nucleophile)-(acid)-(histidine) residues. The definition of this protein superfamily would extend previous studies describing relationships among serine hydrolases [14–16]. However, although most of the PLEES proteins are hydrolytic enzymes, it is remarkable that the members of the PLEES family of proteins are not exclusively restricted to this activity, and the group includes proteins like bacterial peroxidases, with ability to catalyse the synthesis of carbon–halogen bonds rather than any type of hydrolytic reaction [12]. It should also be stressed that although PLEES proteins may adopt an α/β hydrolase fold, not all proteins possessing an α/β hydrolase structure would be PLEES proteins. Thus there are a number of α/β-hydrolase-fold proteins, including wheat carboxypeptidase [17] or cutinase [18], which do not show significant similarity with human Bph-rp, nor with the remaining proteins included in the alignment shown in Figure 1. Finally, the finding that PLEES enzymes include bacterial, fungal, plant and mammalian enzymes suggests that they could have evolved from a common ancestor that became adapted to perform a wide array of biological functions in the different organisms in which they have been identified. The identification of these similarities between proteins from organisms that diverged about 2 billion years ago [19] will be helpful for future structure–function relationship studies of the different family members and, in particular, in those directed to elucidate the role of human Bph-rp in both normal and pathological conditions, including breast cancer.

This work was supported by grants SAF94-0892 (Comisión Interministerial de Ciencia y Tecnología) and Glaxo–Wellcome, Spain. X. S. P. is recipient of a Fellowship from FICYT, Asturias, Spain.

Xose S. PUENTE and Carlos LÓPEZ-OTÍN*

Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad de Oviedo, 33006-Oviedo, Spain

* To whom correspondence should be addressed.

```
                I                        II                              III                                   IV                          V                 VI
bphrp_Hum  VLLLPG..(8)..F..(14)..FTVVAWDPRGYGHS..(22)..MKALKFKKVSLLGWSDGGITALIAAAKYPSYI.....PALIVHGEKDPLVP..(17)..LMPEGKHNLHLR..(8)...AEDFL  X81372
bphD_Ppu   VIMLHG..(7)..W..(14)..YRVILKDSPGFNKS..(20)..MDALGIDRAHLVGNSMGGATALNFAIEYPDRI.....KTFITWGRDDRFVP..(17)..VFSKCGHWAQWE..(8)...AIDFL  C35124
bphD_PspL  VIMLHG..(7)..W..(14)..YRVILKDSPGFNKS..(20)..MDALDIDRAHLVGNSMGGATALNFAIEYPDRI.....KTFITWGRDDRFVP..(17)..VFSKCGHWAQWE..(8)...VIDFL  JN0816
bphD_Pte   VIMLHG..(7)..W..(14)..YRVILKDSPGFNKS..(20)..MDALGIERAHLVGNSMGGATALNFAIEYPERL.....KTLVTWGRDDRFVP..(17)..VFSQCGHWAQWE..(8)...AIDFL  L34338
bphD_PspK  VIMLHG..(7)..W..(14)..YRVLLPDAPGFNKS..(20)..MDVLGIEKAHLVGNSMGGAGALNFALEYPERT.....KTLVTWGRDDRFVP..(17)..VFPRCGHWAQWE..(8)...TLDFL  P17548
bphD_Pce   VIMLHG..(7)..W..(14)..YRVILKDSPGFNKS..(20)..MDALDIDRAHLVGNSMGGATALNFALEYPDRI.....KTFITWGRDDRFVP..(17)..VFSKCGHWAQWE..(8)...VIDFL  P47229
bpdF_RM5   LILLHG..(7)..W..(13)..FRVIAPDLPGWGAS..(17)..MDALDIREAGFVGNSMGGHTSLRMAIERPDRI.....PALLIHGRDDRVVS..(17)..IINRCGHWAQLE..(8)...VMDFV  U44891
dmpD_Ppu   LMMIHG..(7)..W..(13)..RRVIAPDMLGFGYS..(20)..LDALEIEQADLVGNSFGGGIALALAIRHPERV.....ETLVIHGREDQIIP..(17)..VFGQCGHWTQIE..(8)...VGDFL  P19076
xylF_Ppu   ALLIHG..(7)..P..(13)..RRVIAPDMLGFGYS..(20)..LDALGIQQGDIVGNSFGGGLALALAIRHPERV.....ETLVIHGREDRIIP..(17)..VFGQCGHWTQIE..(8)...VENFL  P23106
todF_Ppu   VVLVHG..(7)..W..(13)..RRVIAPDMVGFGFT..(20)..LDALELDRVDLVGNSFGGALSLAFAIRFPHRV.....ETLILHGRDDRVIP..(17)..VFGRCGHWVQIE..(8)...VNDFL  P23133
catD_Aca   PALIFS..(9)..W..(10)..YFVICYDTRGHGAS..(19)..LDHLQIPQATFCGISMGGLTGQWLAIHFPERF.....PVLVIAGAQDPVTT..(16)..EVLEASHISNVE..(12)..MKRFN  M76991
pcaD_Aca   PAIVFS..(9)..W..(10)..YRVVTYDTRGHGQS..(18)..LDALNIEKAHFCGISMGGLTALWLGIYQAARF.....PTLIIAGSADPVTT..(16)..EVIDASHLSNIE..(8)...FSGFV  L05770
cmtE_Ppu   IIFLHG..(7)..Y..(10)..FHVIAYDYVGHGLS..(19)..MDALGIEKAHLSGESLGGWVSGHFAARYPQRV.....AGTLFLWTHDNPIH..(19)..VKGESAHWPQYE..(8)...ALRFF  U24215
bpA1_Sau   VVFIHG..(7)..W..(11)..YRGIAHDRRGHGHS..(19)..LTDLDLRDVTLVAHSMGGGELARYVGRHGTGR.....PTLVVHGDDDQVVP..(18)..VYEGSSHGIAMV..(10)..LLEFL  P33912
bpA2_Sau   VVLIHG..(7)..W..(11)..YRVITYDRRGFGQS..(19)..LETLDLQDAVLVGFSMGTGEVARYVSSYGTAR.....PALILHGTGDRTLP..(18)..EVEGAPHGLLWT..(8)...LLAFL  P29715
cpoL_Sli   VVFHHG..(7)..W..(11)..YRVIAHDRRGHGRS..(19)..TEALDLRGAVHIGHSTGGGEVARYVARAEPGR.....PVLVAHGTDDQVVP..(18)..SYEGLPHGMLST..(8)...LLAFV  P49323
cpoP_Ppy   IVFHHG..(7)..W..(11)..YRVIAHDRRGHGRS..(19)..VEALDLRNAVHIGHSTGGGEVARYVANDGQPA.....PTLVLHGEDDQIVP..(18)..TYPGYSHGMLTV..(8)...LLAFV  P25026
est_Pfl    VLFSHG..(7)..W..(11)..YRTIAFDRRGFGRS..(19)..IEHLDLKEVTLVGFSMGGGDVARYIARHGSAR.....PTLVIHGDGDQIVP..(18)..VYKDAPHGFAVT..(8)...LLAFL  P22862
est5_Psp   IFFIAG..(7)..W..(10)..YRTVAFDNRDSGES..(19)..MDAEGLQKAHIVGHSLGGMIAQELAILAPDRV.....KTHVIWASEDKIVT..(17)..CIEESGHFPMIE..(8)...LSGFI  D14529
est_Ppu    IHFHHG..(7)..W..(11)..YRVVAHDRRGHGRS..(19)..VAHLGIQGAVHVGHSTGGGEVVRYMARHPADK.....PVLVMHGDDDQIVP..(18)..TYQGYPHGMPTT..(8)...LLAFI  JC4161
tpes_Ppu   VLLLHG..(7)..F..(11)..KRYLALDLRGHGGT..(19)..IDKMGLHNTTVIGHSMGSMTAGVLASIHPDKV.....KTLILWGNQNQPMT..(17)..QYNGFGHSMFWE..(8)...LNEFL  P07383
nonR_Sgr   IALTHG..(7)..F..(11)..YRTLRWDVRYHGAS..(20)..DAAGMPRPVVLLGQSMGGNIAQEYLRRRPDEV.....PELLLCGEHDSTGN..(18)..VIPGAGHVANLD..(8)...TVAFL  JH0655
phab_Pol   IFNGIG..(6)..F..(9)..LEVIAFDVPGVGGS..(19)..LDYLDYGQVNVIGVSWGGALAQQFAHDYPERC.....PTLVLAGDDDPLIP..(16)..HIIDDGHLFLIT..(8)...IMKFL  P26495
lip3_Msp   LLLIHG..(7)..F..(9)..YHLIIPDLLGFGNS..(20)..QAKGLASNTHVGGNSMGGAISVAYAAKYPKEI.....PTLVVWGDKDQVIK..(17)..MMNDVGHVPMVE..(8)...YKAFR  P24640
pldB_Eco   VVICPG..(7)..Y..(11)..FDVLIIDHRGQGRS..(31)..VQPGPWRKRYILAHSMGGAISTLFLQRHPGVC.....PTLLLQAEEERVVD..(24)..VIKGAYHEIFFE..(11)..IVDFF  P07000
lip1_Pim   LLLIHG..(7)..F..(16)..YHLIIPDLLGFGES..(21)..QAKGLASNIHVGGNSMGGAISVAYAAKYPKDV.....PTLVVWGDKDQIIK..(17)..MMEDVGHVPMVE..(8)...YKAFR  Q02104
dehh1_Msp  VLMLHG..(7)..W..(10)..HTVVCADLRGYGDS..(24)..MRHIGFERFHLVGHDRGGRTGHRMALDHPEAV.....SKGQMGQLFDIPAE..(10)..ASLPGGHFFVDQ..(8)...LLKFL  Q01398
dhla_Xau   FLCLHG..(7)..Y..(11)..ARVIAPDFFGFGKS..(21)..IERLDLRNITLVVQDWGGFLGLTLPMADPSRF.....GMKDKLLGPDVMYP..(18)..EIADAGHFVQEF..(8)...LKHFA  P22643
bcho_Rco   LLLLHG..(7)..F..(10)..YRVIVPDLPGHGCS..(20)..CQHLNVTPAAVIGHSAGGAIALQLALDTPVPR.....PVFLIAGNGDRAVP..(16)..RRIDGGHLIHEV..(8)...ILDWL  P26174
ephx1_Hum  LLMVHG..(7)..F..(20)..FEVICPSIPGYGFS..(20)..MLRLGFQEFYIQGGDWGSLICTNMAQLVPSHV.....PTGFSAFPFELLHT..(16)..YMVRGGHFAAFE..(8)...IRKFL  P07099
ephx1_Rat  LLMVHG..(7)..F..(20)..FEVICPSIPGYGYS..(20)..MTRLGFQKFYIQGGDWGSLICTNMAQMVPNHV.....PTGFSAFPSELLHA..(16)..YMERGGHFAAFE..(8)...IRKFV  P07687
ephx1_Rab  LLMVHG..(7)..F..(20)..FEVICPSIPGYGFS..(20)..MLRLGFQEFYIQGGDWGALVCTNMAQLVPSHV.....PTGFAAFPCEIMHV..(16)..YMPRGGHFAAFE..(8)...ICKFV  P04068
ephx2_Mus  LCLCHG..(7)..W..(11)..FRVLAIDMKGYGDS..(21)..LDKLGIPQAVFIGHDWAGVMVWNMALFYPERV.....PALMVTAEKDIVLR..(17)..HIEDCGHWTQIE..(8)...LIKWL  P34914
ephx2_Rat  ICLCHG..(7)..W..(11)..FRVLAIDMKGYGDS..(21)..LNKLGIPQAVFIGHDWAGVLVWNMALFHPERV.....PALMVTAEKDIVLR..(17)..HIEDCGHWTQIE..(8)...LIKWL  P80299
ephx2_Hum  VCLCHG..(7)..W..(11)..YRVLAMDMKGYGES..(21)..LDKLGLSQAVFIGHDWGGMLVWYMALFYPERV.....PALMVTAEKDFVLV..(17)..HIEDCGHWTQMD..(8)...LIKWL  P34913
ephx_Ath   VLLLHG..(7)..W..(11)..YRAVAPDLRGYGDS..(21)..LTASEDEKVFVVGHDWGALIAWYLCLFRPDRV.....PTKFVIGELDLVYY..(24)..VMEGVAHFINQE..(8)...ILDFI  D16628
ephx_Stu   ILFLHG..(7)..W..(11)..YRAVAPDLRGYGDT..(21)..AIAPNEEKVFVVGHDWGALIAWHLCLFRPDKV.....PTKFIVGEFDLVYH..(24)..VLEGSAHFVNQE..(8)...IYDFI  U02494
ephx_Nta   VFFLHG..(7)..W..(11)..FRGIAPDFRGYGLS..(21)..LDSLGIHQVFLVGKDFGARVAYHFALVHPDRV.....PCLLVMGEKDYALK..(23)..FLPEGSHFVQEQ..(8)...IITFL  U57350
ephx_Gma   VLFLHG..(7)..W..(11)..YRAVAPDLRGYGDT..(21)..IDSLGVQQVFLVAHDWGAIIGWYLCMFRPDKV.....PVKYITGELDMVYN..(24)..VQKGVAHFNNQE..(8)...IYDFI  D63781
pip_Lde    LLLLHG..(7)..Y..(12)..RQVIMYDQLGCGNS..(23)..REQLGLDQIHLLGQSWGGMLALIYLCDYQPEG.....PALITSGTDDLCTP..(16)..LFAGCGHMPFVQ..(8)...LSDWL  P46544
pip_Ngo    VIFLHG..(7)..P..(10)..FRIVIIDQRGCGRS..(21)..REMLGIGKWLVFGGSWGSTLSLAYAQTHPERV.....PTVIVQGRYDLCTP..(16)..RVVQAGHCAFDP..(9)...VEDIL  P42786
pip_Lhe    LVLLHG..(7)..Y..(12)..RRIIMYDQLGCGES..(21)..REHLALRKMHLLGQSWGGMLAIIYMCDYHPEG.....PTLITSGTDDLCTP..(16)..LFEGCGHMSFVE..(8)...LQEWL  Z56283
prep_Hum   FLYGYG..(9)..Y..(12)..GILAVANIRGGGEY..(29)..EGYTSPKRLTINGGSNGGLLVAACANQRPDLF.....SMLLLTADHDDRVV..(28)..VDTKAGHGAGKP..(10)..MFAFI  P48147
prep_Pig   FLYGYG..(9)..Y..(12)..GVLAVANIRGGGEY..(29)..EGYTSPKRLTINGGSNGGLLVATCANQRPDLF.....SMLLLTADHDDRVV..(28)..VDTKAGHGAGKP..(10)..MFAFI  P23687
prep_Ahy   ILYGYG..(9)..F..(11)..GVYAVANLRGGGEY..(29)..EGYTRTDRLAIRGGSNGGLLVGAVMTQRPDLM.....STLVTTADHDDRVV..(24)..IETNAGHGAGTP..(10)..IYAFT  Q06903
prep_Fme   ILYSYG..(9)..F..(11)..GIYAVPNIRGGGEY..(29)..NGYTSKEYMALSGRSNGGLLVGATMTMRPDLA.....STMVITSDHDDRVV..(24)..IETNAGHGAGRS..(10)..LLSFA  P27028
acph_Pig   VVMPHG  (9)..W..(18)..YRGSTGFGQDSILS..(22)..EEHFDAGRVALMGGSHGGFLSCHLIGQYPETY.....PLLLMLGQEDRRVP..(21)..LYPKSTHALSEV..(9)...AVLWL  P19205
acph_Rat   VVMPHG..(9)..W..(18)..YRGSTGFGQDSILS..(22)..EEHFDARRVALMGGSHGGFLSCHLIGQYPETY.....PVLLMLGQEDRRVP..(21)..LYPKSNHALSEV..(9)...AVLWL  P13676
dpp4_Mus   LLDVYA..(10).F..(13)..IIVASFDGRGSGYQ..(29)..MGFVDSKRVAIWGWSYGGYVTSMVLGSGSGVF.....EYLLIHGTADDNVH..(21)..WYTDEDHGIASS..(6)...MSHFL  P28843
dpp4_Rat   LLDVYA..(10).F..(13)..IIVASFDGRGSGYQ..(29)..MGFVDSKRVAIWGWSYGGYVTSMVLGSGSGVF.....EYLLIHGTADDNVH..(21)..WYTDEDHGIASS..(6)...MSHFL  P14740
dpp4_Hum   LLDVYA..(10).F..(13)..IIVASFDGRGSGYQ..(29)..MGFVDNKRIAIWGWSYGGYVTSMVLGSGSGVF.....EYLLIHGTADDNVH..(21)..WYTDEDHGIASS..(6)...MSHFI  P27487
dap1_Yea   LVNIYG..(6)..F..(17)..VIVLQIEPRGTGGK..(31)..SQHIDESKIAIWGWSYGGFTSLKTVELDNGDT.....RLFIVHGTFDDNVH..(22)..IFPDSDHSIRYH..(9)...LYYWL  P33894
dap2_Yea   FFFAYG..(10).F..(14)..AIVVVVDGRGTGFK..(29)..ITFVDPQKISLFGWSYGGYLTLKTLEKDGGRH.....RFLLMHGTGDDNVH..(22)..VFPDSDHSIRYH..(9)...LLDWA  P18962
                                                                        *                                      *                  *
```

Figure 1 Conserved motifs found in the PLEES family of proteins

The six conserved blocks were aligned using the MACAW multiple sequence alignment program. The number of residues located between conserved blocks is indicated. Conserved residues are colour-coded as described by Gibson et al. [9] with minor changes: hydrophobic residues are *dark blue*; Ser and Thr, *green*; Asp and Glu, *purple*; Arg and Lys, *red*; His, *pink*; Tyr, Trp and Phe, *light blue*. Only conserved Gly (*orange*) and Pro (*yellow*) are coloured. More than 50% occurrence of a property results in colouring. Residues forming the catalytic triad are indicated with an asterisk. The sequences are from SWISS-PROT and GenBank databases with the accession number for each sequence indicated in the right-most column. Functional assignment codes: bphrp_Hum, bphD_Ppu, bphD_PspL, bphD_Pte, bphD_PspK, bphD_Pce, bphF_RM5, dmpD_Ppu, xylF_Ppu and TodF_Ppu, catD_Aca, pcaD_Aca and cmt_Ppu, serine hydrolases; bpA1_Sau, bpA2_Sau, cpoL_Sli and cpoP_Ppy, bromo- and chloro-peroxidases; est_Pfl, est5_Psp, est_Ppu, tpes_ppu, bcho_Rca, nonR_Sgr and phab_pol, esterases; lip3_Msp, pldB_Eco and lip1_Pim, lipases; dehh1_Msp and dhla_Xau, haloalkane dehalogenases; ephx, epoxide hydrolases; pip, prep, acph, dpp and dap, peptidases.

1 Harayama, S. and Timmis, K. N. (1989) in Genetics of Bacterial Diversity (Hopwood, D. A. and Chater, K. F., eds.), pp. 151—174, Academic Press, New York
2 Duggleby, C. J. and Williams, P. A. (1986) J. Gen. Microbiol. **132**, 717–726
3 Puente, X. S. and López-Otín, C. (1995) J. Biol. Chem. **270**, 12926–12932
4 Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990) J. Mol. Biol. **215**, 403–410
5 Pellétier, I. and Altenbuchner, J. (1995) Microbiology **141**, 459–468
6 Polgár, L. (1992) FEBS Lett. **311**, 281–284
7 Rawlings, N. D. and Barrett, A. J. (1993) Biochem. J. **290**, 205–218
8 Schuler, G. D., Altschul, S. F. and Lipman, D. J. (1991) Proteins Struct. Funct. Genet. **9**, 180–190
9 Gibson, T. J., Hyvönen, M., Musacchio, A. and Saraste, M. (1994) Trends Biochem. Sci. **19**, 349–353
10 Blow, D. M., Birktoft, J. J. and Hartley, B. S. (1969) Nature (London) **221**, 337–340
11 Franken, S. M., Rozeboom, H. J., Kalk, K. H. and Dijkstra, B. W. (1991) EMBO J. **10**, 1297–1302
12 Hecht, H. J., Sobek, H., Haag, T., Pfeifer, O. and van Pee, K. H. (1994) Nature Struct. Biol. **1**, 532–537
13 Ollis, D. L., Cheah, E., Cygler, M., Dijkstra, B., Frolow, F., Franken, S. M., Harel, M., Remington, S. J., Silman, Y., Schrag, J. et al. (1992) Protein Eng. **5**, 197–211
14 Arand, M., Grant, D. F., Beetham, J. K., Friedberg, T., Oesch, F. and Hammock, B. D. (1994) FEBS Lett. **338**, 251–256
15 Derewenda, Z. S. and Derewenda, U. (1991) Biochem. Cell Biol. **69**, 842–851
16 Cygler, M., Schrag, J. D., Sussman, J. L., Harel, M., Silman, Y., Gentry, M. K. and Doctor, B. P. (1993) Protein Sci. **2**, 366–382
17 Liao, D. L. and Remington, S. J. (1990) J. Biol. Chem. **265**, 6528–6531
18 Martínez, C., Nicolas, A., van Tilbeurgh, H., Egloff, M. P., Cudrey, C., Verger, R. and Cambillau, C. (1994) Biochemistry **33**, 83–89
19 Doolittle, R. F., Feng, D. F., Tsang, S., Cho, G. and Little, E. (1996) Science **271**, 470–477

Received 4 November 1996