The EMBO Journal vol.10 no.5 pp.1053 - 1059, 1991

A hypothetical model for the peptide binding domain of hsp70 based on the peptide binding domain of HLA

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Communicated by J.J.Skehel

The sequences of the peptide binding domains of 33 70 kd heat shock proteins (hsp70) have been aligned and a consensus secondary structure has been deduced. Individual members showed no significant deviation from the consensus, which showed a $\beta_4\alpha$ motif repeated twice, followed by two further helices and a terminus rich in Pro and Gly. The repeated motif could be aligned with the secondary structure of the functionally equivalent peptide binding domain of human leucocyte antigen (HLA) class I maintaining equivalent residues in structurally important positions in the two families and a model was built based on this alignment. The interaction of this domain with the ATP domain is considered. The overall model is shown to be consistent with the properties of products of chymotryptic cleavage.

Key words: hsp70/HLA peptide binding site/structure prediction

Introduction

Heat shock proteins of the hsp70 class form a highly conserved family found in cells of all types in both constitutive and inducible forms. They appear to function by binding to unfolded segments of peptide chain and play a central role in controlling folding and unfolding of proteins and in their transport across intracellular membranes (Schlesinger, 1990; Rothman, 1989). The structural basis for this general binding property has not yet been defined, but it is known that energy from ATP hydrolysis is required to dissociate the complexes and a peptide dependent ATPase activity has been demonstrated (Flynn *et al.*, 1989; DeLuca-Flaherty *et al.*, 1990). This ATPase has been well characterized using the clathrin uncoating activity of hsp70 as a functional assay in parallel with measurements of clathrin binding (Chappell *et al.*, 1986; Heuser and Steer, 1989). The N-terminal ATP-binding domain has been isolated as a proteolytic fragment, its structure has been determined by X-ray crystallography (Flaherty *et al.*, 1990) and has been shown to be similar to that of actin (Kabsch *et al.*, 1990). The proteolysis experiments also established that peptide binding was associated with the C-terminal 250 residues (Chappell *et al.*, 1987). In this paper we examine the known properties of this segment of the molecule in a search for clues to the mode of action of the protein family. By use of secondary structure prediction, sequence alignment and modelling techniques, we show that this binding domain could have a structure very similar to that of the HLA molecule, another protein which can bind a variety of peptide sequences. The possible interactions of this domain with the ATP binding domain are considered.

Results and Discussion

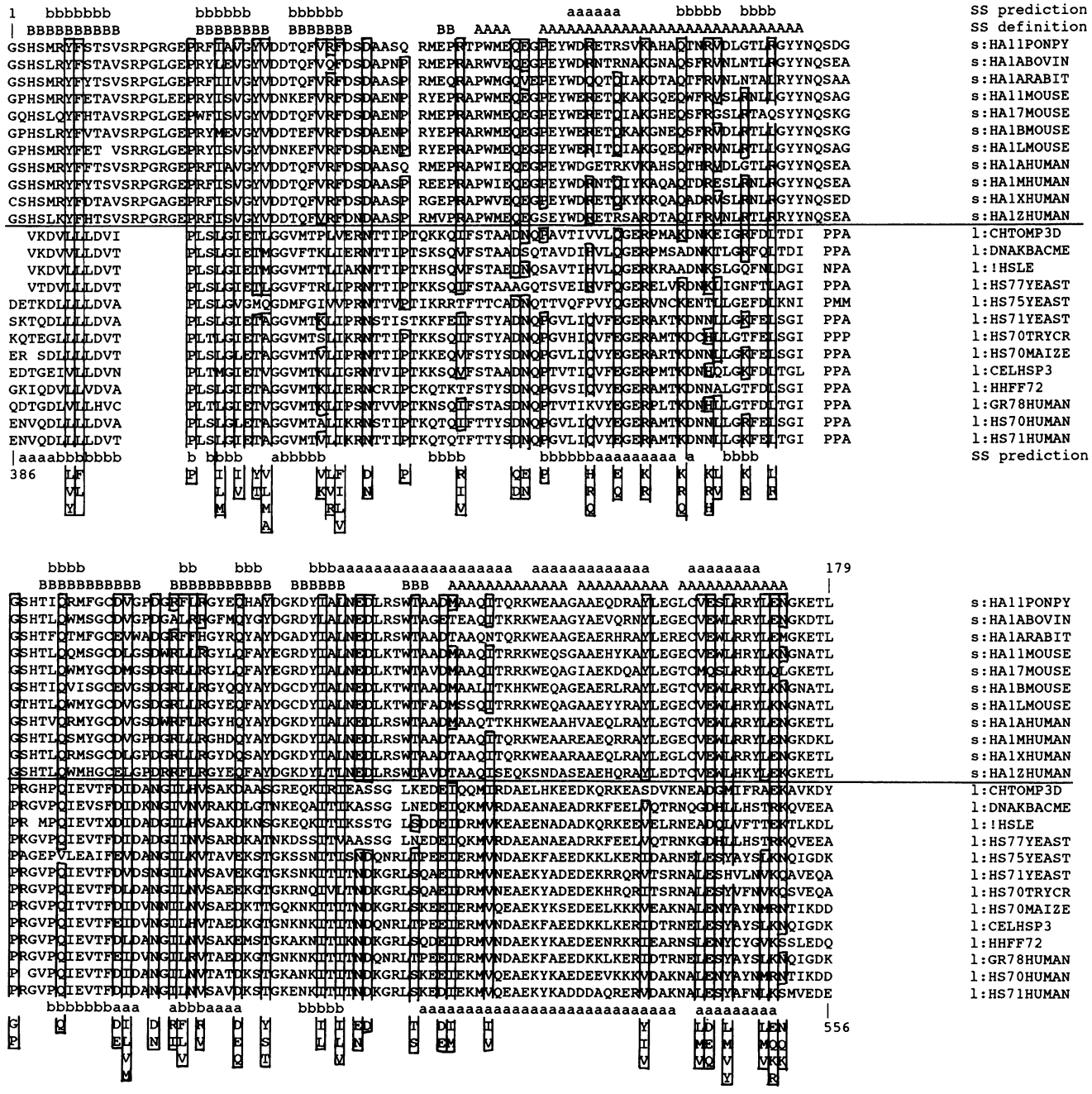
The first 150 residues immediately following the ATP-binding domain were predicted mainly as alternating β-strands and bends and were followed by a region of more variable sequence, predicted as α-helix. Prolines were highly conserved in a number of positions (Figure 1). A more critical examination revealed a consistent helical prediction in the middle of the β-strand region, so that the whole domain could be described approximately as $\beta_4\alpha\beta_4\alpha$, a pattern which agrees with an HLA-type fold. The validity of this tentative parallel was reinforced by the ability of both HLA and hsp70 to bind a wide range of peptide sequences (Flynn *et al.*, 1989). A further interesting clue in this respect is that the human MHC which includes the genes for HLA also contains genes for hsp70 (Sargent *et al.*, 1989).

In the absence of any significant sequence identity we used the secondary structure predictions for the hsp70 protein family and the positions of functionally important amino acids, especially prolines to guide a sequence alignment of the two families (Figure 1) from which a three-dimensional model of the binding domain of the human protein (Hunt and Morimoto, 1985) based on the known structure of the $\alpha_1\alpha_2$ domains of HLA-A2 (Bjorkman *et al.*, 1987) was generated, introducing only a few gaps in existing loop regions. Because of ambiguities in the secondary structure prediction, two alternative models were considered. Both agree in the first half of the protein which comprises four β-strands and a long helix. The first model (Figure 2) tries to satisfy the apparent two-fold symmetry in HLA, but this has the consequence that a relatively well predicted and conserved strand (residues 458–461, RFEL in human hsp70) is incorporated into the end of the first long helix. However, it should be noted that the predicted secondary structure of HLA (shown as top line of the alignments) also includes a β-strand in this position in place of the extension of this helix. The imposition of a helical conformation formed a complete binding cleft on this end. This model has the further advantage that only a few small insertions and deletions are necessary to align the secondary structure elements satisfactorily. By contrast, if one puts more weight on the secondary structure prediction, one then has to accommodate the strand in the succeeding sheet. This second model (Figure 3) in which the cleft is open shifts the residues in the final strand into the second helix (see alignments in the figures). Compared with HLA, the total number of hydrophobic residues in the predicted sheet is lower for hsp, but still large enough to create hydrophobic patches on both sides of the sheet, suggesting that parts of the lower face

of the sheet in hsp70 either interact with the C-terminal helices or the ATPase domain. The hydrophobic residues pointing into the cleft might correspond to the supposed preference of the heat shock proteins to bind hydrophobic regions in incompletely folded polypeptides. This preference would be reinforced by a stripe of five conserved hydrophobic residues in the first α-helix (V, V, M, L, F), all pointing into the binding site. The corresponding residues in the second helix are more polar and only moderately conserved. In HLA these residues (the 'p' positions in the alignment of Brown *et al.*, 1988), vary widely and determine the HLA specificity. By contrast hsp70 is a more highly conserved protein of broad specificity and the variation within a species correlates mainly with the intracellular location (Craig *et al.*, 1989). Too few ATPase activating peptides have been studied to establish a correlation between structure and binding (Flynn *et al.*, 1989; DeLuca-Flaherty *et al.*, 1990) which could be used in the model building, but from the scarce data available it is obvious that not all binding peptides are very hydrophobic (DeLuca-Flaherty *et al.*, 1990). This would not be surprising if the peptide binding is not mediated by side chain interaction, but by interaction with the peptide backbone, as suggested by T.Hubbard and C.Sander (submitted).

The intron positions of the human hsp70 protein (Dworniczak and Mirault, 1987) were checked for compatibility with the models. In both models the intron positions are found in positions outside the well defined β-strand elements in the kink of the first helix; the second intron is in the loop connecting strands seven and eight (model 1)

A



or at the very beginning of the second helix (model 2). The arrangement is plausible but it differs significantly from HLA, where the two exons code exactly for the two halves of the domain. The two main exons coding for the binding site in hsp70 would adopt a more symmetric relationship in model 2 (both exons coding for a helical part and four strands, compared with four and three strands for the two respective exons in model 1). A good test for the right model would be the introduction by site-directed mutagenesis of the disulphide bond present in (nearly) all HLA class I molecules (Cys101−Cys164) into the heat shock protein at the positions predicted by the two models. As the last two helices which are predicted from the heat shock sequences seem not to be involved in protein binding (Chappell *et al.*, 1987), no attempt has been made to include them in the structural models.

The secondary structural and functional relation of the hsp70 proteins and the antigen-presenting MHC class I proteins allowed the generation of the hypothetical models for the former presented above. The secondary structure prediction in itself would not have been sufficient to pinpoint HLA as the basis for the model building as it is usually only

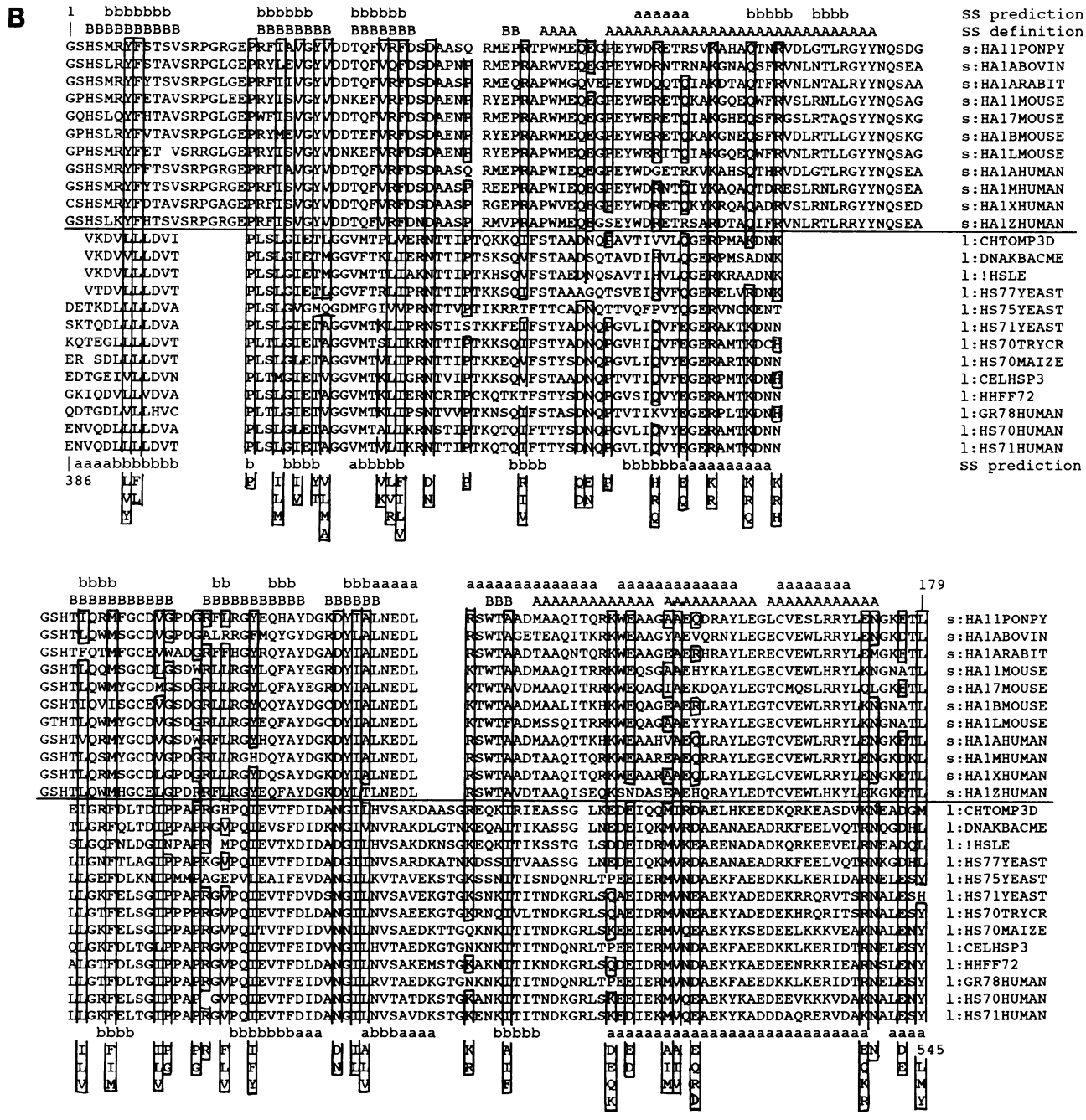


Fig. 1. Alignments of the peptide binding domains of the HLA family and the hsp70 family. Only sequences with high diversity are shown. Conserved structurally important prolines and glycines, hydrophobic and hydrophilic residues are boxed and summarized below the alignments. In order to indicate the positions of the strands and helices, the secondary structure definitions for HLA-A2 are given above the HLA sequences (in capital letters). The secondary structure predictions are given in lower case letters above and below the families for comparison. The letters in front of the databank codes for the sequences indicate from which source they were extracted (s: SWISSPROT database, l: Leeds database). (**A**) Alignment for model 1. (**B**) Alignment for model 2.

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pred.  1   bbbbbbb         bbbbbb    bbbbbb                        aaaaaa      bbbbb  bbbb
def.   | BBBBBBBBBB        BBBBBBBB  BBBBBBB         BB  AAAA   AAAAAAAAAAAAAAAAAAAAAAAAAAAA
HLA:   GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQ RMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRGYYNQSEA
hsp70: ENVQDLLLLDVA       PLSLGLETAGGVMTALIKRNSTIPTKQTQIFTTYSDNQPGVLIQVYEGERAMTKDNNLLGRFELSGI  PPA
pred.  |aaaabbbbbbb       b bbbb   abbbbb           bbbb        bbbbbbaaaaaaaaa a   bbbb
       386

           bbbb          bb    bbb     bbbaaaaaaaaaaaaaaaaaa  aaaaaaaaaaaa    aaaaaaaa       179
         BBBBBBBBBBB   BBBBBBBBBBB  BBBBBB      BBB  AAAAAAAAAAAAA AAAAAAAAAA AAAAAAAAAAAA    |
       GSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETL
       PRGVPQIEVTFDIDANGILNVTATDKSTGKANKITITNDKGRLSKEEIERMVQEAEKYKAEDEVQRERVSAKNALESYAFNMKSAVEDE
           bbbbbbbaaa   abbbaaaa      bbbbb        aaaaaaaaaaaaaaaaaaaaaaaaaaa  aaaaaaaaa     |
                                                                                               556
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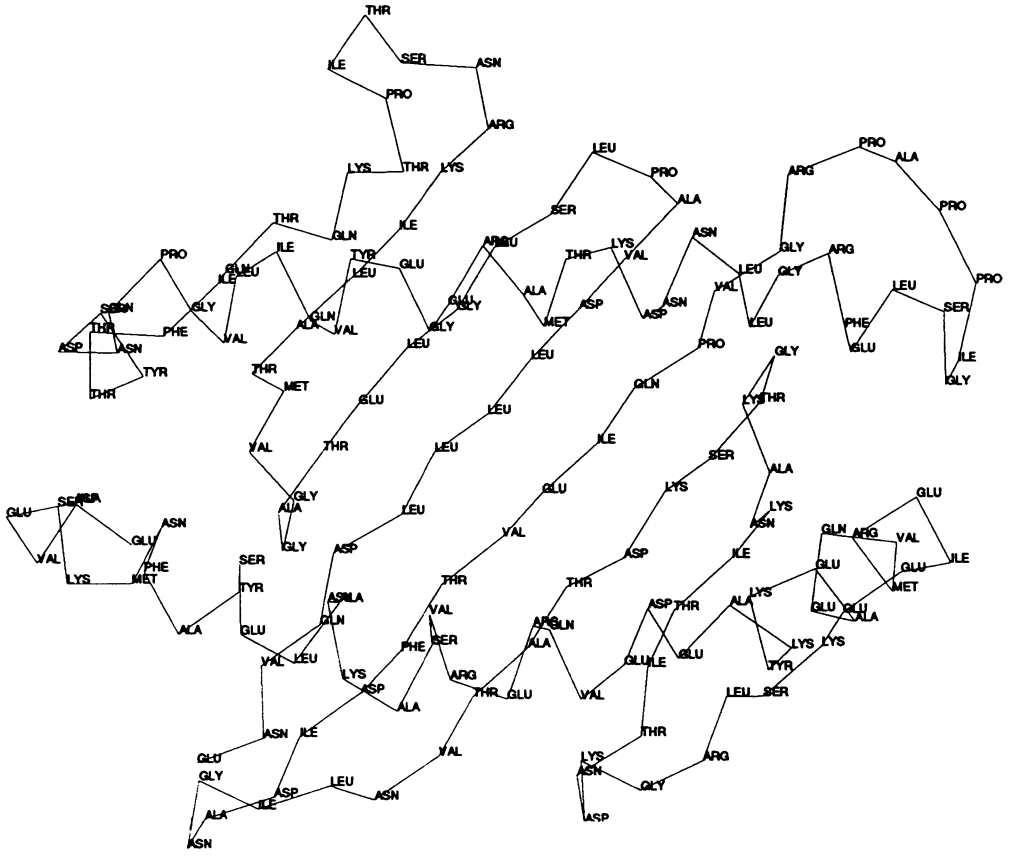

Fig. 2. Hypothetical model of the human hsp70 binding site based on the structure of HLA-A2 maintaining the overall symmetry of the molecule. Secondary structure elements were aligned and small gaps were introduced in loop regions. The alignment (in standard one letter amino acid code) is shown together with the secondary structure definition (Kabsch and Sander, 1983) in capital letters above the HLA sequence; A = α-helix, B = β-strand) and the averaged secondary structure prediction (Garnier *et al.*, 1978; Zvelebil *et al.*, 1987) on top of the HLA secondary structure definition and below the hsp70 sequence in lower case letters.

~60% correct (as can be seen in the comparison of the secondary structure prediction and the respective definition for HLA, Figure 2) and as there are other known structures of similar topology. But taken together with the peptide binding property of hsp70, HLA was the preferred framework for the model building. The procedure of model building without obvious sequence identity is not dissimilar to the generation of the MHC class II model by Brown *et al.* (1988), although the relations among class I and class II molecules are much closer. It is interesting to note that the positions for insertions and deletions in our models are almost identical with the predicted deviations from class I proposed by Brown *et al.* in their model of the class II structure.

In HLA the binding domains (α_1 + α_2) do not appear to be stable in isolation. It is likely that the support of the Ig-like α_3 and β_2m microglobulin domains which interact with the underside of the β-sheet is required to stabilize the correct fold (Williams *et al.*, 1989). The interacting region is not compact and involves the loops and bends which connect the strands of α_3 or β_2m. In the sequence of hsp70 the α_3 domain is replaced by two long (20–25 residue) α-helices followed by a C-terminal tail, rich in prolines and glycines. Although the sequence in this helical region is less well conserved than in the binding domain, the pattern of hydrophobic residues and the strong α-helical prediction is the same in all the hsp70 proteins. These helices could pack beside the C-terminal helix of the α_2-like domain, but it seems more likely that they would adopt a position equivalent to that of α_3 and pack against the underside of the β-sheet. Evidence that this surface may interact with other parts of the molecule comes from the high conservation of a few hydrophobic residues located on the concave lower surface of the sheet. The highly conserved hydrophobic sequence in the first strand (Figure 1) strongly suggests such an interaction.

Since ATP hydrolysis is required to release bound peptide chains from hsp70, there must be close interaction between conserved surface regions of the ATP domain and the peptide

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pred.  1   bbbbbbb          bbbbbb    bbbbbb                          aaaaaa      bbbbb  bbbb
def.   | BBBBBBBBBB         BBBBBBBB  BBBBBBB          BB  AAAA   AAAAAAAAAAAAAAAAAAAAAAAAAAAAA
HLA:   GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQ RMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRGYYNQSEA
hsp70: ENVQDLLLLDVA       PLSLGLETAGGVMTALIKRNSTIPTKQTQIFTTYSDNQPGVLIQVYEGERAMTKDNN
pred.  |aaaabbbbbbb       b    bbbb    abbbbb           bbbb        bbbbbbaaaaaaaaaa
       386

          bbbb          bb     bbb      bbbaaaaa      aaaaaaaaaaaaaa  aaaaaaaaaaaa     aaaaaaaa        179
     BBBBBBBBBBB   BBBBBBBBBB  BBBBBB            BBB  AAAAAAAAAAAAA AAAAAAAAAA AAAAAAAAAAAA     |
GSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDL     RSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETL
   LLGRFELSGIPPAPRGVPQIEVTFDIDANGILNVTATDKSTGKANKITITNDKGRLSKEEIERMVQEAEKYKAEDEVQRERVSAKNALESY
      bbbb          bbbbbbbaaa     abbbaaaa       bbbbb       aaaaaaaaaaaaaaaaaaaaaaaaaaaa  aaaa
                                                                                              545
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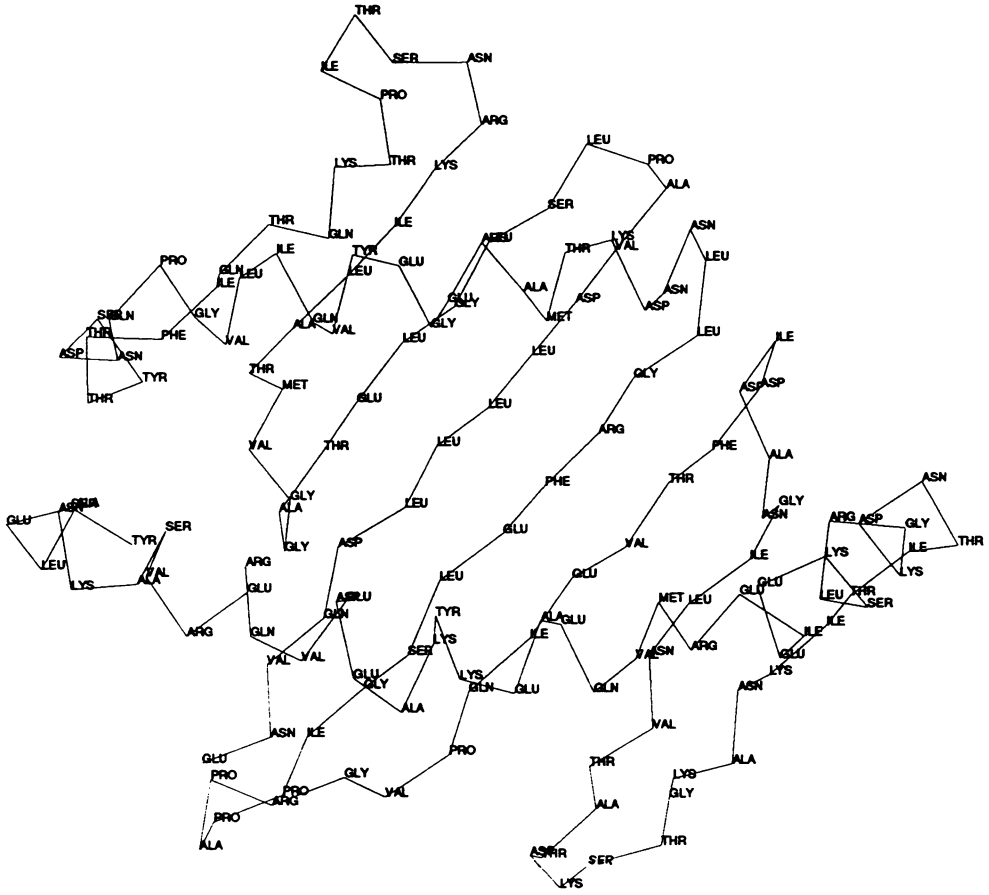

Fig. 3. Hypothetical model of the human hsp70 binding site based on the structure of HLA-A2 but with greater emphasis on the secondary structure prediction. The two helices differ greatly in length and the symmetry of the molecule is less obvious.

binding domain. The conserved surface regions of the ATPase domains include several segments noted by Flaherty *et al.*, (1990) to be on the same face of their domain I as the C-terminus of the final α-helix which connects to the first strand of the HLA-like domain. They proposed this as a possible region for interactions with the peptide binding domain. Combining this with the short (5 residue) connection between the conserved hydrophobic C-terminus of this helix and the first strand of the HLA sheet, the interaction of the binding domain with the ATPase domain is well constrained. Results from proteolysis provide further support for our model. The first product of chymotrypsin action (Chappell *et al.*, 1987) is a 60 kd fragment, cleaved just after the C-terminus of our model, which still binds clathrin but has lost its ATPase activity. A second cleavage removes the binding domain, generating the 44 kd ATPase fragment (Flaherty *et al.*, 1990). This sequence of events is comprehensible if it is assumed that the C-terminal helices, which are removed by the first cleavage, mediate the interaction between the two domains. An interesting feature of the glycine and proline rich tail, which follows these helices, is its resemblance to the peptide from the clathrin light chain which is specifically bound by hsp70 (DeLuca-Flaherty *et al.*, 1990). This suggests that the C-terminus may be recognized by the hsp70 binding site, probably by interaction with the unshielded backbone in this sequence as proposed by T.Hubbard and C.Sander (submitted) and this might account for the oligomerization of hsp70 which is reversed by ATP (Heuser and Steer, 1989). Using the sequences of the clathrin light chain which is recognized by hsp70 and the C-terminal sequences of hsp70 as starting points, peptides could be tested for binding to hsp70 in order to clarify the structural origin for the interaction.

The predicted secondary structure of the hsp70 protein GR78 was recently evaluated in relation to the CD spectra of the protein (Sadis *et al.*, 1990). The predictions were essentially the same as those shown here, but it was concluded that the domain consists of an antiparallel β-

Table I. Comparison of buried residue positions in HLA and the two hsp70 models

Buried HLA residue	Residue at the equivalent position in the hsp70 models	
	Model 1	Model 2
Met5	Asp390 partly exposed	= model 1
Phe9	Leu394 buried	= model 1
Ser11	Val396 buried	= model 1
Phe22	Ser400 nearly buried	= model 1
Ala24	Gly402 buried	= model 1
Gly26	Glu404 exposed	= model 1
Val28	Ala406 buried	= model 1
Phe33	Thr411 nearly buried	= model 1
Val34	Ala412 nearly buried	= model 1
Phe36	Ile414 buried	= model 1
Ala49	Phe428 partly exposed	= model 1
Ile52	Tyr431 partly exposed	= model 1
Thr46	Tyr443 OH exposed	= model 1
Val67	Glu446 partly exposed	= model 1
His74	Asn453 partly exposed	= model 1
Leu81	Glu460 partly exposed	–
Val95	Pro472 buried	Leu458 partly exposed
Gly100	Thr477 partly exposed	Leu461 buried
Cys101	Phe478 buried	Ser462 buried, HB Lys526
Val103	Ile480 buried	Ile464 buried
Gly112	Thr489 partly exposed	Gln473 exposed
Tyr123	Lys500 partly exposed	Gly485 buried
Ile124	Ile501 buried	Ile486 buried
Leu130	Lys507 partly exposed	Thr491 exposed
Ala140	Arg517 partly exposed	Thr502 exposed
Ala153	Glu530 partly exposed	Val519 buried
Leu160	Ser537 buried, HB Thr489	Lys526 partly exposed
Cys164	Ala541 buried	Glu530 partly exposed
Val165	Leu542 buried	Val531 buried
Leu168	Tyr545 partly exposed	Glu534 partly exposed
Tyr171	Asn548 partly exposed	Ser537 buried, HB Tyr431
Leu172	Met549 partly exposed	Ala538 buried
Gly175	Ala552 buried	Ala541 buried

Possible side chain hydrogen bonds (HB) for buried residues are indicated. The accessible area for each residue was calculated using the program DSSP (Kabsch and Sander, 1983).

segment followed by three polar α-helices and the possible significance of the short α-helix following the first set of β-strands was not considered.

In topological terms, three other known protein structures are somewhat similar to HLA and the hsp70 model and could form a special subclass of the $\alpha + \beta$ proteins. The bacteriophage MS2 coat protein (Valegard *et al.*, 1990) is formed of dimers with a four stranded β-sheet topped by a long α-helix. The bovine platelet factor 4 (St Charles *et al.*, 1989) and interleukin 8 (Clore *et al.*, 1990) form dimers with similar topology ($\beta_3\alpha$ for the monomer). In all these structures, the helices lie parallel to each other on top of the sheet exposed to the environment. There is no obvious functional relation of the three proteins to HLA or hsp70. If the duplicated $\beta_4\alpha$ structure constitutes a general motif then our model for hsp70 need not necessarily imply any evolutionary relatedness to the HLA proteins, although it is tempting to assume that both hsp70 and the HLA system are derived from a common peptide binding ancestor.

Materials and methods

In preparation for the model building, 33 sequences of heat shock proteins and 41 HLA class I sequences were aligned using the method of Taylor (1988). The heat shock protein sequences were extracted from the Leeds database (Bleasby and Wootton, 1990), codes !HSLE, A25398, CELHSP3, CHKGR78, CHTOMP3D, DNAKBACME, DNAKBACSU, DROHSP7A2, GR78HUMAN, GR78MESAU, GR78RAT, HHFF72, HHKW7A, HHXL70, HS70CHICK, HS70HUMAN, HS70MAIZE, HS70PETHY, HS70PLAFA, HS70TRYBR, HS70TRYCR, HS71HUMAN, HS71MOUSE, HS71YEAST, HS72MOUSE, HS72YEAST, HS73RAT, HS75YEAST, HS77YEAST, IQECDK, THE70HSP, TRCHSP70A and YSCKAR2. These sequences from a large variety of species have a high divergence but the interesting region could still be unambiguously aligned. The HLA sequences are from the SWISSPROT database (Bairoch, 1990), codes HA1ABOVIN, HA1BBOVIN, HA10HUMAN, HA11HUMAN, HA13HUMAN, HA1AHUMAN, HA1CHUMAN, HA1GHUMAN, HA1JHUMAN, HA1MHUMAN, HA1NHUMAN, HA1OHUMAN, HA1PHUMAN, HA1QHUMAN, HA1VHUMAN, HA1XHUMAN, HA1YHUMAN, HA1ZHUMAN, HA10MOUSE, HA11MOUSE, HA12MOUSE, HA13MOUSE, HA14MOUSE, HA15MOUSE, HA17MOUSE, HA18MOUSE, HA1BMOUSE, HA1DMOUSE, HA1KMOUSE, HA1LMOUSE, HA1UMOUSE, HA1WMOUSE, HA1BPANTR, HA1CPANTR, HA1DPANTR, HA1EPANTR, HA1NPANTR, HA11PONPY, HA1ARABIT, HA1BRABIT and HA11RAT. The HLA sequences, which are found only in multicellular species, show much less variation than hsp70 except for certain residues involved in the specific binding of peptides or T-cell restriction. The secondary structures were predicted for a number of individual sequences using standard prediction procedures (Chou and Fasman, 1978; Garnier *et al.*, 1978) and a prediction for the aligned sequences was then produced by simple averaging (Zvelebil *et al.*, 1987). The predicted β-strand and α-helix sequences were aligned manually with structurally corresponding sequences in HLA (residues 1 – 180) taking structurally important residues, especially conserved hydrophobics, prolines and glycines into account. A

selection of 11 HLA and 13 hsp sequences representing high divergence are aligned in Figure 1 (the alignments of all 41 HLA and 33 hsp sequences are available from the authors on request). The residue type in every position was checked in order to obtain a good correspondence of residue type for the strands and for the phasing of the helices and any gaps in the alignment were introduced in the loop regions and in kink positions in the helices. The residues in the HLA-A2 parent structure were mutated into the corresponding residues maintaining the original side chain orientations. Arg469, which is present in all other aligned hsp sequences, was inserted into the modeled sequence HS70HUMAN. The insertions and deletions were modelled by conformational search and local energy minimization using the modelling program QUANTA (Polygen Corporation, Waltham, MA, USA). Finally the calculated energy for the entire structure was minimized roughly. On the assumption that conserved hydrophobic residues tend to be buried (Bowie *et al.*, 1990), the initial alignment was improved by plotting the accessible area of each residue (Kabsch and Sander, 1983) together with a quantitative measure for conservation against position in the sequence. Inconsistencies were removed by short shifts (1–2 residues) in the sequence alignment. The resulting alignment was used to build a further model and this cycle was repeated until a reasonable agreement between conservation pattern and accessible area pattern was reached. The residues in the equivalent positions to buried residues in HLA were checked individually for hydrophobic interaction and possible hydrogen bonds (see Table I). For the few buried hydrophilic residues in the model, hydrogen bond partners were found. The large, partly hydrophobic side chains of arginine and lysine are equivalent to valine and leucine in some positions (see Figure 1 and Table I). In the predicted strands conserved hydrophobic residues were placed in positions where their side chains would point towards the helices flanking the binding cleft while in the helices unconserved and hydrophilic residues were placed in outward pointing positions. Prolines were either placed in loop or turn regions except for Pro436 which is situated in the kink between the 3,10-helical and the α-helical part of the α_1-domain. Most conserved proline positions in hsp have an equivalent proline or glycine in HLA (see Figure 1).

Acknowledgements

We thank Dr D.B.McKay for information on the structure of the ATPase prior to publication.

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Received on December 10, 1990; revised on February 11, 1991