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Conformational change of chaperone Hsc70 upon binding to a decapeptide: A circular dichroism study

KYUSUNG PARK,[1,3] GREGORY C. FLYNN,[2]
JAMES E. ROTHMAN,[2] AND GERALD D. FASMAN[1]

[1] Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254-9110
[2] Department of Molecular Biology, Lewis Thomas Laboratory, Princeton University, Princeton, New Jersey 08544

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Abstract

The conformation of bovine Hsc70, a 70-kDa heat shock cognate protein, and its conformational change upon binding to decapeptides, was studied by CD spectroscopy and secondary structure prediction (Chou, P.Y. & Fasman, G.D., 1974, *Biochemistry 13*, 222–245). The CD spectra were analyzed by the LINCOMB method, as well as by the convex constraint analysis (CCA) method (Perczel, A., Park, K., & Fasman, G.D., 1992, *Anal. Biochem. 203*, 83–93). The result of the CD analysis of Hsc70 (15% α-helix, 24% β-sheet, 24% β-turn, and 38% remainder) was very similar to the predicted secondary structure for the β-sheet (24%) and the β-turn (29%). However, there is disagreement between the α-helical content by CD analysis (15%) and the predicted structure (30%). In spite of the fact that the decapeptides contained a considerable amount of β-sheet (22%), the interaction of the heat shock protein with the peptide resulted in an overall decrease in the content of β-sheet conformation (-15%) of the complex. This may be due to induction of a molten globule state. The result of the CCA analysis indicated that the Hsc70 undergoes a conformational change upon binding the decapeptides.

Keywords: chaperone Hsc70; circular dichroism; conformational change; decapeptide

Molecular chaperones are defined as a family of proteins that mediate the correct folding of other proteins, and in some cases their oligomerization into polymeric structures, but are not themselves components of the final structures (Gatenby & Ellis, 1990). It is believed that chaperones function by recognizing structural features of the interactive surfaces that are accessible only during stages in the assembly process, or that appear as a result of stress, such as heat, in an already assembled structure (Ellis & Van der Vies, 1991).

The general concept of molecular chaperones was introduced by Laskey et al. (1978) and later extended by Ellis and his colleagues (Ellis, 1987, 1990; Ellis & Van der Vies, 1988) to define a much wider range of proteins whose function is to reduce the probability of incorrect interactions. It became clear that a wide variety of biological processes in many types of cells require chaperones to function properly.

Chaperone use is diverse:

1. In protein synthesis and cytosolic folding. The introduction of the concept of molecular chaperones does not contradict the self-assembly hypothesis (Anfinsen, 1973) by suggesting that in many cases interactions within and between polypeptides and other molecules play a role in reducing the probability of the formation of incorrect structures (Ellis & Van der Vies, 1991).
2. Proteins are transported into the endoplasmic reticulum, mitochondrion, plastid, and bacterial periplasm, in an unfolded or partially folded form. Chaperones solubilize and prevent precipitation of the protein (Nilsson & Anderson, 1991) during transport.
3. The normal functioning of oligomeric polypeptide complexes involves changes in subunit–subunit interactions, so that regions previously involved

Reprint requests to: Gerald D. Fasman, Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254-9110.
[3] Present address: Lineberger Cancer Research Center, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514.

in subunit contacts are transiently exposed to the intracellular environment, e.g., DNA replication, recycling of clathrin cages, and the assembly of microtubules.

4. During organelle biogenesis, subunits of some oligomeric proteins are synthesized in the cytosolic compartment and are transported to the organelles (Ellis & Van der Vies, 1991).
5. Environmental stresses such as excessive heat often cause the denaturation of proteins and the formation of aggregates. Chaperones protect against such stresses.

The most fundamental concept in the function of chaperones would be their "interactive surface." The term interactive surface refers to regions of intramolecular or intermolecular contact that are significant in maintaining the structure, such as the conformation of monomeric proteins and the quaternary structure of oligomeric proteins (Gatenby & Ellis, 1990). Transient exposure of such a surface involves changes in protein-protein interactions.

A 70-kDa heat shock cognate protein (Hsc70), the clathrin uncoating ATPase, is a member of the Hsp70 family, found in both cytosolic and nuclear matrices and believed to be involved in the folding of nascent proteins (Beckmann et al., 1990). Unlike the members of the Hsp70 family, however, Hsc70 is a constitutive stress protein that functions in normal cellular physiology (Hightower, 1991). The eukaryotic cytosolic Hsc70 protein facilitates posttranslational translocation of proteins into mitochondria and the endoplasmic reticulum by interacting with the protein to be translocated (Chirico et al., 1988; Deshaies et al., 1988). The translocation process is believed to involve unfolding of the protein, based on the observation that translocating proteins were in an incompletely folded state (Eilers & Schatz, 1986), and that preincubation of proteins with urea can mimic the Hsc70's stimulatory effect on translocation (Eilers et al., 1988).

Data collected indicate that Hsc70 forms a reversible complex with a heat shock (transcription) factor (HSF), which is released when Hsc70 forms aggregates with denatured proteins. The free HSF, in turn, turns on heat shock genes by interacting with heat shock elements (Hightower, 1991; Sorger, 1991).

Because a given chaperone acts on a number of different proteins, chaperones must recognize features present in incompletely folded polypeptide chains and not strictly dependent on primary structures (Landry & Gierasch, 1991; Nilsson & Anderson, 1991). Even though contradictory to the "framework model" of protein folding (Ptitsyn, 1973, 1991; Kim & Baldwin, 1982), Landry and Gierasch (1991) proposed that GroEL binding can induce a helical conformation. Martin et al. (1991) suggested that GroEL binds to a protein in a molten globule state, which is less specific in terms of the binding region of the unfolded proteins. The induced α-helix of a peptide upon binding to GroEL is an interesting observation, but it is premature to suggest it as a universal binding motif for chaperones.

Bychkova et al. (1988) were the first to propose that the molten globule state, a compact state having secondary but not rigid tertiary structure, was involved in translocation of proteins across a variety of membranes. A similar state has been shown to accumulate on the folding pathway of globular proteins.

In this report, the binding of random decapeptides to Hsc70 has been studied by CD spectroscopy to investigate whether the binding causes alteration in the conformation of Hsc70.

Results and discussion

The CD spectrum of bovine Hsc70 is shown in Figure 1. This spectrum is similar to that reported by Palleros et al. (1991). The ellipticity values are approximately one-half the magnitude of those reported by Sadis et al. (1990), with a ratio of $[\theta]_{208}/[\theta]_{222} < 1$ in contrast to the >1 value previously reported (Sadis et al., 1990). Sadis et al. (1990) used a different buffer (20 mM 4-(2-hydroxyethy)-1-piperazineethanesulfonic acid [Hepes]-KOH, 25 mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1 mM EDTA, 1 mM dithiothreitol [DTT], pH 7.50) than that used by Palleros et al. (1991) (20 mM KCl, 50 mM Tris-HCl, pH 7.5) and that

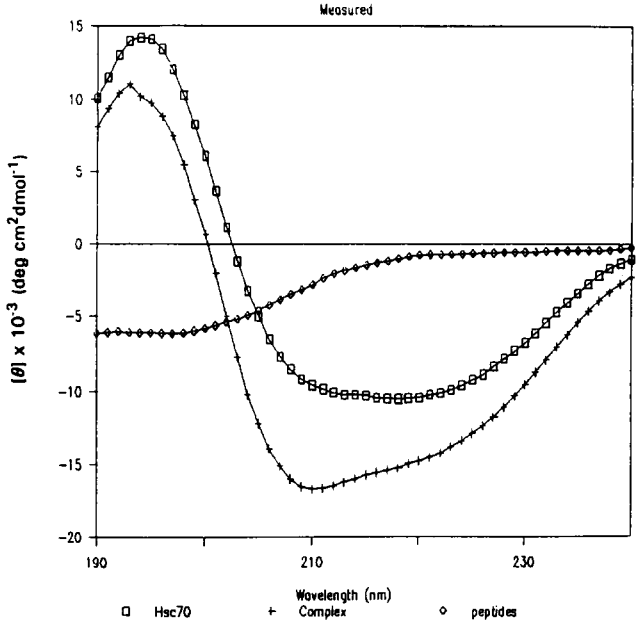

Fig. 1. The CD spectra of Hsc70 (□—□), the decapeptides (◇—◇), and the complex of Hsc70 and the decapeptides (+—+) at a molar ratio of 5.89:1 (peptide:protein). The protein concentration was 0.38 mg/mL in 20 mM KCl, 50 mM Tris, pH 7.5. The peptide concentration used to measure the CD of the peptides alone was 1 mg/mL and 36 μg/mL in the complex. At this concentration, the peptide CD signal was very weak, coinciding with the noise level.

used herein (20 mM KCl, 50 mM Tris-HCl, pH 7.5). Such differences in buffer may affect the state of oligomerization and conformation of Hsc70. The CD analyses, by the LINCOMB method (Perczel et al., 1992a), yielded a lower α-helical content (15%) (Table 1) than the 40–42%, reported by Sadis et al. (1990). The content of β-sheet (24%) and β-turn (24%) were more similar to the predicted secondary structure (Table 2) than the values reported by Sadis et al. (1990). The predicted secondary structure, determined by the Chou-Fasman prediction algorithm (Chou & Fasman, 1974; Prevelige & Fasman, 1989), yielded values of 30% α-helix, 24% β-sheet, 29% β-turn, and 17% remainder (Table 2), which was similar to the prediction by Sadis et al. (1990) by the Chou-Fasman method. Using the GOR method (Garnier et al., 1978), Sadis et al. (1990) obtained a higher α-helix content. These differences cannot be attributed to lack of purity of the Hsc70 used herein. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2) indicated that the Hsc70 was at least 95% pure.

The content of α-helix (15%), determined by the CD analysis and the secondary structure prediction, is comparable to the structure determined by X-ray crystallography of the N-terminal half of the Hsc70 (Flaherty et al., 1990). The secondary structural prediction, however, suggested 30% α-helix.

The CD spectrum of the complex of Hsc70 and the decapeptides (Fig. 1) showed increases in magnitudes of the two negative peaks at 208 and 222 nm ($[\theta]_{208}$ and $[\theta]_{222}$) and the ratio of $[\theta]_{208}/[\theta]_{222}$, whereas the magnitude of the positive peak at 198 nm was smaller and blue shifted compared to Hsc70 alone. The change in the CD spectrum indicated that the β-sheet content was decreased (−15%), while the α-helical content remained constant (13%) (Table 1).

The random decapeptides (Flynn et al., 1991), which contained every combination of amino acids in each position, were shown to contain a sizable amount of β-sheet conformation (22%) according to the LINCOMB analysis of the CD spectrum of the peptides (Fig. 3). The β-sheet conformation was relatively stable. At both 22 °C and 30 °C, it contained 19% β-sheet (Fig. 3; Table 1).

To determine whether the decrease in β-sheet content arose from the addition of the mostly random conformation of the decapeptide (Fig. 1), the convex constraint analysis (CCA) (Perczel et al., 1989, 1991) was applied to the three CD spectra shown in Figure 1. The result of the CCA analysis is shown in Figure 4. The spectra of the first component (Fig. 4) and the second component (Fig. 4) were correlated with the CD spectra of the Hsc70:decapeptide complex and the decapeptide, respectively. Because the CD spectrum of the complex was not a simple

Table 2. *Secondary structure prediction of the bovine Hsc70 amino acid sequence*[a]

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MSKGPAVGID LGTTYSCVGV FQHGKVEIIA NDQGNRTTPS YVAFTDTERL  50
cttteeeeee eettteeee ccceeeeecc cttteettt teeecttttc

IGDAAKNQVA MNPTNTVFDA KRLIGRRFDD AVVQSDMKHW PFMVVNDAGR 100
hhhhttttcc cttttchhhh heeeccccce eeettttcce eeeeccttt

PKVQVEYKGE TKSFYPEEVS SMVLTKMKEI AEAYLGKTVT NAVVTVPAYF 150
ceeeecttttt eeecttttee hhhhhhhhhh hhheeeeeee eeeeeecccc

NDSQRQATKD AGTIAGLNVL RIINEPTAAA IAYGLDKKVG AERNVLIFDL 200
ttttthhhhht tttcceeeee eecchhhhhh eeehhhhhhh hheeeeecct

GGGTFDVSIL TIEDGIFEVK STAGDTHLGG EDFDNRMVNH FIAEFKRKHK 250
ttteeeeeee ecceeeeccc cttttctttt cctttteeee eehhhhhhhh

KDISENKRAV RRLRTACERA KRTLSSSTQA SIEIDSLYEG IDFYTSITRA 300
ccctttthhh hhhhhhhhhh hcccttttth hhhhccccce eeeeeeeehh

RFEELNADLF RGTLDPVEKA LRDAKLDKSQ IHDIVLVGGS TRIPKIQKLL 350
hhhhhtttte eecchhhhhh hhhhhhtttt hhhhccttt ccchhhhhh

QDFFNGKELN KSINPDEAVA YGAAVQAAIL SGDKSENVQD LLLLDVTPLS 400
hhcchhhhht ttttttcch hhhhhhhhcc ttttccchhh hhhheeecee

LGIETAGGVM TVLIKRNTTI PTKQTQTFTT YSDNQPGVLI QVYEGERAMT 450
eeecttttee eeecceeeee ceeeeeeett ttcctttee eehhhhhhht

KDNNLLGKFE LTGIPPAPRG VPQIEVTFDI DANGILNVSA VDKSTGKENK 500
ttthhhhhhh ccccccttt cceeeeeecc tttteeeeec cttttcccee

ITITNDKGRL SKEDIERMVQ EAEKYKAEDE KQRDKVSSKN SLESYAFNMK 550
eeecttttth hhhhhhhhhh hhhhhhhhhh httttttth hhhchhhhhh

ATVEDEKLQG KINDEDKQKI LDKCNEIINW LDKNQTAEKE EFEHQQKELE 600
hhhhhhhttt tcttttthhh httteeecc cttthhhhh hhhhhhhhh

KVCNPIITKL YQSAGGMPGG MPGGFPGGGA PPSGGASSGP TIEEVD     646
ccceeeeeee ecttttttt ttttttttcc ttttttttt ttcccc
```

[a] The prediction was performed by the method of Chou and Fasman (1974) with the 64-protein database. h, α-helix; e, β-sheet; t, β-turn; c, remainder.

Table 1. *The secondary structure of bovine Hsc70, decapeptides, and their complex determined by CD analyses and secondary structure prediction*

Analyzed	α-Helix (%)	β-Sheet (%)	β-Turn (%)	Remainder (%)
Hsc70 alone				
CD analysis[a]	15	24	24	38
Prediction[b]	30	24	29	17
Decapeptides (CD)[a,c]				
At 22 °C	0	22	0	79
At 30 °C	0	19	0	81
Hsc70:decapeptide (CD)[a,d]	13	9	48	30
Difference spectra (CD)[a,e]	10	0	43	47

[a] The LINCOMB method (Perczel et al., 1992a) was used.
[b] The secondary structure prediction used the Chou-Fasman algorithm (Chou & Fasman, 1974; Prevelige & Fasman, 1989).
[c] The peptide concentration used for the CD analysis of the thermostability of the peptides was 1 mg/mL.
[d] The molar ratio of the protein:peptide was 1:5.89 at a peptide concentration of 36 μg/mL.
[e] Hsc70:decapeptide complex − Hsc70.

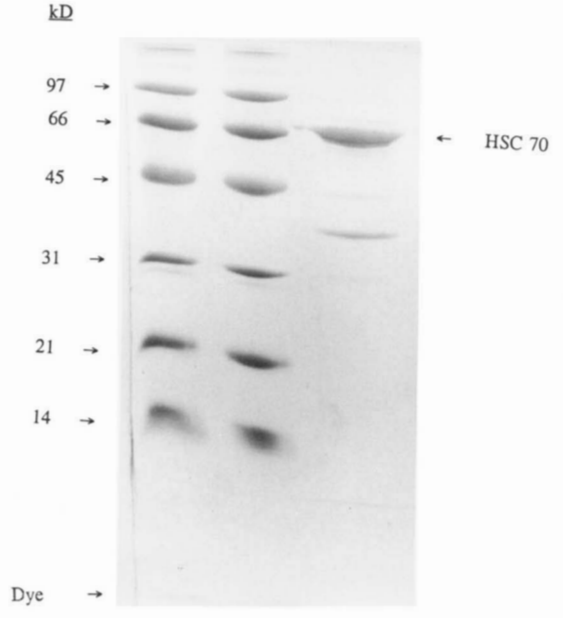

Fig. 2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of Hsc70. Run on a 10% acrylamide/0.2% bis-acrylamide gel and stained with Coomassie blue R-250. Bio-Rad low molecular weight standards were used.

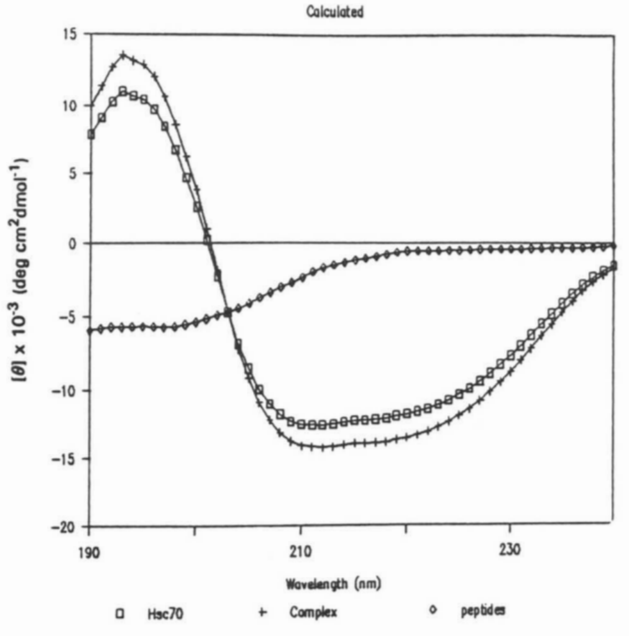

Fig. 4. The component spectra obtained by convex constraint analysis (CCA). The spectra of the first component (+—+) and the second component (◇—◇) were correlated with the CD spectra of the Hsc70:decapeptide complex and the decapeptide, respectively, using the CCA method (Perczel et al., 1989, 1991). The CD spectrum of the Hsc70 (□—□) was obtained by a combination of the two component curves (87% of the first component curve, +—+, and 13% of the second component curve, ◇—◇).

linear combination of the protein and the peptide, the CD spectrum of the Hsc70 (Fig. 4) was obtained by a combination of the two component curves (87% of the first component curve, +—+, and 13% of the second component curve, ◇—◇). Therefore, there appears to be a conformational change in the components of the complex upon complex formation. As the decamer, because of its low concentration (Fig. 1), contributes but a small CD contribution to the complex, it would appear that the Hsc70 undergoes a significant conformational change.

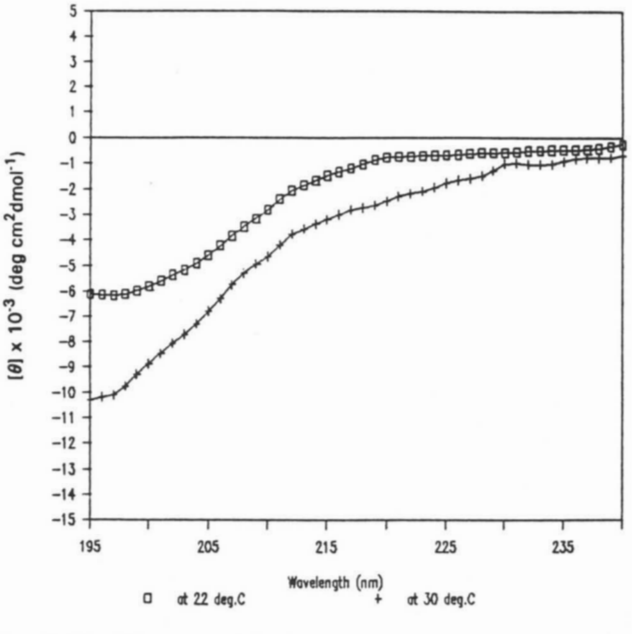

Fig. 3. The CD spectra of the decapeptides measured at 22 °C (□—□) and at 30 °C (+—+), at a peptide concentration of 1 mg/mL in 20 mM KCl, 50 mM Tris, pH 7.5.

The GroEL chaperone, upon binding to an extended peptide, caused the peptide to adopt an α-helical conformation (Landry & Gierasch, 1991; Landry et al., 1992). However, the present study of the complex of Hsc70 and the decapeptides indicates a different conformational change. While the α-helical content remained constant upon binding the decapeptides, a noticeable decrease in β-sheet conformation (−15%) was detected by the CD analysis, although the decapeptides alone contained a considerable amount of β-sheet conformation (Fig. 3). The CD analysis also indicated an increase in β-turn conformation. Because conformational motifs of the β-turn and the 3_{10}-helix are similar to each other, continuous β-turns are equivalent to a 3_{10}-helix, which is frequently found at the terminal of an α-helix. This study has shown that the Hsc70 chaperone undergoes a significant conformational change upon binding to the decapeptides. This conformation, containing less secondary structure, may be the molten globule state previously suggested by Bychkova et al. (1988).

Materials and methods

Materials

Hsc70 from bovine brain in 25 mM KCl, 25 mM Hepes, pH 7.0, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, and decapeptides were prepared as previously reported (Flynn et al., 1989). The decapeptides were dissolved in 0.2 M NaF, 50 mM Tris, pH 7.0 by sonicating for 30 s. The alteration of the buffer used with Hsc70 was performed by reiteration of concentration and dilution with the final buffer as follows. Concentration of the stock solution was done in a Centricon 10 microconcentrator (Amicon) by centrifuging at 6,000 rpm in a JA-20 rotor ($4{,}600 \times g$), followed by dilution with the final buffer (20 mM KCl, 50 mM Tris-HCl, pH 7.5). This was a nondenaturing procedure, as the Scatchard analysis indicated that ≈ 1 mol of peptide bound 1 mol of protein. As the peptide-binding studies were performed in this Tris-HCl buffer (unlike the ATPase assays), the Tris buffer was compatible with the binding procedure. There were no indications that the Hsp70 aggregated in this buffer system. Protein concentration was determined by the BCA method (Pierce). The purity of Hsc70 was checked by SDS-PAGE electrophoresis (Laemmli, 1970) on a 10% acrylamide/0.2% bis-acrylamide gel and stained with Coomassie blue R-250 (Fig. 2).

Circular dichroism measurements

All CD measurements were performed on a Jovin-Yvon Mark V autodichrograph using circular quartz cells with pathlengths of 0.02 cm and a sensitivity setting at 1×10^{-5} to 5×10^{-6}. The response time setting for the spectrometer was 2 s with a data acquisition time of 5 s. A computer connected to the spectrometer ensured noise reduction as well as full-magnitude signal conversion from analog to digital. The CD spectra are reported in terms of $[\theta]_M$, molar ellipticity, deg cm^2 dmol^{-1}. Each measurement was the average of five repeated scans in steps of 0.2 nm at 22 °C unless otherwise indicated. The temperature of the sample was controlled by a circulating water bath (Lauda, type K2R) linked to the outer jacket of the cuvette (Helmann).

For CD measurements of the complex of Hsc70 and decapeptides, a molar ratio of 5.89:1 (peptide:protein), at a peptide concentration of 36 μM, was used, which is comparable to the K_m value of the Hsc70 to the peptides (Flynn et al., 1991). The differential spectrum of Hsc70 was obtained by subtracting the CD spectrum of the decapeptides from the CD spectrum of the complex at the same concentration as in the complex before the conversion to the ellipticity values.

Analysis of the CD spectra

All the CD spectra were analyzed by the CCA algorithm (Perczel et al., 1989, 1991).

The LINCOMB method (Perczel et al., 1992a) was used for the analysis of the CD spectra, derived from 25 globular proteins by the CCA method (Perczel et al., 1992b). The CCA utilizes three constraints. The sum of the secondary structure is 100%, and each component must have a positive value (weight coefficient). In addition, each coefficient value for a given protein should be placed in a simplex of the P-dimensional Euclidean space with the smallest volume (volume minimization), where the value P is the number of the conformational secondary structures. The CCA method has been put to extensive tests for its utilization and efficiency (Perczel et al., 1992a). However, direct comparison with other methods for accuracy is not feasible, as each algorithm has its own advantages and disadvantages.

Secondary structure prediction

The prediction of the secondary structure of the bovine Hsc70 was performed by the method of Chou and Fasman (1974) using a 64-protein database. The prediction algorithm was written in C language by Dr. Peter E. Prevelige and allows the operator's experience and intuition as an input (Prevelige & Fasman, 1989).

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