The predicted secondary structure of enolase

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The results of several secondary-structure prediction programs were combined to produce an estimate of the regions of α -helix, β -sheet and reverse turn for both chicken skeletal-muscle and yeast enolase sequences. The predicted secondary-structure content of the chicken enzyme is 27% α -helix and less than 10% β -sheet, whereas in the yeast enolase a similar helix content but virtually no sheet are predicted. These results are in fair agreement with published experimental estimates of the amount of secondary structure in the yeast enzyme. The enzyme appears to be formed from three domains.

INTRODUCTION

Estimates of the secondary-structure features of enolase have been made by using o.r.d., c.d. and laser Raman spectroscopy applied to the yeast enzyme. Westhead (1964) reports the b_0 value of the Moffitt equation for yeast enolase as $-205^\circ\text{cm}^2\cdot\text{dmol}^{-1}$ at pH 7.5 at 30 °C, although he expresses some doubt as to its reliability. Chen & Yang (1971) provide a means of converting this value into an estimate of α -helix content, which yields for enolase about 35%, considerably higher than the value of 18.5% quoted by Chin *et al.* (1981) as derived from the same data. However, Rosenberg & Lumry (1964) estimate the helix content as 25% on the basis of a b_0 value of $-140^\circ\text{cm}^2\cdot\text{dmol}^{-1}$ at pH 6.3 at 19.6 °C. Collins & Brewer (1982) have measured the c.d. spectrum of the yeast apoenzyme and reach a value of 26.6% for the helix content. This is somewhat lower than the value of 31% obtained by use of the $[\theta]_{222} = -11700^\circ\text{cm}^2\cdot\text{dmol}^{-1}$ from the diagram of Jirgensons (1970) for the rabbit enzyme. Brewer *et al.* (1978) have determined values of 'less than 40%' for the helix content and 'little (less than 5%)' β -sheet structure by consideration of the Raman spectra in several solvents.

From a theoretical point of view, applying the algorithm of Krigbaum & Knutton (1973) to the composition of the chicken enzyme values of 44, 13 and 39% respectively for helix, sheet and reverse turn. The corresponding values for the yeast enzyme are 48, 6 and 28%. Chin *et al.* (1981) have applied the criteria of Levitt (1978) to the yeast enzyme sequence and arrive at values of about 15% helix and 6% sheet on the basis of runs of at least ten residues that are, at worst, indifferent to the formation of the secondary-structure type.

Thus the picture that emerges from these studies is of a protein with somewhere between one-quarter and one-third of its residues present in a helical conformation and less than 10% as sheet.

With these values in mind, we have undertaken a secondary-structure prediction based on the combination of seven separate methods [Burgess *et al.* (1974); Chou & Fasman (1974), as modified by Lenstra *et al.* (1977) and with the updated indices of Geisow & Roberts (1980);

Dufton & Hider (1977); Garnier *et al.* (1978); Lim (1974); McLachlan (1977); Nagano (1973)] as suggested by Eliopoulos *et al.* (1982). Because we have added in the method of McLachlan (1977), our rules for the final prediction are the following: (a) a residue is considered likely to adopt a helical or sheet conformation if at least four methods predict it to be so; (b) a sheet or helix requires at least four consecutive residues before it is deemed to exist, whereas for a turn two adjacent predictions of at least three of the methods are required; (c) any regions not predicted as helical, sheet or reverse turn are considered to be irregular.

Listings of the individual predictions may be obtained from the authors.

RESULTS AND DISCUSSION

The histogram in Fig. 1 shows the results of the joint prediction with heavy horizontal bars demarking the regions of likely secondary structure. It can be seen that the protein can be roughly divided into three parts, which it is tempting to identify as domains. Within the first part, residues 1–140, the major structural element predicted is α -helix with helices for residues 2–9, 42–48, 60–68, 75–81 and 83–93 for the chicken enzyme. Reverse turns appear to be present in the regions 11–16, 36–40 and 96–98. The second part, residues 150–260, appears to form a smaller, mainly helical, domain (residues 159–167, 176–181, 215–232 and 242–249) with a strand of sheet at residues 237–243 and turns around residues 152–155 and 198–200. The third part predicts strongly as alternating segments of sheet and helix (helices at residues 297–305, 324–334, 352–356, 376–382 and 399–420, sheets: at residues 286–292, 309–316, 337–343, 364–369 and 383–388). Reverse turns are predicted around residues 252–257, 262–266, 361–364 and 396–398. Fig. 2 shows that similar predictions are obtained with the yeast enzyme sequences (Chin *et al.*, 1981; Holland *et al.* 1981) although the helices at residues 2–9, 60–67 and 242–249 are absent and the sheet predictions in the latter part are much less convincing. Indeed, by our criteria only one strand is predicted in the yeast enzyme sequence at residues 108–112 (not in the chicken), and in the same region an

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Fig. 1. Combined predictions for α -helix (top), β -sheet (centre) and reverse turn (bottom) for chicken skeletal-muscle enolase

The vertical axis represents the number of separate predictions for each structural element and the horizontal axis gives the residue number. The heavy horizontal bars represent those regions that are deemed to be in the specification as described in the text.

extra helix (114–123) appears. The criteria of Levitt (1978) as applied by Chin *et al.* (1981) predict helices at residues 221–235, 333–349, 356–365 and 404–419, all of which agree with the results presented here. However, of the β -sheet conformation these workers predict (residues 109–123 and 156–167), only the first agrees with our results. There is only a weak indication for the second strand in the yeast enzyme sequence and we find no predicted sheet in the chicken enzyme sequence.

It is striking how similar the combined predictions are for chicken and yeast enolases, and how well the overall amounts of helix and sheet agree with the solution studies

referred to above. It is also worth noting that the two 'inter-domain' regions correspond to less-well-conserved regions of the sequence, including the two insertions, near residues 140 and 265. Finally, we consider that, by combining the various prediction methods and applying rather strict criteria, those regions predicted are likely to be underestimated.

The activity of the yeast enzyme has been shown to be associated with the binding of bivalent cations to three distinct classes of site. One is a conformational metal-ion-binding site, whose occupation by Mg^{2+} allows the binding of the catalytic ion to the second class of site

Fig. 2. Combined predictions for α -helix (top), β -sheet (centre) and reverse turn (bottom) for yeast aldolase

The comments from the legend to Fig. 1 also apply here.

after the substrate has bound. The third class of site seems to have an inhibitory function (see Collins & Brewer, 1982, and references cited therein). Both o.r.d. (Westhead, 1964) and c.d. (Collins & Brewer, 1982) measurements indicate that there is little change in the secondary structure associated with the conformational changes, and it is tempting to conclude that these changes are brought about by the relative movements of the tentative domains that we have predicted.

The lack of convincing β -sheet predictions between residues 160 and 220, the featureless run at residues 240–270 and the variability of the sequences in these regions are the reasons for our assigning the structure to three domains. Because enolase and pyruvate kinase are

adjacent enzymes in the glycolytic pathway, both having a binding site for phosphoenolpyruvate, the possibility of a recurrence of the α/β -barrel as found in pyruvate kinase, triose phosphate isomerase and KDPG-aldolase cannot be ruled out. Although WORDSEARCH (Devereux *et al.*, 1984) found no sequence matches with these enzymes, the possibility of the barrel being a stable structure to which several distinct enzymes have converged has been discussed by Muirhead (1983). Resolution of the various intriguing possibilities must await the results of current X-ray analysis of the chicken skeletal-muscle enzyme crystals (H. C. Watson & P. J. Shaw, personal communication).

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