Circular dichroism studies on the signal sequence of *E. coli* alkaline phosphatase indicate the presence of both α-helix and β-structure in hydrophobic environments

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The conformations of a synthetic peptide corresponding to the signal sequence of *E. coli* alkaline phosphatase, Lys-Gln-Ser-Thr-Ile-Ala-Leu-Ala-Leu-Leu-Pro-Leu-Leu-Phe-Thr-Pro-Val-Thr-Lys-Ala-OCH$_3$, have been examined in different environments by circular dichroism spectroscopy. In trifluoroethanol, methanol and aqueous mixtures of these solvents, the signal peptide has largely random conformation (~80%) with small amounts of α-helix and β-structure. However, in micellar environment, there is a significant increase in ordered conformation with both α-helix and β-structure being present, unlike in other signal sequences reported in the literature, where only the α-helical conformation has been observed. Hence, an α-helical conformation may not be as stringent a requirement as overall hydrophobicity for recognition of signal sequences by the cell's export machinery.

CD   *Signal sequence*   *Helical conformation*   β-Structure

1. INTRODUCTION

In eukaryotic as well as in prokaryotic cells, proteins destined for secretion are synthesized as precursors [1,2] with amino-terminal extensions (signal sequences) varying between 15 and 25 residues [3]. Both eukaryotic and prokaryotic signal sequences have an amino-terminal basic region within, followed by a region consisting of 12–15 hydrophobic amino acids [3]. As there is no primary structure homology amongst signal sequences, it is conceivable that they have similar secondary structural features which are recognized by the components of the cell's export machinery like the signal recognition particle [4,5]. Circular dichroism (CD) studies on synthetic signal sequences [6–10] have indicated that these sequences tend to adopt α-helical conformation, particularly in hydrophobic environments, with ~40–50 α-helices and no β-structure. An examination of the

primary structure of signal sequences [3] reveals the presence of Pro, a helix-breaking amino acid [11], at a central position, in several signal sequences. In an effort to determine whether such signal sequences also have high α-helical content, we have carried out CD studies on a synthetic peptide corresponding to the signal sequence of *E. coli* alkaline phosphatase [12]:

Lys-Gln-Ser-Thr-Ile-Ala-Leu-Ala-Leu-Leu-Pro-Leu-
1   5   10

      Leu-Phe-Thr-Pro-Val-Thr-Lys-Ala-OCH$_3$ (AP)
             15   20

which has two Pro residues, one situated centrally at the 11th position and the other at the 16th position at the carboxy-terminus, in different environments.

2. EXPERIMENTAL

Peptide AP was synthesized by the solid-phase method. The resin employed for the synthesis was

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chloromethylated polystyrene-co 1% divinyl benzene. The transesterification method of cleavage was employed [13] and the crude protected peptide was purified by column chromatography on silica gel followed by partition chromatography on Sephadex LH-20. The purified peptide AP showed a single peak on HPLC analysis and yielded satisfactory amino acid analysis. The details of the synthesis are described elsewhere [22].

CD studies were performed on a Jobin Yvon Dichrograph V spectropolarimeter in 1 mm cells at 25°C. Mean residue ellipticities were calculated using the equation:

$$[\theta]_M = \frac{A \times 3300 \times M}{cl}$$

where A = observed dichroic absorbances; l = path length in cm; M = mean residue weight taken as 110; c = concentration of the peptide in g/l.

The peptide was weighed (approx. 2 mg) to an accuracy of 0.01 mg and 2 mM stock solutions were prepared in the appropriate solvent. An aliquot of this solution was subjected to quantitative amino acid analysis.

3. RESULTS AND DISCUSSION

The CD spectrum of AP in trifluoroethanol (TFE) and 1:1 (v/v) TFE/H$_2$O are shown in fig.1. Two negative bands with extrema at ~205 and 220 nm are observed in both solvents. Estimation of secondary structure parameters based on the method of Chen et al. [14] yields 12% α-helix, 8% β-structure and 80% random conformation in TFE, and 10% α-helix, 14% β-structure and 76% random conformation in 1:1 (v/v) TFE/H$_2$O. In methanol (MeOH) and MeOH/H$_2$O mixtures (fig.2), two negative bands at ~204 and 220 nm are observed. With increasing H$_2$O concentration, the 220 nm band remains unchanged whereas there is a slight increase in intensity as well as a blue shift of the ~204 nm band. The positions and intensities of the peaks suggest that the peptide has largely random conformation with small amounts of α-helix and β-structure.

In micelles of SDS the CD spectrum (fig.3) is characterized by negative bands at ~206 and 222 nm. Estimation of secondary structure [14]
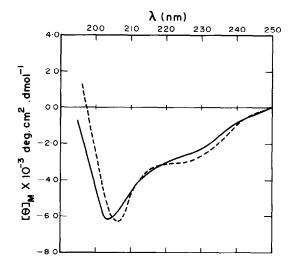

Fig.1. CD spectra of AP in TFE and TFE/H$_2$O: (- - -) TFE, (——) 1:1 (v/v) TFE/H$_2$O. Peptide concentration, 0.1 mM.
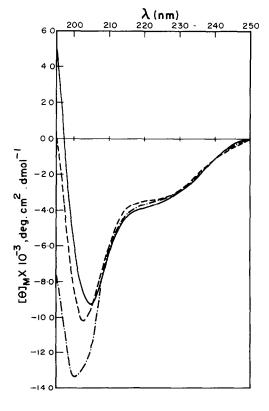

Fig.2. CD spectra of AP in MeOH and MeOH/H$_2$O: (——) MeOH, (- - -) 3:1 (v/v) MeOH/H$_2$O, (-----) 1:1 (v/v) MeOH/H$_2$O. Peptide concentration, 0.1 mM.

Table 1

CD data for the alkaline phosphatase signal peptide

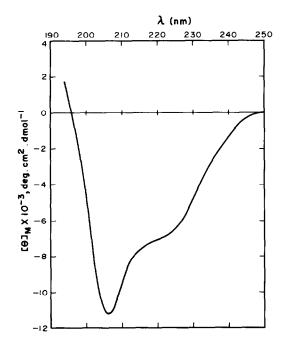| Solvent	CD extrema	
	λ (nm)	$[\theta]_M \times 10^{-3}$
TFE	207	-6.3
	225	-3.1
TFE/H$_2$O (1:1)	203	-6.2
	225	-2.6
MeOH	205	-9.2
	225	-3.4
MeOH/H$_2$O (3:1)	202.5	-10.2
	225	-3.3
MeOH/H$_2$O (1:1)	200	-13.4
	225	-3.4
30 mM SDS	206	-11.2
	220	-7.1

Fig.3. CD spectrum of AP in 30 mM SDS. Peptide concentration, 0.1 mM.

yields 24% α-helix, 19% β-structure and 57% random conformation. The spectra in organic solvents and SDS were independent of concentration and the range 0.1–0.4 mM. The $[\theta]_M$ values at the peak extrema in different environments are summarized in table 1.

Application of the Chou-Fasman rules for prediction of secondary structure of peptides [11] yields a $\langle P_\alpha \rangle$ (average helix parameter) of 1.15 for residues Ser(3)–Leu(10) and $\langle P_\beta \rangle$ (average β-structure parameter) of 1.12 for residues Gln(2)–Leu(10) and 1.18 for residues Leu(12)–Thr(15) (see fig.4). Thus theoretical analysis indicates that both α-helix and β-structure are probable.

While there are limitations in the quantitation of secondary structure from the CD spectra of relatively small peptides based on the methods of Chen et al. [14] and Greenfield and Fasman [15] and also secondary structure prediction according to Chou and Fasman [11], it is evident that the signal sequence of *E. coli* alkaline phosphatase has a significant amount of β-structure compared to the signal sequences of M 13 coat protein [9] and parathyroid hormone [6]. However, it is unlikely that the β-structure observed is due to the presence of the central Pro residues as this amino acid disrupts both helix and β-structure. Clearly further experimental studies on the conformational preferences of signal sequences is necessary before it can be generalized that α-helical conformation is a common structural feature in signal sequences.

Protein translocation across membranes in eukaryotes is initiated by binding of the signal recognition particle (SRP) to the signal sequences of nascent secretory proteins [4,5]. This interaction results in arrest of translation of the remaining polypeptide chain which is relieved by interaction of the SRP-nascent peptide-ribosome complex with a receptor on the endoplasmic reticulum membrane, the docking protein [16]. Genetic studies have indicated the presence of proteins as components of the cell's export machinery in prokaryotes as well [1,2]. Once the targeting to the

Lys-Gln-Ser-Thr-Ile-Ala-Leu-Ala-Leu-Leu-Pro-Leu-Leu-Phe-Thr-Pro-Val-Thr-Lys-Ala-OCH$_3$

Fig.4. Probable secondary structures of AP based on Chou-Fasman rules: (——) region of α-helix; (– – –) regions of β-structure.

membrane site is achieved, the mechanism by which proteins actually cross the membrane barrier is still a subject of debate. According to the signal hypothesis by Blobel and co-workers [16] the polypeptide chain traverses the bilayer through a protein channel. Other models are based on direct interaction of signal sequences with the phospholipid bilayer [17–19]. In any event since specific interactions between signal sequences and protein 'receptors' are involved at some stage of protein export, it is pertinent to look for common features in signal sequences in the absence of primary structure homology. Since signal sequences have a similar hydrophobic profile [20] it is conceivable that this structural feature is one of the recognition elements [21]. In fact introduction of charged or hydrophilic amino acids in the hydrophobic region renders a signal sequence 'non-functional' (i.e. unable to initiate export) [1,2]. On the basis of CD studies of a few signal sequences, an α-helical conformation as another recognition element has been suggested. However, we have observed that the signal sequence of *E. coli* alkaline phosphatase has a significant amount of β-conformation even in hydrophobic environments like in micelles. Hence an α-helical conformation may not be as stringent a requirement as overall hydrophobicity for recognition of signal sequences by the cell's export machinery.

ACKNOWLEDGEMENT

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