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The amphiphilicity of ACP helices: a means of macromolecular interaction?

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ACP interacts with diverse proteins in an unknown way. Possibly there is a similar mode of interaction between ACP and all ACP-binding proteins, the amphiphilic helix. The hydrophobicities of helices from 4 different ACPs were compared. Hydrophobic moment plots were prepared for ACP helices and those of many EF hand calcium-binding proteins. Both groups of proteins occupied the same region of the plot.

Acyl carrier protein; Amphiphilic helix; Ca^{2+}-binding protein; Hydrophobic moment plot; Hydrophobicity projection; Protein interaction

1. INTRODUCTION

Acyl carrier protein (ACP) interacts with numerous proteins in its capacity as a carrier of substrates in fatty acid and lipid biosyntheses. ACP binding to proteins is not limited to enzymes for which it functions as a substrate carrier; it inhibits (K_i 5 μM) an NADH-dependent acetoacetyl-CoA reductase from *Euglena* that is not part of the fatty acid synthetase [1]. The way that ACP and proteins to which it binds recognize one another is not known. Possibly there are similar recognition sites or domains on all ACP-binding proteins; this would imply that ACP binds in the same way to different proteins. A possible general mechanism for interaction among diverse proteins is by way of amphiphilic helix faces [2]. The amphipathic helix concept was proposed originally to explain certain features of protein-lipid interactions in plasma lipoproteins. Extensions of the concept have included protein-membrane [3,4] and protein-protein interactions [5,6].

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In addition to interaction with a diversity of proteins, ACP binds Ca^{2+} [7]. Although a regulatory role has not been shown, ACP Ca^{2+} binding does result in enhanced biological activity and in pH-dependent conformational changes. Calcium binding is a common feature of many proteins known to be interactive with other proteins. Calcium binding EF hand regulatory proteins interact with a variety of specific proteins in a way that is not fully understood. Investigation of the binding between calmodulin and several peptides suggested the involvement of amphiphilic helices with possibly some electrostatic attractions [5,6]. Dissociation constants ranging from about 0.1 μM to 5 μM were measured for the binding between calmodulin and several biologically active peptides that may be subject to calcium second-messenger regulation [8]. This is comparable to the K_i for ACP inhibitory-binding to the reductase described above.

Escherichia coli ACP secondary structure has been refined from two-dimensional ^1H NMR spectroscopy, and a 4 α-helical, 5-turn secondary structure has been deduced [9]. Given the high helical content of the protein, it is possible that ACP may interact with other biological molecules by way amphiphilic helix faces [2]. We have examined the

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amphiphilicities of ACP helices from several different sources and compared them among themselves and with helices from numerous EF hand Ca^{2+} binding proteins.

2. METHODS

ACP sequences and homologies were taken from a compilation by Kuo and Ohlrogge [10]. Assignment of *E. coli* ACP helices were those designated by Holak and Prestegard [9] in a refinement of ACP secondary structure by two-dimensional ^1H NMR spectroscopy. Helices for other ACPs were assigned to residues homologous with those comprising helices in *E. coli* ACP. EF hand calcium binding sequences and helix residue assignments were the following: rabbit troponin C, 73–84, 52–62, 36–44, 12–28, 112–120 [11]; synthetic rabbit troponin C, 90–101, 112–119 [12]; carp muscle calcium binding protein, 38–51, 60–70 [12]; pike muscle calcium binding protein, 79–88, 99–107, 43–50, 59–70 [11]; bovine intestinal calcium binding protein, 3–14, 25–35, 46–53, 69–75 [13]; Tetrahymena calmodulin (sequences listed in [14]); and bovine brain calmodulin (sequences listed in [14]).

Helical wheel projections of the various α-helices were formulated as described by Schiffer and Edmundson [15]. The amphiphilic properties of the helical wheels were quantified by calculating mean residue hydrophobicities, $\langle H \rangle$, and hydrophobic moments $\langle \mu_H \rangle$ [16]. The effective hydrophobicity of each amino acid residue was defined in terms of a hydrophobic moment vector, H_i; H_i values were from the consensus hydrophobicity scale for amino acid residues [17]. The direction of the vector H_i, is determined by the orientation of the side chain to the helix axis. The mean vector for a helix is defined as the hydrophobic moment, $\langle \mu_H \rangle$. In hydrophobic moment plots, $\langle \mu_H \rangle$ versus $\langle H \rangle$, different classes of proteins plot in different regions of the graph [18].

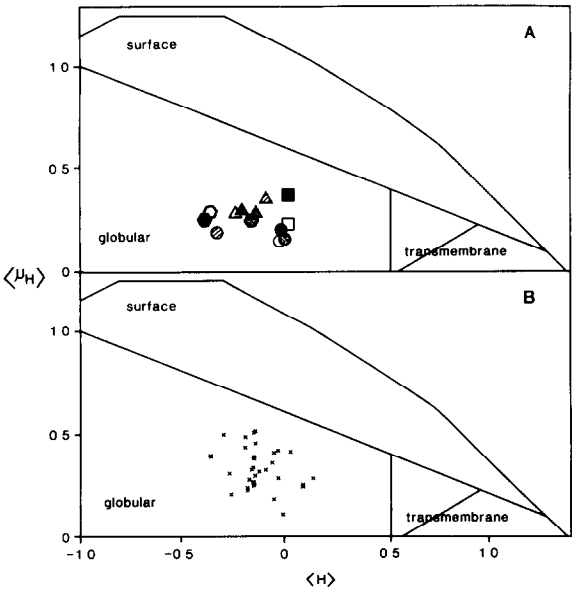
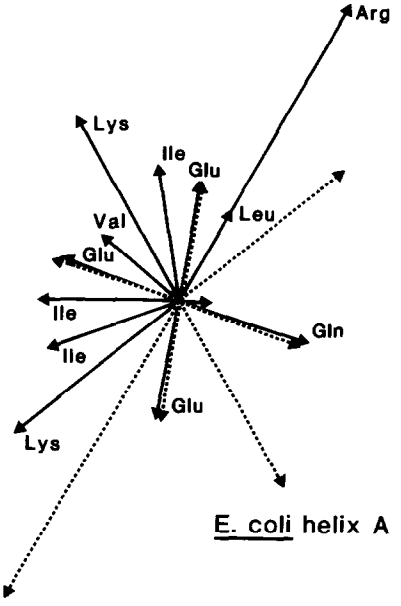


Fig.1. Hydrophobicity plots of *E. coli* helices (A) and EF hand calcium binding protein helices (B). *E. coli* ACP helix assignments included the following residues: 3–15, helix A; 37–51, helix B; 56–63, helix C; and 65–75, helix D [6]. Homologous helices for ACPs from other sources were designated to the following residues [8]. Spinach: A, 5–17; B, 39–53; C, 58–65; D, 67–77. Barley: A, 12–24; B, 46–60; C, 65–72. Rabbit: A, 5–17; B, 39–53. The $\langle \mu_H \rangle$ and $\langle H \rangle$ were calculated for each helix and plotted as explained in the text. The plot divisions for various classes of proteins was from Eisenberg et al. [16]. ACP helices are represented as follows: helix A (△) *E. coli*, (▲) spinach, (◭) barley, (◬) rabbit; helix B (○) *E. coli*, (●) spinach, (◍) barley, (⊘) rabbit; helix C (◌) *E. coli*, (◉) spinach, (◉) barley; and helix D (□) *E. coli*, (■) spinach. EF hand calcium binding protein helix residues are designated in the text.

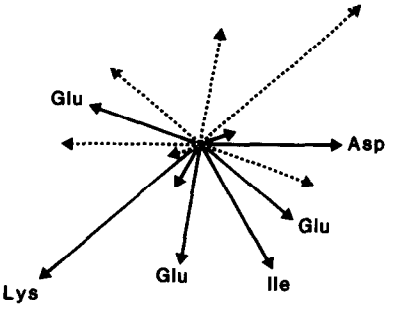
3. RESULTS AND DISCUSSION

Homologous portions of the primary structures of ACPs from *E. coli*, spinach, barley and rabbit were assumed to have homologous secondary

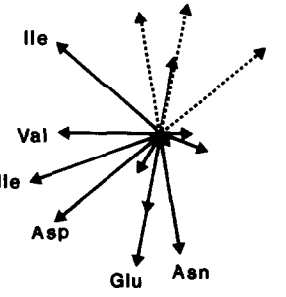
Fig.2. Graphical representations of residue contributions to the hydrophobic moments of different ACP helices. Calculation methods and representations are described in the text and references [14,16]. Residue compositions of the various ACP helices are given in the legend to fig.1. Hydrophobic residues have positive values for hydrophobicity, H_i and are shown as solid line vectors extending from the center of the helical wheels. Hydrophilic residues have negative values of H_i and are shown as dashed vectors extending from the center; their vector contributions are represented by solid lines 180° away. All residues are represented by vectors but only the major residue contributions are labelled.



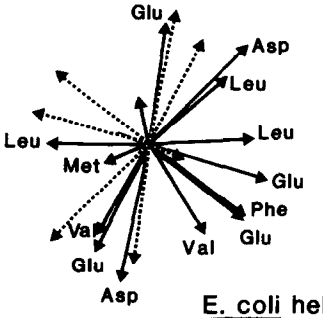
E. coli helix A



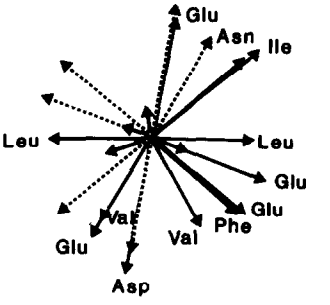
E. coli helix C



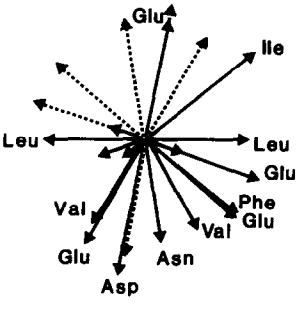
E. coli helix D



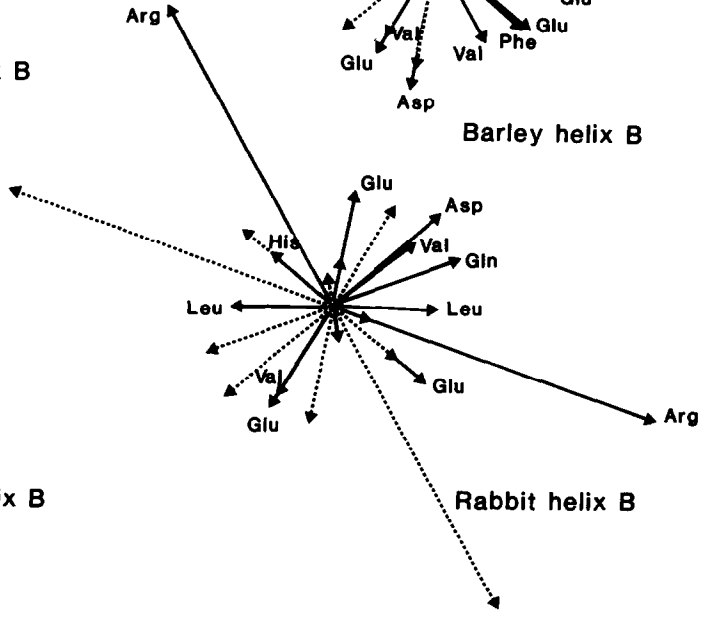
E. coli helix B



Barley helix B



Spinach helix B



Rabbit helix B

structure. Thus α-helix content was assigned to all ACPs at residues homologous to those determined for *E. coli* ACP secondary structure by two-dimensional ^{1}H NMR spectroscopy. The hydrophobicities of the helical wheels constructed from the homologous ACP sequences were quantified. Helical wheels constructed from comparable helices of homologous ACPs had similar $\langle H \rangle$ and $\langle \mu_H \rangle$ values. $\langle \mu_H \rangle$, in particular, varied little from one organism to the next while $\langle H \rangle$ exhibited a wider latitude. This is shown in fig.1A where similarly shaped symbols representing homologous helices are clustered together. Although spinach and barley exhibited the highest primary sequence homology, about 70%, among the 4 ACPs [10], *E. coli* and spinach helices were the most similar in amphiphilic parameters. Barley and rabbit ACP helical wheels, to the extent that they could be formulated from available primary sequence, were slightly more scattered in amphiphilic properties. Rabbit ACP has the least primary structure homology to the other 3, and it varied more than the others in amphiphilic parameters.

Residue hydrophobicity projections on the helical wheels of the B helices of the different ACPs are shown in fig.2 along with those for *E. coli* helices A, C and D. The length of each vector is H_i, the hydrophobicity of the residue, H_i is a signed quantity; hydrophobic residues on one face of the helix reinforce contributions of hydrophilic residues on the opposite face. Helix B had the lowest $\langle \mu_H \rangle$ of all the helices in each of the ACPs. It does, nevertheless, show clustering of polar (dashed lines) and apolar (solid lines) residues within portions of the helical projections, and this grouping tends to be similar from one ACP to the next for the free ACPs. The rabbit ACP helical wheel residue hydrophobicity shows much more variation.

Unlike the 3 other ACPs shown, rabbit ACP is fixed in the fatty acid synthetase protein aggregate by covalent bonding. The other ACPs must rely upon noncovalent interactions to ensure their biological reactivity. If amphiphilic helical interactions are important in this respect, then less latitude for variance in the bonding area might be expected for the free ACPs whereas the helix composition may be less critical for the covalently bonded rabbit ACP. *E. coli* helices A, C and D have higher $\langle \mu_H \rangle$ values than B, and, upon projection, display a greater segregation of ionic and nonionic residues (fig.2).

Another group of proteins that also has a preponderance of acidic residues is the calcium-binding proteins. Like the ACPs [10,19], the EF hand calcium binding proteins are structurally related to one another [20]. The amphiphilic qualities of the helices that participate in Ca^{2+} binding were examined for a number of EF hand Ca^{2+} binding proteins and are summarized on a hydrophobic moment plot (fig.1B). Proteins with different functions or general characteristics, e.g. integral membrane proteins, tend to group in distinct regions of a hydrophobic moment plot [18]. The EF hand calcium binding protein helices also segregate. As a group, the helices involved in Ca^{2+} binding show a moderately high amphiphilicity and a low mean residue hydrophobicity. They occupy a relatively small and circumscribed portion of the plot which is different from the regions where other classes of proteins have plotted (for examples, see [18]).

For the EF hand calcium binding proteins plotted on fig.1B, the mean $\langle H \rangle$ and $\langle \mu_H \rangle$ were -0.13 and 0.34. (The respective standard deviation values were 0.11 and 0.12.) The mean location on the hydrophobic moment plot for the EF hand calcium binding proteins and the ACP helices (average $\langle H \rangle = -0.15$, average $\langle \mu_H \rangle = 0.25$) are similar. The ACP helices group in the hydrophobic moment plot in the same region as the EF hand calcium binding proteins. It is not clear whether this be coincidental or a structural feature essential for similar undefined functions. Both types of protein groups bind Ca^{2+} with conformational changes, but there are many proteins that bind Ca^{2+} that are not Ca^{2+} modulated proteins [20]. A possible EF hand configuration had been speculated for *E. coli* ACP based on secondary-structure-prediction algorithms [11], but the helices predicted do not match those defined by two-dimensional NMR analysis of the secondary structure [9]. Both types of proteins interact with other proteins with comparable affinities [1,21]. Perhaps the extent of the similarity of the two groups is a common structural feature that permits efficient reversible interaction with numerous different proteins and/or membranes, the amphiphilic helix.

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