# The contribution of α-helices to the surface activities of proteins

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The amphilicity of an α-helical segment in a protein may be quantitated by calculating its mean helical hydrophobic moment ($\mu_H$). For proteins whose hydrophobic interactions with interfaces are mediated by α-helices, the surface pressures exerted at the air–water interface correlate with the product ($\overline{\mu_H} \times F$) where $\overline{\mu_H}$ is the mean helical hydrophobic moment averaged over all helices in the entire molecule, and $F$ is the fraction of α-helix in the protein. Knowledge of $\mu_H$ permits a description of the contribution of amphipathic α-helices to the surface activities at the air–water interface of serum apolipoproteins, surface-seeking peptides, and globular water-soluble proteins.

*Apolipoprotein* *α-Helix* *Amphipathic protein structure* *Surface activity* *Hydrophobic moment*

## 1. INTRODUCTION

Soluble proteins in biological systems are variously located in aqueous solution, or associated with interfaces in structures such as cell membranes and serum lipoproteins. The α-helices in proteins may be classified by a $\mu_H$-$H_i$ plot as described in [1]; $\mu_H$ is the mean helical hydrophobic moment which gives a measure of the separation of apolar and polar amino acid side chains on the opposite faces of an α-helix, and $H_i$ is the average residue hydrophobicity of the helix. Interestingly, α-helices from membrane proteins and globular proteins cluster in different regions of such a plot. The application of this concept has been extended to the amphiphilic helices of the serum apolipoproteins [2,3]. The apolipoproteins are of interest due to their importance as structural components of lipoprotein surfaces, ligands for cell receptors, and cofactors for enzymes involved in lipoprotein metabolism. These functions are modulated in part as a result of apolipoprotein transfer and exchange between lipoprotein particles during metabolism. The relocation of a protein molecule is a function of its surface activity or relative affinity for the various lipoprotein surfaces [4,5].

The surface activity and lipid-binding capabilities of the apolipoproteins are thought to be mediated by the amphipathic α-helices within the protein [6], the amphiphilicities of which may be quantitated by $\mu_H$ [2,3]. Given that the interfacial interaction, where hydrophobic effects are dominant, is mediated by amphiphilic α-helices, the quantitation of $\overline{\mu_H}$ for different proteins permits a prediction of their relative surface activities. Here we demonstrate that knowledge of $\mu_H$ and the α-helix content allows a prediction of the intrinsic surface activities of water-soluble proteins.

## 2. MATERIALS AND METHODS

The chromatographically pure serum apolipoproteins A-I, C-II and C-III were isolated as described in [7,8] and desalted from 3 M guanidine hydrochloride immediately prior to use, insuring that monomeric protein was being studied. Bovine serum albumin (BSA) (Cohn fraction V), bovine

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ribonuclease A in a 0.2 M sodium phosphate solution, and melittin were obtained from Sigma (St. Louis, MO). LAP-20 (a synthetic lipid-associating peptide) [9], human apo A-II, and reduced and carboxymethylated apo A-II (RCM-A-II) were generous gifts from Drs J.T. Sparrow and H.J. Pownall (Baylor College of Medicine, Houston, TX). The data for hen egg-white lysozyme and horse heart cytochrome *c* were taken from [10]. Protein concentrations were determined by a sodium dodecyl sulfate–Lowry protein analysis [11] using BSA as a standard, or by absorbance of the protein solution at 280 nm.

The surface pressures ($\pi$) exerted at the air–water interface by the pure proteins were determined using a surface balance with a Wilhelmy plate as in [10]. For comparison of the relative surface activities of the proteins, $\pi$ was measured at an initial substrate protein concentration of $5 \times 10^{-5}$ g/100 ml. Protein injections were made into a Teflon dish containing 100 ml of a phosphate buffer substrate (pH 7.0, 5.65 mM $Na_2HPO_4$/3.05 mM $NaH_2PO_4$/0.08 M NaCl) at 22 ± 2°C. Surface pressure measurements were accurate to $\pm 1$ mN·m$^{-1}$.

The mean $\alpha$-helical hydrophobic moment for individual helices ($\mu_H$) was calculated by the vector addition method as in [1] using the amino acid hydrophobicity scales in [12] or [13]. The predicted sections of amphipathic $\alpha$-helix for the human apolipoproteins A-I, A-II, C-II, and C-III were taken from [14]. The fractions of $\alpha$-helix ($F$) are maximum values measured for each protein [9,14–19]. To permit comparison of the surface activities of different proteins on the basis of the helical hydrophobic moments, the average value ($\overline{\mu_H}$) was calculated over all helical segments in the protein from the equation $\overline{\mu_H} = [\Sigma_{i=1}^{n} \mu_H]/n$ where $n$ = the number of helical segments in the protein, and $\mu_H$ is the mean helical hydrophobic moment for a given helical segment. The contribution of the amphiphilic $\alpha$-helices to the overall interfacial interaction of a protein molecule is given by the product ($\overline{\mu_H} \times F$).

## 3. RESULTS AND DISCUSSION

### 3.1. *Hydrophobicity scales for calculation of* $\mu_H$

An important consideration in the calculation of $\mu_H$ for an $\alpha$-helix is the free energy of transfer ($\Delta G_t$) of the amino acid side chain from a polar to an apolar environment. Historically, $\Delta G_t$ values have been derived from the partition coefficient for monomeric amino acids between aqueous and organic solvents, given by the equation $\Delta G_t = RT \ln f$, where $f$ = the partition coefficient of the given amino acid between the aqueous and organic phases. Such hydrophobicity scales are an approximation when applied to proteins because the derivation of partition coefficients for individual amino acids does not take into account all of the interactions involved in the transfer of a segment of polypeptide into a given environment. Consequently for this study we have selected two differently derived hydrophobicity scales for the calculation of $\mu_H$.

The hydrophobicity scale in [13] is experimentally derived from the ability of an amino acid to lower the surface tension of an air–water interface. The hydrophobicity scale in [12] is a theoretical scale derived from the partitioning of given amino acids between the surface and interior of globular proteins; X-ray crystallographic data for globular proteins provide the structural information. The advantage of the Bull and Breese scale is that it is experimentally derived, and the ability of amino acids to lower surface tension may be particularly relevant to the ability of proteins to lower the surface tension of an air–water interface. In contrast, the advantage of the Janin scale is that it considers the amino acid as a constituent of a polymeric unit, and the $\Delta G_t$ value is determined as a function of protein folding. The forces that are important in protein folding are likely to be important in the orientation of amino acid residues at an interface upon adsorption. Although the two hydrophobicity scales are derived by different means, there is a correlation between the two scales (correlation coefficient, $r = 0.64$) which is significant at the level $P < 0.01$ for 20 amino acids; this demonstrates the relationship between the partitioning of amino acids between aqueous and organic solvents, and their distribution in protein folding.

### 3.2. *Contribution of protein hydrophobicity to surface activity*

A consideration of the surface pressure exerted at the air–water interface by the 11 proteins mentioned in fig.1 as a function of the average residue

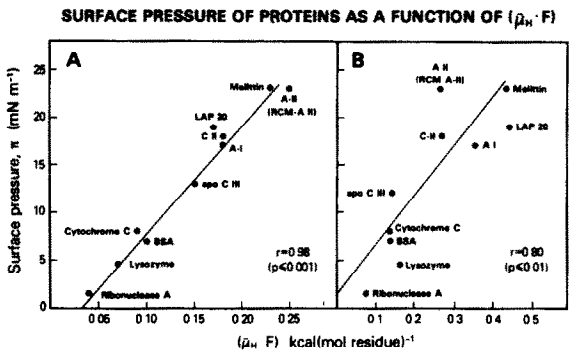


Fig.1. Surface pressures exerted by proteins at the air–water interface as a function of the product $\bar{\mu}_H \times F$ (mean helical hydrophobic moment averaged over all $\alpha$-helices in the protein molecule × fraction of secondary structure present as $\alpha$-helix). Proteins were injected beneath an air–water interface at an initial substrate protein concentration of $5 \times 10^{-5}$ g/100 ml, and allowed to adsorb to a steady-state surface pressure as determined by a Wilhelmy plate surface balance. The steady state surface pressures ($\pi$) are plotted (●) as a function of the product ($\bar{\mu}_H \times F$). See section 2 for details of the proteins and peptides. Human apo-A-II and RCM-A-II give the same value indicating that breaking the disulfide bond between the two identical chains and thereby halving the molecular mass has no influence on the surface activity. The solid lines are linear regression lines whose fits to the experimental points are given by the correlation coefficients $r$. The probability of the fit being due to a random occurrence is given by the confidence level $P$. (A) $\bar{\mu}_H$ was calculated using the hydrophobicity scale of [12], based on the distribution of amino acids between the surface and interior of globular proteins. (B) $\bar{\mu}_H$ was calculated using the hydrophobicity scale of [13], based on the surface tension lowering capability of individual amino acids.

hydrophobicity of the entire protein molecule, calculated from the amino acid composition of the protein and by the hydrophobicity scale in [13], gives a correlation of 0.81, which is significant at the level $P < 0.01$ (not shown). Thus, increasing the content of apolar residues tends to confer more surface activity without regard for the details of the secondary and tertiary structures of the protein molecules. Interestingly, a plot of $\pi$ against the hydrophobicities ($H_i$) of all the residues in all the $\alpha$-helices in each molecule in the same group of proteins gives a correlation coefficient of 0.65, which is not significant at $P < 0.01$. This relatively poor correlation is not improved if only the residues on the apolar sides of the amphipathic helices are considered; the basis of this particular calculation is that the hydration of polar residues might be the same whether they are adsorbed to the surface or not. It should be noted that the presence of $\alpha$-helix is not essential for a protein to be surface active, and it is known that other aspects of protein structure may contribute. Thus, the largely random coil bovine $\beta$-casein molecule is extremely hydrophobic and exhibits a high degree of surface activity [21].

### 3.3. *Contribution of α-helices to protein surface activity*

The 11 proteins and peptides mentioned in fig.1 have $\alpha$-helix contents of 25–90% (i.e., $F$ = 0.25–0.9) and those molecules with higher $F$ exert higher $\pi$ under the conditions of fig.1; the correlation coefficient for the linear regression line through the $\pi$–$F$ data points is 0.82. As demonstrated in fig.1, this correlation with $F$ of the surface pressures exerted at the air–water interface by lipid-associating peptides, apolipoproteins, and globular proteins is improved if $\pi$ is plotted as a function of the product ($\bar{\mu}_H \times F$). It is apparent that, regardless of the hydrophobicity scale used in the calculation, the product ($\bar{\mu}_H \times F$) gives a good prediction of surface activity for all of the various types of proteins examined in this study. In fig.1B, $\bar{\mu}_H$ was calculated using the hydrophobicity scale as in [13]; a correlation coefficient of 0.80, significant at $P < 0.01$ for 11 proteins, is obtained between ($\bar{\mu}_H \times F$) and $\pi$. However, this is not better than the correlation between $\pi$ and $F$ because the Bull and Breese hydrophobicity scale ignores the polymer structure of proteins.

Fig.1A represents the regression line for $\pi$ as a function of ($\bar{\mu}_H \times F$) calculated using the hydrophobicity scale of Janin. The equation for the regression line is $\pi = 107\ (\bar{\mu}_H \times F) - 2.36$ and the correlation coefficient is 0.98; this $r$ value is significant at the level $P < 0.001$, and represents a marked improvement over the correlations mentioned above. It follows that when $\alpha$-helices are involved in the hydrophobic interaction of a protein molecule with an interface, the surface activity apparently is determined by both the fraction of secondary structure which is $\alpha$-helix and the amphiphilicities of the helices. This observation holds for proteins with different tertiary structures.

Thus, bovine serum albumin, cytochrome *c*, lysozyme, and ribonuclease A are water-soluble globular proteins that fit to the same $\pi - (\overline{\mu_H} \times F)$ regression line as LAP-20 and melittin which are low molecular mass lipid-associating peptides. Furthermore, the $\pi - (\overline{\mu_H} \times F)$ data for serum apolipoproteins fit the same regression line, although they are a distinctive class of lipid-binding proteins which have different tertiary structures than the above proteins. The serum apolipoproteins of fig.1 are postulated to assume a conformation at the air–water interface with $\alpha$-helices located in the plane of the surface, and connected by segments of random coil about which free rotation may occur to optimize the interaction with the interface [22]. The greatest free energy decrease on adsorption should result from an interfacial conformation such as this when the individual amphipathic $\alpha$-helices are oriented with their mean helical hydrophobic moments perpendicular to the interface, and directed into the apolar phase. The fact that the correlation illustrated in fig.1 holds for lipid-assocating peptides, apolipoproteins, and globular water-soluble proteins implies that the secondary structure of proteins may be more important than the tertiary structure in conferring surface activity, when hydrophobic interactions of $\alpha$-helices are dominant.

We conclude that the helical hydrophobic moment provides a method for quantifying the degree of amphiphilicity of an $\alpha$-helix and, since the interaction energy of an amphipathic $\alpha$-helix with an interface is proportional to the magnitude of $\mu_H$, the product $(\overline{\mu_H} \times F)$ may be used to predict the surface pressures exerted at the air–water interface by monomeric, water-soluble proteins which contain $\alpha$-helices. This provides some basis for a molecular understanding of the interfacial behavior of proteins.

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