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A STUDY OF PHOSPHOLIPID ASSOCIATING PEPTIDES

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ABSTRACT

This thesis describes the solid-phase synthesis of a series of 5 peptides and their subsequent purification by conventional chromatography and semipreparative reversed-phase HPLC. The efficiencies of these 2 methods of purification have been compared. The peptides are: peptide 208, VSSLLSSLKEYWSSLKESFS; peptide 199, RALASSLKEYWSSLKESFS; peptide 202, LESFLKSWLSALEQALKA; peptide 203, LESFKVSWLSALEEYTKA; and peptide 209, LESFLLSWLSAKEQALKA. The peptides were chosen so that each would exhibit a slightly different non-polar face when it adopted an α -helical conformation. Peptides 202 and 209 have exactly the same amino acid composition but differ in that a leucine and a lysine residue have changed positions. This results in the non-polar face of peptide 209 containing one less leucine relative to peptide 202.

The retentions of the series of peptides in several reversed-phase HPLC systems were measured by gradient elution. These systems utilised the following solvent system: Solvent A = 1% triethylammonium phosphate, pH 3.2, Solvent B = 80% 2-propanol, 20% solvent A. Radial-PAK CN, Radial-PAK C18 and μ Bondapak alkylphenyl columns were used. When a linear gradient from 0 to 100% Solvent B was used the retention of the peptides on the Radial-PAK CN column were: peptide 202, 54.75; peptide 208, 51.5; peptide 209, 49; peptide 203, 48; and peptide 199, 44; (expressed as a percentage of the gradient). The isocratic elution of the peptides were studied in the same solvent system on a μ Bondapak alkylphenyl column by varying the organic solvent content of the mobile phase. The retention of the peptides could not be correlated with the total hydrophobicity of the peptides but could be correlated with the total hydrophobicity of the non-polar side of each peptide when in the α -helical conformation. This result suggests that the peptides adopt an α -helical conformation when binding to the reversed-phase and suggest an adsorption rather than a partitioning mode of binding.

The isocratic elution of peptide 202 in the same system was studied at 4 different temperatures. Construction of van't Hoff plots allowed the calculation of the standard enthalpies of association of peptide 202 with the reversed phase. The standard enthalpy of association of peptide 202 at 39% Solvent B was -12 kcal/mol.

The affinity of the peptides for dimyristoyl phosphatidylcholine (DMPC) was determined by monitoring turbidity clearance and by determining fluorescence emission wavelength changes of the tryptophan residues upon binding of the peptides to phospholipid vesicles. The peptide affinities for DMPC could be correlated with their retention on the HPLC systems detailed above and with their number of cationic residues.

Application of this relationship to the total number of synthesised apolipoprotein fragments allows a very accurate division (92% correct) between those fragments which will and those which will not bind to phosphatidylcholines. This relationship also appears to be applicable to peptides which are not apolipoprotein in origin and may also be useful in modelling β -endorphin - opiate receptor interactions.

The hydrophobic effect is discussed in relation to simple systems and to RP-HPLC and phospholipid binding. The conclusion is drawn that the hydrophobic effect is not always entropy driven.

PREFACE

This thesis examines the process of peptide interactions with reversed-phase bonded silicas as a model for the interaction of proteins and peptides with lipids. As such, the work is relevant to the study of lipoproteins (and hence to atherosclerosis), membrane proteins, cell receptor binding and perhaps to protein structure in general since proteins are synthesised in the presence of phospholipids in the endoplasmic reticulum.

The work is set out in three main sections. Section A is an introduction outlining the importance of peptide-lipid and protein-lipid interactions to the functioning of biological systems. It also outlines the aims and philosophy of this work. Section B relates the synthesis and purification of a series of 5 peptides used to model the lipid-protein interactions. Section C discusses the hydrophobic effect, relates an investigation of the binding of a peptide series to the nonpolar surfaces of reversed-phase bonded silica and dimyristoyl phosphatidylcholine and discusses the relationship between these two processes. On the basis of these results a modified theory of protein-phospholipid interactions is proposed.

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