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Basic amphipathic model peptides: Structural investigations in solution, studied by circular dichroism, fluorescence, analytical ultracentrifugation and molecular modelling

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ABSTRACT

A twenty amino acid residue long amphipathic peptide made of ten leucine and ten lysine residues and four derivatives, in which a tryptophan, as a fluorescent probe, is substituted for a leucine, are studied. The peptides in water are mainly in an unordered conformation (~ 90%), and undergo a two state reversible transition upon heating, leading to a partially helical conformation (cold denaturation). Time resolved fluorescence results show that fluorescence decay for the four Trp containing peptides is best described by triple fluorescence decay kinetics. In TFE/water mixture, peptides adopt a single α-helix conformation but the Leu-Trp9 substitution leads to an effective helix destabilizing effect. In salted media, the peptides are fully helical and present a great tendency to self associate by bringing the hydrophobic faces of helices into close contact. This proceeds in non-cooperative multisteps leading to the formation of α helix aggregates with various degrees of complexation. Using modelling, the relative hydrophobic surface areas accessible to water molecules in n-mer structures are calculated and discussed.
Key words : amphipathic peptides, aggregates, optical spectroscopy, modelling.

RÉSUMÉ

Nous avons étudié un peptide amphipathique composé de dix lysine et dix leucine, ainsi que quatre dérivés comportant un résidu tryptophane pour les études par fluorescence. Dans l'eau, les peptides ne sont pas structurés (~ 90%), et se structurent partiellement en hélice α par chauffage (dénaturation froide). Les mesures de déclin de fluorescence font apparaître une cinétique à trois temps de vie. Dans un mélange eau/TFE, les peptides adoptent une conformation en hélice α, mais la substitution Leu-Trp9 possède un effet déstabilisant. En milieu salin, les peptides sont totalement hélicoïdaux et ont tendance à s'agréger de façon à regrouper leur face hydrophobe. Ce processus se fait en plusieurs étapes avec des agrégats de taille variable. L'existence de tels agrégats est discutée sur la base de la modélisation moléculaire complétée par des calculs d'accessibilité des surfaces hydrophobes.
Mots clés : peptides amphipathiques, agrégats, spectroscopie optique, modélisation.

INTRODUCTION

Since the early papers by DeGrado [1] and Kaiser [2] concerning peptide synthesis used to elucidate the role of secondary structures in their interactions with biological membranes or membrane models, many papers have been devoted to these investigations, and among them more specifically those dealing with amphipatic cytolytic peptides [3], [4], [5].

In earlier studies [6], [7], [8], we investigated amphiphilic peptides such as $(LKKL)_4$ and acetyl-LKKLLKKLLKLLKKLLK-KLK-NH_2, comprising solely leucine and lysine residues, which can mimic the behaviour of cytolytic peptides when interacting with membranes [9],[10]. Among other conclusions, we have shown that whatever the media (pure water or salted media), wherein peptide conformation alteration could be expected, the peptide binding on lipid membrane models always took place ; such a result differs from those obtained with other various peptides. Thus, it has been shown [11], that a flexible structural intermediate of a lactalbumin in solution is a prerequisite for its association with membranes. Likewise the melittin binding to phospholipid bilayers depends on the ionic strength, that is on the equilibrium between monomers and tetramers in solution [12].

In this paper we report on the behaviour of the twenty amino acid residue long peptide : Acetyl-LKKLLKKLLKLLKKLLKKLK-NH_2 in various aqueous media, and we propose a reaction pathway for explaining the reason why these peptides can always bind lipid membrane models whatever the conformation in solution be. Different experimental techniques were used, circular dichroism spectroscopy, analytical ultracentrifugation, fluorescence (steady state and time resolved). As the latter requires the presence of an intrinsic chromophore in the molecule, we therefore designed and synthesised four other peptides where a tryptophan residue was substituted for a leucine at different locations near either the middle or the ends of the matrix peptide, as indicated in figure 1A. Although under certain experimental conditions this

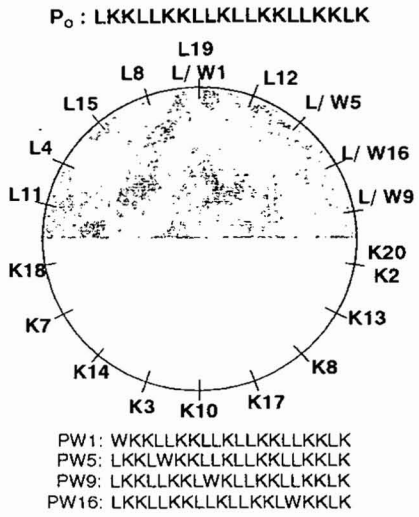

Figure 1. Amino acid sequences of five designed amphiphilic peptides and helical wheel representation of the studied peptides and location of tryptophan residues substituted for leucines. The grey area represents the hydrophobic part of the α helix.

substitution gives rise to an α helix destabilizing effect when Trp residue is located in the middle of the sequence, we show that peptides are mainly in random coil conformation in water, in monomeric α helices in water/TFE mixture and in α helix aggregates in salted media. Molecular modelling studies supplement the experimental results and enable us to propose a model of aggregate.

MATERIALS and METHODS

Chemicals

All reagents purchased from MERCK were of analytical grade and used without further purification.

Peptide synthesis

Peptides were prepared by solid-phase synthesis using a Fmoc/Pam resin strategy in an automatic synthetisor (Applied Biosystem model 433A). The amino-acid functions of lysine and tryptophan residues were selectively protected by BOC moieties.