Exploiting the putative binding sites of DGAT1 enzyme: a fluorescence spectroscopy and synchrotron radiation circular dichroism study

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Diacylglycerol acyltransferase 1 (DGAT1) is one of the enzymes responsible for the final step in the triacylglyceride synthesis pathway, which is seen as a potential target for treating obesity. Although little structural information is available for DGAT1 or any close homologue enzyme, some recent studies have predicted the substrate binding sites of the bovine DGAT1 to be in a large luminal extramembranous loop. In the present study, the conformation and dynamics of synthetic peptides corresponding to the putative binding sites of the DGAT1 were investigated using synchrotron radiation circular dichroism (SRCD) and tryptophan fluorescence (polarization, quenching, and time-resolved) spectroscopies, both in aqueous solution and in the presence of substrates (oleoyl-CoA and dioleoylglycerol) and membrane models. The high flexibility and unordered conformation of the peptides in aqueous solution were confirmed by their typical disordered SRCD spectra and by the fluorescence parameters (low polarizations, high accessibility to water-soluble quencher, fast rotational correlation times of the Trp residues). However, upon binding to the lipid systems, one of the peptides (Sit1) which includes common motifs with acyl-CoA cholesterol acyltransferase enzymes, changes its dynamics and conformation, suggesting its capability to bind the acyl chains of membrane models and both substrates, whilst the other peptide (Sit2), which includes a putative diacylglycerol binding domain found in protein kinases, appears to interact with the charged headgroup region of the substrates/membrane models. A new time-resolved fluorescence approach known as the phasor plot was employed to analyze intensity decays of peptides during lipid binding. These plots yielded a data trajectory that lies along the line connecting the points of the free and bound peptide, which permits one to calculate an association constant. The different hydrophobic and electrostatic interactions observed in the binding of the peptides to the model membranes may be involved in the interactions of the enzyme with membrane surfaces during the triacylglycerol synthesis, in which a synergistic work between the binding sites might play a role to bring the triacylglycerol substrates into close proximity to the membrane.