BINDING OF THE N-ACYLETHANOLAMINES WITH SERUM ALBUMIN

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Ethanolamides of saturated or unsaturated free fatty acids are called N-acylethanolamines (NAEs) and are minor lipids, which have high biological activity. At the tissue and organ level, the blood circulation integrates metabolism. Based on the wide distribution of the exogenous NAEs in the organism one can assume the existence of NAE transport in blood circulation. For the moment, nothing is known about such transport. It was decided that serum albumin is the most prominent candidate because it is the major transport protein in blood and only one that transports free fatty acids. The aim of the present study was: to estimate the probability of NAE binding to human serum albumin by means of computer modeling of human serum albumin-N-stearoylethanolamine (HSA-$NAE_{18:0}$) complex; to investigate the binding of radiolabelled N-palmitoylethanolamine ($NAE_{16:0}$) to proteins contained in the rat serum; to study complex formation between HSA and $NAE_{16:0}$, $NAE_{18:0}$, N-oleylethanolamine ($NAE_{18:1n9}$) and N-arachidonoylethanolamine ($NAE_{20:4n6}$) by mass spectrometry and fluorimetry.

It was shown that [9,10-^{3}H]-$NAE_{16:0}$ was easily assimilated and found in the blood as soon as 20 min after *per os* feeding of rats. The isolation of albumin by affinity chromatography followed by measurements of isolated fraction radioactivity showed that radioactivity was associated with albumin fraction. The measurement of intrinsic Trp-fluorescence of HSA showed that $NAE_{16:0}$, $NAE_{18:0}$, $NAE_{18:1n9}$ and $NAE_{20:4n6}$ caused the quenching of Trp-fluorescence. In addition the $NAE_{20:4n6}$ induces the small fluorescence shift, possibly due to four double bounds in the $NAE_{20:4n6}$ molecule. Computer modeling was made by Dock and Autodock programmes. We have found five sites for $NAE_{18:0}$ molecules on albumin molecule, which are the best positions for the binding. The principal site of the binding is localized near the Trp-214 residue. MALDI-MS spectra of HSA complexes with different NAEs demonstrate small molecular weight shift between pure albumin and HSA-NAEs. The energy of $NAE_{18:0}$-HSA binding in this site has the following values: -8.47 kcal/mol. The molecular dynamic simulations session by GROMACS program was performed for this complex. The results shown significant complex stability without sizeable changes of conformation and pointed at junctions between $NAE_{18:0}$ and HSA molecule. Simultaneously, potential energy of this complex was essentially lower than that for $NAE_{18:0}$ molecules in water box.

Our findings allow supposing that namely albumins are the transport form for saturated NAEs in the organism. According to computer modeling $NAE_{16:0}$ and $NAE_{18:0}$ have high affinity for serum albumin and create a stable complex with it. At the same time, there are a few sites for $NAE_{18:0}$ binding to albumin molecule and the most stable of them is localized in a focal point of the hydrophobic binding pocket of subdomain IIa near the residue of Trp-214 of human serum albumin. MALDI-MS and fluorescence spectra demonstrate the binding of NAEs, especially $NAE_{20:4n6}$ to HSA.