ABSTRACT

The use of modern nanotechnology techniques and atomic force microscopy (AFM) in particular, in the study of some of the most interesting nanoparticles met in human, lipoproteins, remains a scientific challenge. The aim of the present study was: 1. to image LDL particle with the use of AFM without special sample preparation with fixation and 2. to investigate its interaction with material surfaces. Blood plasma was obtained from four healthy normolipidemic subjects (mean age 29,7±5,5 years). LDL fraction was isolated by ultracentrifugation. Experiments were performed: 1. on two different smooth and clean substrates of different hydrophobicity [HOPG and c-Si(100)], 2. with varying LDL concentrations (5, 15 and 50 μg/ml) and 3. at different incubation times (1h, 2h and 12h). Therefore, comparisons of results were made on three directions, each time keeping two of them constant. Measurements were performed with Solver P47H Pro (NT-MDT) using tapping mode in air. AFM images of LDL on HOPG were obtained and dimensions attributed to single LDL particles were determined. The lateral dimensions of these molecules were approximately 60-65 nm and their height 10-12 nm. Various LDL aggregates were discernible and some of them had a specific motif (tetramers). The particles demonstrated preferential accumulation at the HOPG steps and clean areas on the surface were observed. The adsorption increased with the increase of the solution concentration while no significant changes in the amount of LDL as a function of time was observed for the selected time periods. On the other hand, full c-Si surface coverage from the adsorbed LDL layer was observed after 1h and 2h of incubation. Lipoproteins formed clusters of different shape and size, without being aggregated on special morphology features. LDL solution concentration was positively correlated with grain size. After 12h of incubation the grain size seemed to be reduced and small uncovered areas were revealed. This may be due to the sample preparation before imaging which leads to the layer's removal after being rinsed and dried. In all cases, the lipoproteins were displaced by the action of the tip which may be a manifestation of the weak LDL-substrate interactions. Using the AFM, the appropriate substrate and sample solution and a simple surface immobilization procedure, images allowing identification of LDL molecules can be obtained. Their dimensions are overestimated and their shape is changed due to the forces applied to them by the AFM tip. The adsorption seems to be dependent on the substrate wetting properties. It is also affected by LDL concentration and surface morphology. Further research is required in order to determine the effect of incubation time, AFM parameters (tip geometry, applied force, in ambient or liquid environment) and various pathological conditions on LDL shapes, sizes and biological behavior.