conferenceseries.com SciTechnol

A I Archakov, J Nanomater Mol Nanotechnol 2017, 6:6
DOI: 10.4172/2324-8777-C1-013

19th Nano Congress for Next Generation

August 31- September 01, 2017 Brussels, Belgium

A I Archakov

Institute of Biomedical Chemistry, Russia

AFM physicochemical properties and activity of single protein molecules of CYP 102A1 (BM3)

Atomic force microscopy (AFM) is a nanotechnological multifunctional molecular platform for measuring of physicochemical and functional properties of single proteins molecules. AFM was used for visualization of oligomeric state, activity, elasticity and electron transfer of single molecules of CYP 102A1 (BM3). It was shown that BM3 in water solution exists as monomer and different oligomers by use of sharp and super sharp AFM probes. Functional activity of single monomers and oligomers of BM3 was measured by AFM as well. The BM3 height fluctuations amplitude (HFA) during catalytic cycle is much larger than the HFA of the enzyme molecules in the resting state. It was found that an average HFA of dimers P450 BM3 during catalytic cycle increased up to 5.0±2Å•s^{-1} that was 2.5 times larger than a HFA of P450 BM3 in the resting state. It was obtained that the HFA of immobilized on mica cytochrome P450 BM3 depends on temperature, and 22˚C is a peak of this temperature profile. Mass spectrometry (MS) measurements were used to obtain a time course of a hydroxylation product of lauric acid oxidation during the enzymatic reaction of P450 BM3 in two cases: when enzyme was solubilized in the volume and when it was immobilized on the mica chip. In both cases the number of enzyme molecules was ≈10^{10}, and the kinetics was linear during the first 10 minutes. It was shown that in the case of solubilized enzyme k_{cat}=10^{-3} s^{-1}, and in the case of immobilized enzyme k_{cat}=0.4•10^{-3} s^{-1} that was 2.5 times less than the first one. The purpose of our work was to find a relationship between enzyme HFA and its catalytic activity. Therefore, AFM data was analyzed together with MS data and the following equation was obtained: k_{cat}= β• (exp(ΔA/ A0)$^{-1}$, where k_{cat} – is a catalytic rate constant (s^{-1}); β is a proportionality factor (s^{-1}); ΔA = Δ Ā – A0 (Å), where Ā and A0 are the average amplitudes of P450 BM3 height oscillations in the active and resting states, respectively. The value β = 5•10^{-6} s^{-1} was calculated from time dependence of reaction curves measured by AFM and MS. Elasticity of single protein was measured based on deformation of this protein under AFM probes with various radii of curvature. Young's modulus of BM3 molecules depends on AFM modes. Based on the obtained data, the following conclusions may be made: the enzyme catalytic activity of single molecules can be measured as a HFA of BM3 oscillation during catalytic cycle.

Biography

A I Archakov is a Full Member of the Russian Academy of Sciences and Professor & Scientific Advisor at Institute of Biomedical Chemistry. He has organized scientific school to study molecular organization and functioning of oxygenase cytochrome P450-containing systems, molecular mechanisms of the structure and function of membranes and biological oxidation. He has guided the institute's members in developing a fundamentally new pharmaceutical composition "Phosphogliv" with antiviral activity for the treatment of liver diseases of various etiology. He is the pioneer in the development of proteomics in Russia. Currently, he is the International "Human Proteome" Project Coordinator in Russia. He is one of the Russia's top 100 scientists with Hirsch number 27. He is the author of more than 700 scientific works including about 482 scientific articles, 6 monographs, 30 patents and author's certificates. He was Scientific Advisor for 15 Doctors' and more than 60 PhD theses. He is the winner of three state prizes of the USSR, the RSFSR and of the Russian Federation, the Russian Federation Government Award, the Bach Prize of the USSR Academy, and the Order "For Merit to the Fatherland" (IV, III, II class).

inst@ibmc.msk.ru