The inner dynamics of focal adhesions

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Alex G. F. de Beer

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There are billions of conscientious body cells oxidating away day and night like dumb animals at their complicated job of keeping me alive and healthy, and every one is a potential traitor and foe.

FROM: CATCH-22, JOSEPH HELLER

Abstract

Protein clustering effects play a major role in cell adhesion and cell signaling. The density of these clusters is likely to cause a hindering to diffusion, in contrast with models assuming free movement of proteins. We have used total internal reflection microscopy in combination with continuous photobleaching to determine protein mobilities inside focal adhesions. Following from our adaptation of continuous photobleaching theory, β3 integrin inside a focal adhesion is shown to be subject to limited exchange with integrin molecules outside the adhesion sites. These results indicate a diffusional hindering of integrin within a focal adhesion. Previous studies have shown a dependence of cell adhesion characteristics on lateral ligand spacing. This thesis provides further insight into this effect: the shape and temporal integrity of individual focal adhesions were studied using nanopatterned substrates. The previously observed, ligand spacing dependent, transition from stable to unstable cellular adhesion was confirmed, and it was observed that this loss of adhesion stability correlates with a loss of spatial and temporal focal adhesion coherence. A model based on the force balance between ligand-integrin bond and the cytoskeleton demonstrated that a possible source for this transition is a force-induced unbinding of proteins due to an increased force, which in turn is the result of a total force distributed over a lowered number of bonds. In combination with the hindrance of diffusion measured using continuous photobleaching, the model suggests a phase diagram consisting of three regimes. Depending on force and ligand spacing, an adhesion may either be stable, unstable or unable to form. The presence of gold particles in the substrates used may lead to an additional effect: when fluorophores move within 10 nm of a gold particle, fluorescence quenching may occur. We have shown that this fluorescence quenching is due to an increase in both radiative and non-radiative emission rate. The increase in nonradiative rate causes a decrease in emission intensity and shortening of the excited state lifetime, while the increase in radiative rate causes a decrease in photobleaching chance per excitation-emission cycle. We demonstrate that these effects can be used to generate a contrast between fluorophores bound to gold particles and unbound fluorophores.

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List of abbreviations

CP	Continuous Photobleaching
DMEM	Dulbecco's Modified Eagles's Medium
ECM	ExtraCellular Matrix
GFP	Green Fluorescent Protein
FA	Focal Adhesion
FAK	Focal Adhesion Kinase
FBS	Fetal Bovine Serum
FRAP	Fluorescence Recovery After Photobleaching
N.A.	Numerical Aperture
PBS	Phosphate Buffered Saline
PEG	Poly(EthyleneGlycol)
REF	Rat Embyonic Fibroblast
rms	root-mean-square
SA	StreptAvidin
TIRF	Total Internal Reflection Fluorescence (microscopy)
YFP	Yellow Fluorescent Protein