细胞分子生物力学

Rapid kinetics of human neutrophil β_2 integrin upregulation induced by e-selectin engagement

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Using a micropipette adhesion frequency assay, we measure the frequency of neutrophil adhesion to red blood cells coated with E-selectin and intercellular adhesion molecule-1 as a function of contact time. This binding curve exhibits a biphasic pattern that begins with a fast ascending phase to a low plateau, which, after a 5-s delay time, follows by a slow ascending phase to a high plateau. The first phase is mediated by E-selectin binding to its neutrophil ligands only whereas the increased binding in the second phase is due to upregulated β_2 integrin binding to intercellular adhesion molecule-1. The affinity upregulation of β_2 integrins requires crosslinking of E-selectin ligands on neutrophils and requires Syk. The dose-dependence curve of β_2 integrin upregulation exhibits a threshold at ~12 E-selectin/m^2 on red cell surface or ~1 g/ml E-selectin in solution above which the level of β_2 integrin upregulation becomes saturated, which represents an intermediate affinity state. Moreover, E-selectin is the only selectin able to upregulate the binding affinity of human β_2 integrins; P- and L-selectins failed to induce the noticeable upregulation of β_2 integrin binding affinity under current experimental conditions. These findings elucidate the kinetic requirements for β_2 integrin upregulation induced by engagement of E-selectin ligands on human neutrophils.

Acknowledgement: This work was supported by US National Institutes of Health grant R01 AI44902.

光镊技术热运动方法直接测量二维正反应率

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细胞粘附分子间相互作用介导了炎症反应、肿瘤转移、动脉硬化和创伤愈合等重要生物学过程，定量描述为受体-配体结合和解离的二维反应动力学。反应动力学参数-分子结合时的正反应率和解离时的负反应率-决定着分子反应发生的可能性、快慢和键的强弱，故反应动力学参数的测量是理解生理现象本质的关键。现有定量测量技术包括微管吸吮、原子力显微镜、微悬臂梁等，均是从键的解离过程出发测量粘附频率或动力谱，拟合得到正、负反应率参数。对于二维正反应率的测量而言，均为间接方法。近期报道的平板流动腔[1]、离心[2]和生物膜力探针[3]技术开始关注正反应率的直接测量，但没有把面积因素排除。

二维正反应率的直接实验测定目前很少报道，原因在于很难直接观察到分子结合的过程。本文采用高力灵敏度（$\sim 10^{-1}$ pN）、高位移灵敏度（~100 nm）、低刚度系数（$\sim 10^{-4}$-10^{-2} pN nm^{-1}）的光镊技术，建立了直接测量受体-配体二维正反应率的实验方法。分子系统采用三种选择素重组蛋白（sPs、PLE、sLs）与其糖蛋白配体 PSGL-1，分别包被在直径为 2.32 μm 和 5.66 μm 的玻璃微珠表面；大微珠固定于底板，小微珠在弱光阱约束下作布朗运动；当分子调整取向形成分子键时，物理连接将约束小球的布朗运动，其振幅、频率、中心位置将发生变

化;通过记录小微珠布朗运动的位移曲线,可观测到分子键的结合-解离交替过程。受体-配体键作为弱的非共价键,其结合和解离事件是独立的随机事件。由一系列独立的分子解离状态(即等待键形成所需时间)、分子结合状态(即键的寿命)的时间分布,根据一阶不可逆动力学模型,可分别得到分子键的正、负反应率。

结果表明:1:三种不同选择素正反应率大小依次为 sPs > sLs > PLE,与分子长度正相关;负反应率大小依次为 sLs > PLE ≥ sPs,与其生物学功能相适应;2:不同的数值分析方法对结合事件起止时间的判断略有差异,对结果略有影响,但不影响不同选择素分子的正负反应率大小趋势;3:分子载体间初始距离、扩散、表面拓扑对分子结合过程有影响;4:对于热涨落方法,实验技术的探针刚度对分子结合率和分子复合物刚度的测量有影响。上述结果为深入认识受体-配体结合动力学及其结构-功能关系提供新的分子生物力学与生物物理信息,对阐明由受体-配体动力学介导的细胞粘附有重要的生理意义。

致谢:本文得到科技部"蛋白质科学"国家重大研究计划(2006CB910303)、国家自然科学基金项目(10332060/30730032)、中国科学院海外杰出学者基金(2005-1-16)以及科技部863项目(2007AA02Z306)的支持。

参考文献

[1] Yago, T., Zarnitsyna, V. I. et al.. Biophys. J. 92, 330-342 (2007).
[2] Liang, S., Fu C. et al.. Am. J. Physiol Cell Physiol 294, C743-C753 (2008).
[3] Chen, W., Evans, E. A., Mcever, R. P. & Zhu, C.. Biophys. J. 94, 694-701 (2008).

Flash sniper: automated detection and analysis of superoxide flash in single mitochondrion of cardiac myocytes

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Mitochondrial "superoxide flashes" reflect transient bursts of mitochondrial superoxide production, which plays a vital role in regulating various cellular processes based on its own homeostasis.[1] We can observe the superoxide level of single mitochondrion and capture its changes in time series scanning of confocal microscope by expressing a mitochondrial matrix-targeted redox-sensitive fluorescent protein. Here we develop a numerical algorithm to detect and analyze the flash events from time sequences of confocal image stack. Our algorithm consists of three steps.

Pretreatment of raw images is to auto-define cell region based on histogram analysis approach, eliminate the fluorescent background, and perform smooth filtering. It also model the degradation caused by photobleach and Photoconversion and restore the original signal.

Flash detection algorithm is designed based on the general notion of detecting connective areas whose fluorescent intensity changes are above certain threshold, which relies on the noise level. The first phase of flash detection is basically aiming at classify spatial points belonging to tentative flash sites on 2D features, which are calculated by decomposing the fluorescent signal into noise, baseline and signal change terms following a linear model. Sites that are spatially or temporally adjacent can be separated using morphological segmentation. The second phase further sieves true flash sites from the detected tentative ones according to the statistic property of their local noise level[2]. The detection implementation avoids 3D searching thus reduce complicity and the time consumed, and also make full use of information by adding local-based sieving to global classification.

Flash measurement is finally implemented according to the trace of determined flash sites, to compute spatiotemporal features such as amplitude, duration and kinetics.