11th International Conference on Advanced Materials
Rio de Janeiro Brazil
September 20 - 25
ICAM2009

Specific affinity between hexokinase and carbohydrates studied by atomic force microscopy spectroscopy

D. F. S. Petri[(1)*], L. B. R. Castro[(1)] and M. Kappl[(2)]

(1) Instituto de Química, Universidade de São Paulo, SP, Brazil, dfsp@usp.br*, lizandra.castro@celanese.de
(2) Max Planck Institut für Polymerforschung, Mainz, Germany, kappl@mpip-mainz.mpg.de
* Corresponding author.

Abstract – Atomic force microscopy force spectroscopy was used to determine the specific affinity between proteins and carbohydrates. Adhesion forces between protein (hexokinase or concanavalinA) covered colloidal probe and Si wafers were measured in the absence and in the presence of sugar. In the absence of sugar strong attraction between colloidal probe and Si wafers brought about large adhesion forces and pull-off distances. In the presence of fructose, glucose or mannose the mean adhesion forces and distances decreased with different intensities, indicating different affinities between proteins and each sugar. These findings were supported by liquid-air surface tension measurements.

Atomic force microscopy has been often used as a tool for measuring adhesion forces between biomolecules [1]. Affinity between enzyme and substrate is often indicated by Michaelis-Menten constant (K_M), which might be determined by conventional methods (espectrophotometric, calorimetric). In this work adhesion forces between hexokinase covered colloidal probe and Si wafers were measured in the presence of fructose, glucose or mannose in order to gain insight about the affinity between enzyme and substrate. To the best of our knowledge this is the first time that AFM force spectroscopy has been applied for such purpose. HK covered PS/CH particles were glued with epoxy glue onto the apex of the tipless V-shaped silicon nitride cantilevers (Veeco NP-OW) (see Figure 1). The AFM cantilevers with attached colloidal particle were mounted in the fluid cell that allows measurements of the interaction forces in liquids in the Multimode Nanoscope IIIa AFM with Picoforce add-on from Veeco/Digital Instruments operating in the force mode. The cell was filled with about 50µL of Tris-HCl buffer solution (pH 7.5) containing $MgCl_2$ 0.010 and cantilever deflections versus piezo position curves against Si/SiO_2 surfaces were acquired using the AFM software of the manufacturers at a scan rate of 1 Hz. Recorded deflection versus piezo position data were converted into force versus distance data. In the absence of sugar strong attraction between positively charged patches on HK and negatively charged Si wafers gave rise to large adhesion forces and pull-off distances. In the presence of 0.025 mol/L fructose, glucose or mannose the mean adhesion forces and distances decreased 70%, 55% and 40%, respectively, indicating different affinities between HK and each sugar (Figure 2). These findings were supported by liquid-air surface tension measurements and literature K_M values.

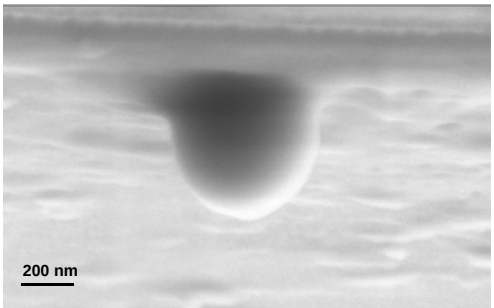
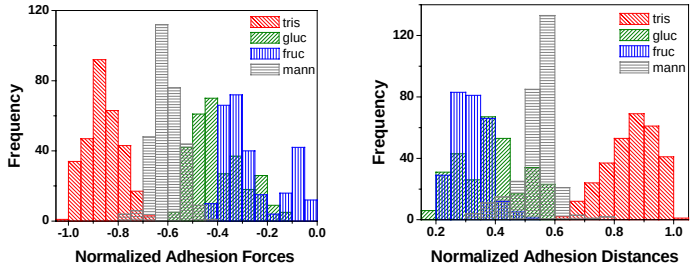


Figure 1. Scanning electron microscopy of HK covered PS/CH colloidal probe glued onto the apex of the tipless V-shaped silicon nitride cantilever.



(a) **(b)**

Figure 2. Histograms of normalized adhesion **(a)** forces and **(b)** distances obtained from force-distance curves (retract) determined for PS/CH/HK particle probing against Si/SiO_2 surfaces under four different media: red for Tris-HCl buffer, green for glucose, blue for fructose and gray for mannose.

[1] Morris, V. J., Kirby, A. R., Gunning, A. P. Atomic Force Microscopy for Biologists, Imperial College Press, London, 2004.