Atomic force microscopy of biological macromolecules and their assemblies

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SUMMARY

The Thesis is concerned with the investigation of structure and dynamics of biological macromolecular assemblies by atomic force microscopy (AFM). The technique of AFM enables the investigator to visualize the surface topography of an object with up to atomic resolution. It also offers an exciting possibility to study a biological system in a near-native environment: i.e., in fluid, and to visualize processes in real time at sub-molecular resolution. These, and other characteristics of the technique, are discussed in detail in *Section I* of the Thesis. *Section I* also contains a review of the relevant literature.

In *Section II*, Chapter 1 the formation of supported phospholipid bilayers (SPBs) on mica surface is investigated. While SPBs have been used by the scientific community since their introduction in mid-80's, the mechanism of their formation has remained obscure. It was possible to visualize, in buffer, intact individual liposomes adsorbed to the mica surface and follow their behavior. This allowed the experimental data to be compared with the theoretical predictions available in the literature, resulting in a consistent picture of the process, which was found to be consistent with the results obtained by spectroscopic means in other groups. The results of this Chapter provide the foundation for the three subsequent *Section*s. Chapter 2 is concerned with the topography structure and with the dynamics of SPBs composed of phospholipid mixtures.

Section III is concerned with the surface structure of membrane-binding proteins. Annexin A5, described in the first Chapter of this *Section*, was used as a model to demonstrate, for the first time, the ability of SPBs to support the growth of protein 2D crystals. This was achieved by extending the well-established procedure for growing protein 2D crystals on phospholipid monolayers at the air-water interface to SPBs. The equivalence of SPBs and lipid monolayers insofar as the crystallization of annexin A5 is concerned was demonstrated through the comparison of high-resolution structural data on the two crystal forms annexin A5 crystallizes in (both on SPBs and monolayers) obtained by electron crystallography and AFM. The crystal forms observed by AFM and electron microscopy (EM) were shown to be identical and formed under equivalent conditions. This allowed, firstly, a detailed investigation of the surface structure of annexin A5, including the comparison of AFM topography with that expected from the X-ray

structure of the protein, and secondly, a study of the relationship between the two crystals forms in *Section IV*. In the second Chapter, the topography structure of another protein crystallized on an SPB – streptavidin - is discussed.

The two 2D crystal forms which annexin A5 crystallizes in have been known through the EM studies of this protein for some time. Evidence that they are related by a direct phase transition is provided, and the mechanism of the transformation is proposed in this *Section* (*Section IV*) based on AFM and EM observations.

Section V discusses in some detail the issue of contrast mechanism in AFM. This question has been voiced since the very conception of AFM, and, in spite of significant advances, had not been resolved satisfactory. The data accumulated throughout the work discussed in the preceding sections is compared critically with the literature, and possible contributions to the contrast are discussed.