

Structural transitions of concanavalin A adsorbed onto bare mica plates: surface force measurements

J.-P. Gallinet, B. Gauthier-Manuel

Groupe pluridisciplinaire d'étude des interfaces, Laboratoire d'Electrochimie des solides, UA 436, CNRS UFR des Sciences & Techniques, F-25030 Besancon Cedex, France

Received: 4 August 1992 / Accepted in revised form: 19 March 1993

Abstract. The structure of the β -sheet rich molecule, concanavalin A (Con A), shows complicated behaviour when mechanical stress is imposed on adsorbed Con A layers. In the pH range 3.9 to 7.4, the structural changes are dependent on the pH and ionic strength of the solution at the liquid/solid interface. Several molecular shapes are observed ranging from a highly unfolded state characterized by a 1.25 nm thickness of each expelled layer at pH 3.9 to other states with various layer thicknesses at pH 6–7.4 and at a low ionic strength ($<10^{-4}$). In the pH range 6 to 7.4, different molecular species appear to be present.

Key words: Concanavalin A – Structural transition – Plant glycoprotein – Protein adsorption – Mica plate – Force surface apparatus

Introduction

The behaviour of a protein upon adsorption-desorption onto a solid surface depends on various types of interaction (Ivarsson and Lundström 1986) such as the location and nature of hydrophobic patches, charge distribution and dynamics of the adsorbed macromolecule, solid surface potential, interfacial dynamics and hydrodynamics (Andrade 1985).

Thus, the structure of a protein molecule often undergoes a rearrangement upon adsorption on a solid surface (Andrade 1985; Norde and Favier 1992). The structural changes are generally attributed to intramolecular electric fields; however the detailed mechanism of adsorption of a protein onto a solid surface and the behaviour of the macromolecule at the solid/liquid interface are not fully understood (Andrade 1985; Horbett 1986; Adamson 1990; Fitzpatrick et al. 1992; Norde and Favier 1992).

In addition to analyses by various spectroscopic methods (Andrade 1985; Horbett 1986), the investigations of

adsorbed layers of proteins with the force surface apparatus (Tabor and Winterton 1969; Israelachvili 1992) have revealed several types of attractive and repulsive short-range forces which can arise in liquids (solutions) and at solid/liquid interfaces (Smith et al. 1991). An automated force surface apparatus, recently developed in the laboratory, has been used not only to estimate the magnitude of the interactions between several layers of the protein BSA during adsorption-desorption behaviour induced by a compressive stress, but also to obtain an estimate of the shape of the molecules in each layer (Gallinet and Gauthier-Manuel 1992). The automation allows accurate control of speed and position of the mica surfaces. Thus we can observe the size variations of layers of the adsorbed macromolecules during changes of pH and ionic strength in the surrounding solution. To illustrate the performance of such an automated device, we report data obtained with concanavalin A, a β -sheet rich macromolecule.

Concanavalin A or Con A is a lectin, a saccharide-binding protein of the jackbean *Canavalia ensiformis* (Sharon and Lis 1990). This macromolecule binds carbohydrates, agglutinates a variety of cells and can serve as a histochemical probe (Bowles and Pappin 1988; Sharon and Lis 1989; Kery 1991; Lin and Levitan 1991). From the X-ray crystallographic structure, the Con A molecule consists of identical protomers of molecular weight 25,000 folded into dome-like structures ($4.2 \times 4.0 \times 3.9$ nm) (Reeke et al. 1975; Derewenda et al. 1989). Each subunit contains only two large antiparallel pleated β -sheets with three binding sites for Mn^{++} , Ca^{++} and carbohydrate respectively (Bowles and Pappin 1988; Sharon and Lis 1990). The native three-dimensional structure (tertiary structure) and the capacity to form functional higher-order assembly structures (quaternary structure) in solution depend on the pH and the ionic strength of the solution: two protomers may form an ellipsoidal dimer (8.0×4.0 nm) stable at a $pH < pHi = 5.5$ (Reeke et al. 1975). When $pH < 5$, the protomer releases its ions, Mn^{++} and Ca^{++} , and undergoes a transition from a "locked" conformation in which these ions are tightly

bound, to an "unlocked" state in which the protomer molecule binds metals weakly (Auer and Schilz 1984; Reeke et al. 1978). The surrounding physicochemical conditions affect the dimer-tetramer conversion (Kanoh and Maeda 1987).

We observe complicated behaviour of the adsorbed Con A layers in the pH range 3.9 to 7.4, where different molecular species are present, and compare the results with data obtained by several spectroscopic methods (e.g. Auer and Schilz 1984; Kanoh and Maeda 1987; Fitzpatrick et al. 1992).

Materials and method

Several modifications shown in Fig. 1 allow easier use of the force surface apparatus developed from the original measuring device (Tabor and Winterton 1969). The experimental procedure has been extensively documented in recent reviews (Smith et al. 1991; Israelachvili 1992). Only a few instrumental details will be presented here.

The mica plates are glued on hemicylindrical glass supports and oriented in a crossed-cylindrical geometry. The positions of the mica surfaces are controlled by a servo-looped piezoelectric ceramic in the range 0.1 nm–5,000 nm. This device eliminates the hysteresis and the creep of the piezoelectric material. A CCD Camera (Array of 4096 pixels; 7 μ m wide) performs the measurement of the fringes of equal chromatic order (F.E.C.O). The geometric profiles of the surfaces are obtained by driving the array in the z axis with a stepping motor (each step 7 μ m).

The F.E.C.O method offers a high sensitivity (0.1 nm from the experimental analysis in Smith et al. 1991) in the direction perpendicular to the plane of contact area and a lower sensitivity (500 nm) in the direction of the plane of contact. An automatic data acquisition system yields the interaction curves (Fig. 2). A very low thermal drift is achieved because the experimenter is not near the device during the experiment.

The mica plates are cleaved muscovite mica sheets, silvered on the back side, and glued to cylindrical silica lenses (mean radius = 2 cm). In Fig. 2, the true distance between the mica plates measured by the optical method is plotted as a function of the displacement of the piezoelectric ceramic. A straight line of slope = 1 is obtained at a long distance ($D_1 > 100$ nm) where no force of interaction occurs. The reference position is determined by direct contact of the two mica surfaces in air and controlled with 2 different fringes with an accuracy ± 0.1 nm. Because the F.E.C.O fringes close to the molecular contact produce a change in the curvature radius of the mica sheets induced by the interaction at short distance (Gallinet and Gauthier-Manuel 1992), it is necessary to plot the force as a function of the distance rather than the energy (force over radius) as usually done (Afshar-Rad et al. 1988; Smith et al. 1991; Israelachvili 1992).

After the determination of the reference position, the mica surfaces are separated. A drop (30 μ l) of an aqueous Con A solution (40 μ g \cdot ml $^{-1}$ of Con A, type IV, Sigma,

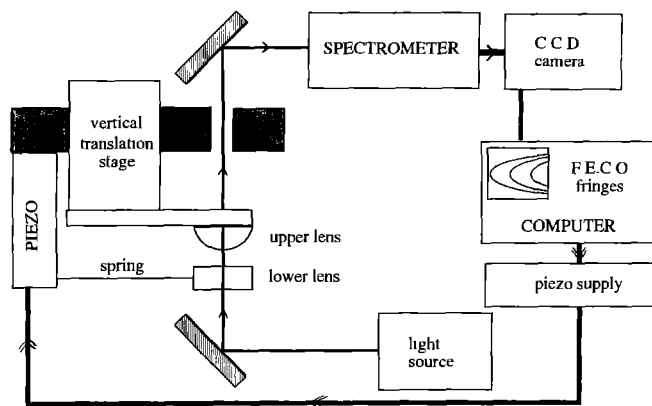


Fig. 1. Schematic drawing of the automated surface force apparatus. Upper lens/lower lens: 2 mica plates silvered on the back are glued onto two mutually perpendicular cylindrical silica lenses (mean radius = 2 cm). Vertical translation stage: support of the upper mica plate glued on the upper silica lens. Piezo/spring: mechanical servo-looped device for controlling the position of the mica surfaces in the range 0.1 nm–5,000 nm. Light source: tungsten-halogen lamp. \rightarrow : optical path through the two silica lenses coated with the silvered mica plates and then through a spectrometer. The signal is recorded by a CCD camera (Array of 4096 pixels, 7 μ m wide) interfaced with a microcomputer. \rightarrow : servo-looped device (piezo supply) of the piezoelectric ceramic (piezo)

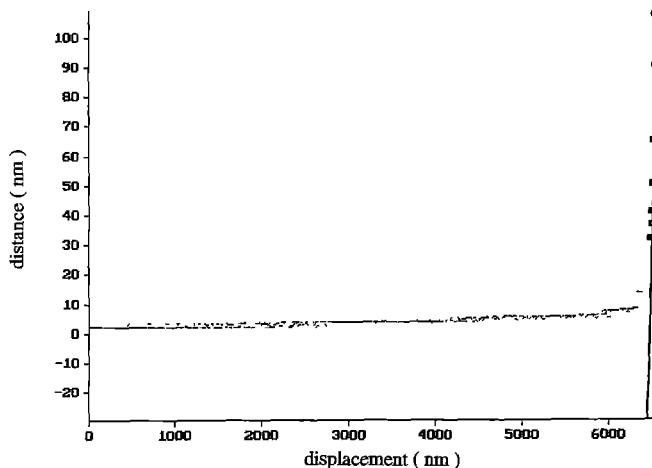


Fig. 2. Evolution of the true distance in nm between the 2 mica plates as a function of the displacement of the support of the mica plates. This displacement is automatically controlled by the servo-looped piezoelectric ceramic (see Fig. 1). The approach velocity of the plates is 0.1087 nm \cdot s $^{-1}$ at a long distance where no force is measured. When the two mica plates approach, a decrease of the approach velocity is induced by the repulsive forces. Con A solution (40 μ g \cdot ml $^{-1}$) in 10 $^{-4}$ M acetate buffer, pH 3.9. Fringe order = 15 at 247.2 nm (F.E.C.O method explained in Smith et al. 1991). At a distance > 30 nm, no force is measured and the true distance between the 2 mica plates is equal to the displacement of the support: this relation is represented in the diagram by a straight line of slope 1

Ref. C-2010) is fed between the two mica plates and allowed to adsorb. The pH of the solution is imposed by a buffer solution at a given ionic strength. The adsorption of the Con A molecules is complete after 60 min and then the measurement cell is filled up with the chosen buffer solution. The two mica surfaces are slowly brought together and the force is recorded continuously. Following

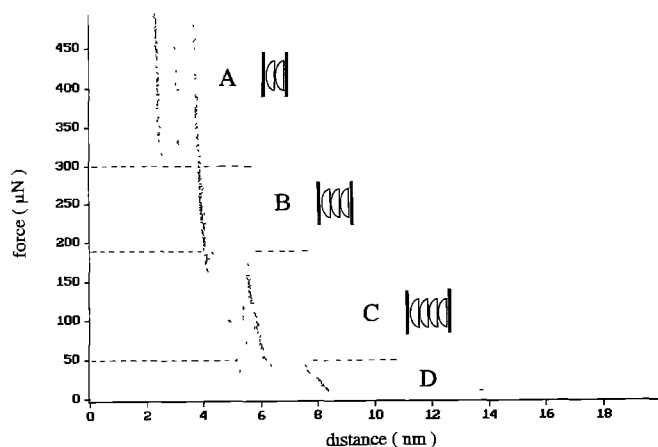


Fig. 3A–D. Change of the interaction force between 2 silvered mica plates coated with Con A molecules. Force as a function of the true distance between the 2 mica surfaces. Same conditions as in Fig. 2. The diagram shows the shape of the Con A molecules located between the mica surfaces before ejection of the layers. **A** The last 2 layers of Con A which do not desorb with a force $> 300 \mu\text{N}$; **B** 3 layers when $190 \mu\text{N} < \text{force} < 300 \mu\text{N}$; **C** 4 layers when $50 \mu\text{N} < \text{force} < 190 \mu\text{N}$; **D** > 5 layers when the force $< 50 \mu\text{N}$. The number of adsorbed Con A layers is determined by assuming that the mean thickness of each layer is 1.25 nm (see Discussion)

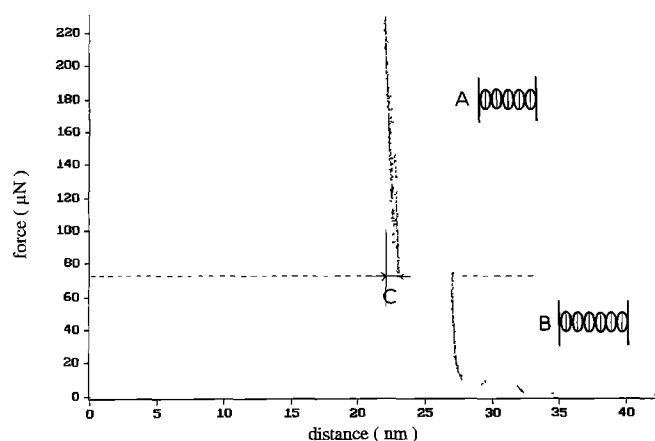


Fig. 4A–C. The interaction force as a function of the true distance between 2 silvered mica plates coated with Con A molecules in a 10^{-5} M phosphate buffer, pH 6.0. Approach velocity of the mica plates $= 0.1090 \text{ nm} \cdot \text{s}^{-1}$. Fringe order $= 15$ at 247.2 nm. Sketched profiles of Con A molecules with the assumption of a mean layer thickness of 3.9 nm: **A**: 5 resting layers when the imposed force $> 70 \mu\text{N}$; **B**: 6 layers when $10 \mu\text{N} < \text{force} < 70 \mu\text{N}$; **C**: magnitude of the compression stress of the 5 Con A layers when the imposed force increases from $70 \mu\text{N}$ to $220 \mu\text{N}$

the experiment, the buffer is removed and the mica surfaces are separated to a large distance. The cell is refilled with an another buffer before a new slow approach is made. The pH of the buffer is measured before and after each experiment, which is performed at atmospheric pressure and 293 K. The data are recorded from 2 different experiments; at a given pH and ionic strength, the thickness measurements on the adsorbed layers are made for 2 or 3 h for each slow approach (total duration: 4–5 days; 5 to 15,000 data points with 2 F.E.C.O fringes for one approach).

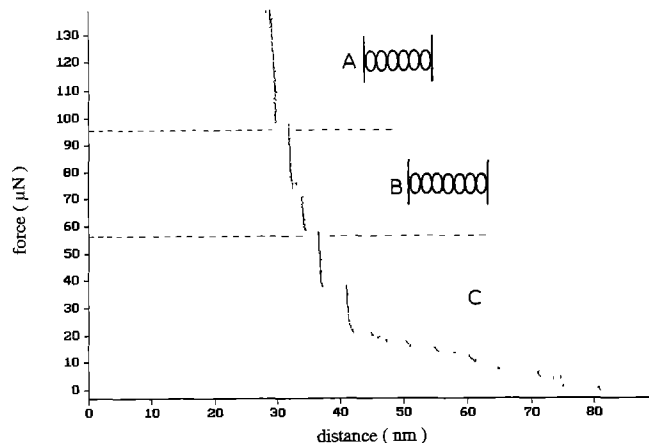


Fig. 5A–C. The interaction force as a function of the true distance between 2 silvered mica plates coated with Con A molecules in a 10^{-3} M phosphate buffer, pH 7.4. Approach velocity of the two mica plates $= 0.1122 \text{ nm} \cdot \text{s}^{-1}$. Fringe order $= 15$ at 270.0 nm. On the assumption that the mean thickness of the adsorbed Con A layers is 4.6 nm, sketched profiles of Con A molecules are: **A** 6 resting layers when the imposed force $> 95 \mu\text{N}$; **B** 7 layers when $55 \mu\text{N} < \text{force} < 95 \mu\text{N}$; **C** > 8 layers when the imposed force $< 55 \mu\text{N}$

Results

Figure 3 shows the force-distance relationship for Con A adsorbed onto the mica plates at pH 3.9 (10^{-4} M acetate buffer). When the pressure stress increases, different layers are peeled away. The thickness of each expelled layer is about 1.25 nm and the first two adsorbed layers corresponding to a thickness of 2.35 nm, are not desorbed by an imposed force $< 500 \mu\text{N}$ (Fig. 3A). The central layer is eliminated with a force $< 50 \mu\text{N}$ (Fig. 3D), the following one with a force $< 190 \mu\text{N}$ (Fig. 3C) and the last layer to be expelled with a force $< 300 \mu\text{N}$ (Fig. 3B).

When the approach of the two mica plates is performed in 10^{-5} M phosphate buffer pH 6.0 (Fig. 4), only one apparent layer (thickness $= 3.9 \text{ nm}$) is eliminated by a force $< 70 \mu\text{N}$ (Fig. 4B). The slope of the repulsive wall means that the compressive phenomenon of the five layers of protein adsorbed occurs with a shift approximately equal to 0.25 nm when the imposed force increases from $70 \mu\text{N}$ to $220 \mu\text{N}$ (Fig. 4C). The pH of the bulk solution is stabilized to 6.0. If one assumes that the mean layer thickness is about 3.9 nm, the separation between the mica surfaces corresponds to 5 molecular layers (Fig. 4A).

After prolonged contact with a 10^{-3} M phosphate buffer at pH $= 7.4$ (Fig. 5), the approach of the surfaces ejects 2 layers and if the thickness is about 4.6 nm (Fig. 5B, C), 6 layers remain between the mica surfaces (Fig. 5A). We also observe intermediary displacements (ca. 2.3 nm) during expelling (Fig. 5B).

Discussion

In the acid pH range 2 to 5 and in the presence of a relatively high ionic strength, the Con A molecule retains its hydrodynamic shape and the dimer does not dissociate

to protomers (Auer and Schilz 1984). At pH 3.9 and with a low ionic strength (about 10^{-4} M), the layers expelled by the imposed mechanical force show a mean thickness of 1.25 (Fig. 3B, C). Such a molecular shape does not correspond to the crystallographic data of the monomer ($40 \times 40 \times 39$ nm from Reeke et al. 1975). "Clipped monomers" described by Phillips et al. (1983) may be present in an aqueous acid solution of Con A molecules at low ionic strength. From our observation, the adsorbed Con A molecules are probably in a highly unfolded state and an imposed force of 500 μ N is not sufficient to desorb the first monolayer adsorbed onto each bare mica plate (Fig. 3A). However, further experimental information will be needed about the true surface shape of the adsorbed Con A monolayer.

When the pH exceeds the isoelectric point of Con A (pHi = 5.5, Righetti and Caravaggio 1976) without increasing the ionic strength, a clustering of the molecules forms several stabilized layers (Fig. 4). Only the intermediary Con A layer is ejected by an imposed force of 80 μ N, the thickness of this layer being 3.9 nm. That is approximately the crystallographic thickness of the dimer (Reeke et al. 1975) and the dimer is presumably stabilized at a pH near the isoelectric point of the molecule. The adsorbed molecules are not desorbed by a force in the range 80 to 220 μ N. If one assumes a mean thickness for each Con A layer of 3.9 nm, there are 5 layers between the mica surfaces (Fig. 4A). The approach of the 2 mica plates produces a slight displacement of 0.25 nm, which may correspond to an increase of the molecular packing (Fig. 4C).

It is difficult to compare these data to the data obtained with the Israelachvili's apparatus (Afshar-Rad et al. 1988). When the mechanical setup is not automated, the approach by stepping and the number of the measurements do not permit observation of the possible ejection of molecules such as is possible during a very slow regular approach of the mica plates. In the pH range 5–8, Auer and Schilz describe a "disruption of intersubunit interactions" with loss of rigidity. This phenomenon is strongly dependent on Ca^{++} and Mn^{++} concentrations (Auer and Schilz 1984). In our experimental conditions, Ca^{++} and Mn^{++} concentrations are very low and the macromolecular packing probably becomes rigid when the compression stress increases (Israelachvili 1992). In fact, the other data have been obtained with relatively high ionic concentrations (> 1 mM) (e.g. Kanoh and Maeda 1987; Afshar-Rad et al. 1988; Fitzpatrick et al. 1992).

The molecule accumulation observed here is difficult to reconcile with known theories. Concentrated protein solutions may show two types of interaction, either repulsive or attractive, dependent upon ionic strength and temperature (e.g. Tardieu et al. 1992). Such a transition, from a "liquid-like" distribution (repulsive interaction) to a "gas-like" distribution (attractive interaction) where the proteins may form clusters, is possible in our experimental conditions. Indeed, it is likely that the effective concentration of the proteins is very high between the two mica plates.

When the Con A molecules are in 10^{-3} M phosphate buffer pH, 7.4 for several hours (Fig. 5), the layer thick-

ness is changed and we observe 2 types of layers: one with a size of 4.6 nm (Fig. 5C) and another with a size of 2.3 nm. At this pH, Con A forms tetrahedral tetramers (Reeke et al. 1975). In fact, in the pH range 6 to 7, the dimer-tetramer equilibrium is pH-dependent and is hardly affected by ionic strength (Kanoh and Maeda 1987). It is possible that molecules located between the strongly adsorbed layers and not ejected by a force < 160 μ N (Fig. 5A), area mixture of the different molecular types (monomer, dimer) (Fig. 5B, C). With a mean thickness of one adsorbed layer of 4.6 nm, we find about 6 layers (Fig. 5A). A perturbed profile of the interaction force as a function of mica surface distance appears between 80 and 45 nm (Fig. 5C), indicating a possible heterogeneity in the properties of the adsorbed layers. Such heterogeneity is also obtained with Con A in $5 \cdot 10^{-2}$ M potassium hydrogen phthalate buffer, pH 7.0 and at various concentrations of potassium nitrate: the thickness of the protein layer varies from 1.6 nm to 4.1 nm for both angle-resolved and energy-resolved experiments (Fitzpatrick et al. 1992).

Acknowledgements. This work is supported by the CNRS (GDR 936: "mesures des forces de surface en milieu liquide").

References

- Adamson AW (1990) The solid-liquid interface. Adsorption from solution. In: Adamson AW (ed) Physical chemistry of surfaces. Wiley, New York, pp 420–459
- Afshar-Rad T, Bailey AI, Luckham PF, Mc Naughtan W, Chapman D (1988) Forces between model polypeptides and proteins adsorbed on mica surfaces. *Colloids Surf* 31:125–146
- Andrade JD (1985) Principles of protein adsorption. In: Andrade JD (ed) Surface and interfacial aspects of biomedical polymers, vol 2, Plenum Press, New York, pp 1–80
- Auer HE, Schilz T (1984) pH-dependent changes in properties of concanavalin A in the acid pH range. *Int J Pept Protein Res* 24:462–471
- Bowles DJ, Pappin DJ (1988) Traffic and assembly of concanavalin A. *TIBS* 13:60–64
- Derewenda Z, Yariv J, Helliwell JR, Kalb AJ (Gilboa), Dobson EJ, Papiz MZ, Wan T, Campbell J (1989) The structure of the saccharide – binding site of concanavalin A. *EMBO J* 8:2189–2193
- Fitzpatrick H, Luckham PF, Eriksen S, Hammond K (1992) Use of X-ray photoelectron spectroscopy to study protein adsorption to mica surfaces. *J Colloid Interface Sci* 149:1–10
- Gallinet J-P, Gauthier-Manuel B (1992) Adsorption – desorption of serumalbumin on bare mica surfaces. *Colloids Surf* 68:189–193
- Horbett TA (1986) Techniques for protein adsorption studies. In: Williams DF (ed) Techniques of biocompatibility testing, vol 2. C.R.C Press, Boca Raton, pp 183–214
- Israelachvili JN (1992) Adhesion forces between surfaces in liquids and condensable vapours. *Surf Sci Rep* 14:109–159
- Ivarsson B, Lundström I (1986) Physical in vitro characterization of protein adsorption on metal and metal oxide surfaces. *Crit Rev Biocompat* 2:1–96
- Kanoh H, Maeda H (1987) Effects of CuCl_2 on the dimer-tetramer equilibrium of concanavalin A. *Int J Pept Protein Res* 30:102–107
- Kéry V (1991) Lectin-carbohydrate interactions in immunoregulation. *Int J Biochem* 23:631–640
- Lin SS, Levitan IB (1991) Concanavalin A. A tool to investigate neuronal plasticity. *Trends Neurosci* 14:273–278
- Norde W, Favier JP (1992) Structure of adsorbed and desorbed proteins. *Colloids Surf* 64:87–93

- Phillips PG, Horowitz P, Mac Nab J (1983) Molecular heterogeneity of the concanavalin A tetramer: effects on binding to human red blood cells. *Biochem Int* 7: 549–555
- Reeke GN, Becker JW, Cunningham BA, Wang JL, Yahara I, Edelman GM (1975) Structure and function of concanavalin A. *Adv Exp Med Biol* 55: 13–33
- Reeke GN, Becker JW, Edelman GM (1978) Changes in the three-dimensional structure of concanavalin A upon demetallization. *Proc Natl Acad Sci, USA* 75: 2286–2290
- Righetti PG, Caravaggio T (1976) Isoelectric points and molecular weights of proteins. *Anal Biochem* 127: 1–28
- Sharon N, Lis H (1989) Lectins as cell recognition molecules. *Science* 246: 227–235
- Sharon N, Lis H (1990) Legume lectins. A large family of homologous proteins. *FASEB J.* 4: 3198–3209
- Smith CP, Snyder SR, White HS (1991) Measurement of surface forces. In: Abruna HD (ed) *Electrochemical interfaces: modern techniques for in – situ interface characterization*. V.C.H, New York, pp 156–194
- Tabor D, Winterton RHS (1969) The direct measurement of normal and retarded Van der Waals forces. *Proc R Soc London, Ser A* 312. 435–450
- Tardieu A, Vêrétout F, Krop B, Slingsby C (1992) Protein interactions in the calf eye lens. Interactions between β -crystallins are repulsive whereas in γ -crystallins they are attractive. *Eur Biophys J* 21: 1–12