WATER WETTABILITY OF ANTIGEN AND ANTIGEN–ANTIBODY LAYERS ON SOLID SURFACES STUDIED BY THE CONTACT ANGLE MEASUREMENT TECHNIQUE

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1. Introduction

Thin layer immunoassay (TIA) is a method for analysis and quantification of antigen–antibody reactions on solid surfaces [1,2]. Elaboration of TIA was based on the observation that a polystyrene surface coated with a double layer of antigen and antibody is more wettable than a surface coated with antigen only. The increased wettability is visualized as a distinct hydrophilic condensation pattern when the surface is exposed to water vapour [vapour condensation on surface (VCS) technique].

The immunobiological relevance of the large difference in wettability on antigen and antigen–antibody surfaces is largely inknown although the wettability parameter is a fundamental characteristic of a solid surface and its adsorbed molecules. Several of the antibody-mediated immunological effector mechanisms, e.g., opsonization or complement fixation, occur on the surface of bacteria or other micro-organisms.

This investigation used the contact angle measurement technique in [3] to characterize and quantitate wettability of antigen and antigen–antibody layers adsorbed on polystyrene surfaces in greater detail. The contact angle measurement technique is most appropriate since, in contrast to the VCS technique used in TIA, it registers objectively and quantitatively the wettability of a solid surface.

2. Material and methods

Polystyrene petri dishes (8.5 cm diam., NUNC A/S Roskilde) were used for adsorption of antigen and antibodies. The plates were rinsed with 95% ethanol prior to use.

Antigen and antiserum: Bovine serum albumin (BSA), grade V (Sigma Chemical Co., St Louis, MO) were used. Anti-BSA was prepared by subcutaneous immunization of rabbits as in [1]. Specific anti-BSA antibodies were determined to be 3.5 g/l by quantitative precipitation according to [4]. Antigen and antiserum were diluted in 0.15 M NaCl prior to use.

2.1. *Quantitation of BSA-adsorption on polystyrene surfaces*

BSA was labelled with ^{125}I according to [5]. The labelled BSA was incubated in the plates at various concentrations for 1 h at room temperature. After rinsing with distilled water and drying of the surfaces, pieces of determined size were cut out of the plates. The radioactivity of the pieces was determined in a γ-spectrophotometer type Packard and the radioactivity was then calculated to μg adsorbed BSA/cm^2. The possible difference in adsorption of the labelled BSA, as compared to unlabelled BSA, was checked in competition experiments in which labelled and unlabelled BSA, at various proportions were adsorbed to the plates.

2.2. *Contact angle measurements [3,6]*

NaCl (18 ml, 0.15 M) was poured into the plates followed by admixing with 2 ml protein solution. After incubation for 1 h at room temperature the plates were rinsed with distilled water. Surfaces with double layer of antigen and antibody were prepared by incubating the wet antigen-coated plates with the corresponding antiserum for 1 h at room temperature followed by rinsing with distilled water. All plates were finally dried by means of filtered compressed air. One piece of plastic material was then cut out of each plate.

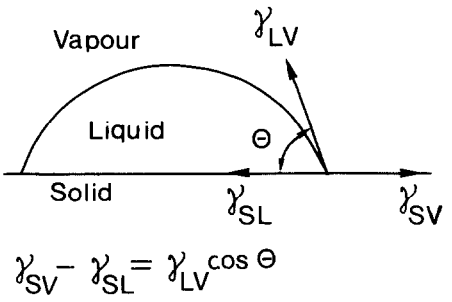

Fig.1. Contact angle (θ) between a liquid and a plane solid surface. Young's equation for the wettability of a surface [3]: γ_{sv}, surface tension of the interface between the solid and vapour; γ_{lv}, surface tension of the interface between the liquid and vapour; γ_{sl}, surface tension of the interface between the solid and the liquid.

Three drops of distilled water (surface tension 72.0 dyn/cm^2 as determined with a tensiometer type Krüss T 52) were applied on the surface of each piece of plastic material. Two contact angles were then determined on opposite sides of each drop with use of a goniometer (a modification of the goniometer in [6]). Contact angles were determined on triplicate surfaces. The mean value and the standard deviation of the contact angles were then calculated. According to Young's equation [3] (fig.1) the wettability was calculated from the cosine value of the contact angle.

3. Results

3.1. *Relations between adsorbed amounts of BSA on polystyrene surfaces and contact angles*

Ten-fold serial dilutions of isotope-labelled BSA at 10 μg/l–10 g/l were used for coating of the plates. When the adsorbed amount of BSA versus concentration of BSA in the solution was plotted in a diagram (fig.2) it was noted that with increasing concentration of BSA in the solution there was a corresponding increase of adsorbed BSA. At higher concentrations ($\gtrsim$0.1 g/l) a levelling of the adsorption was noted indicating saturation.

Plates were also coated with serial dilutions of unlabelled BSA at 10 μg/l–10 g/l followed by determination of contact angles of the various surfaces. As shown in fig.3, the wettability, expressed as Cos for the contact angles, increased with increased coating concentration until a plateau was gradually reached at ~0.1 g BSA/l.

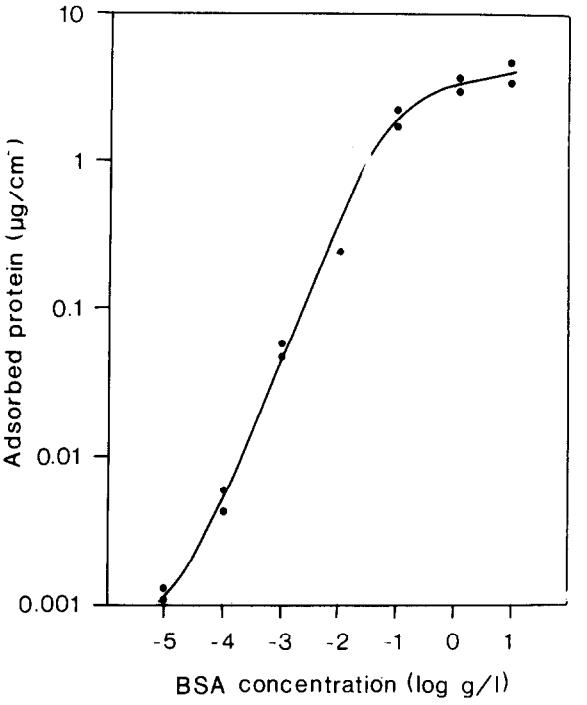

Fig.2. Relations between concentration of labelled BSA used for coating of polystyrene surfaces and amount of adsorbed BSA. The result is based on duplicate determinations.

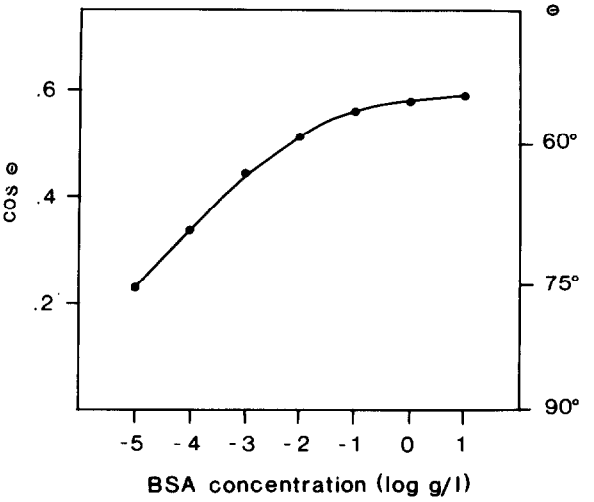

Fig.3. The relationship between concentration of BSA used for coating of the polystyrene surfaces and contact angles. The result is based on triplicate determinations. Reference, contact angle 79.2° for uncoated polystyrene surface.

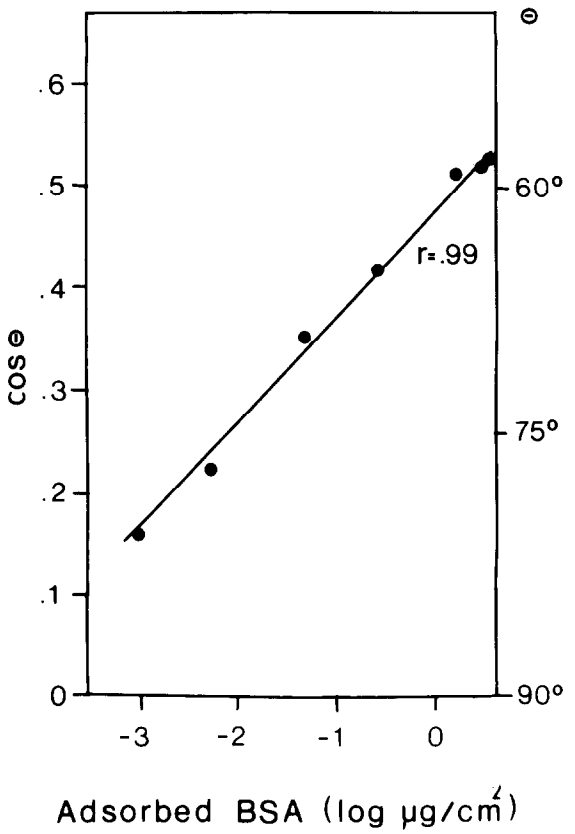

Fig.4. The relationship between adsorbed amount of BSA on polystyrene surfaces and contact angles. For futher explanation, see text.

By combination of results given in fig.2,3 the relation between contact angles and adsorbed amount of adsorbed BSA was obtained (fig.4). It was thus noted that a rectilinear relationship between these parameters existed.

3.2. *Contact angles of surfaces coated with double layers of BSA and anti-BSA*

Plates were coated with constant amounts of BSA (1 g/l) followed by incubation of the plates with anti-BSA at various dilutions. The relationship between contact angles and concentration of antiserum used for incubation is shown in fig.5. This experiment shows that the Cos for the contact angles increased sigmoidally with increasing amounts of anti-BSA exposed to the BSA-coated plates. BSA-coated plates treated with normal rabbit serum did not give rise to a significant lowering of the contact angles.

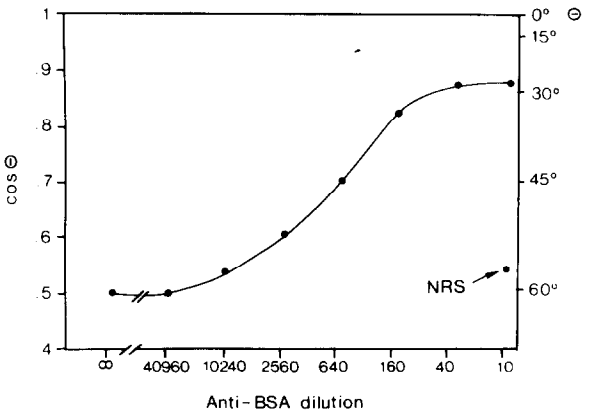

Fig.5. The relationship between concentration of anti-BSA applied to a BSA coated surface and the wettability of the surfaces as determined by contact angle measurement. Control, application of normal rabbit serum (NRS) diluted 1/10. The result is based on triplicate determinations.

4. Discussion

The contact angle measurement technique can be successfully used in studies of antigen adsorption on polystyrene surfaces as well as in studies of antibody binding on layers of antigen. The contact angle measurement technique is, in addition, fairly simple to perform and wettability determinations with this technique can be obtained with high precision provided that the solid surfaces are carefully prepared and that a goniometer with good quality is used for measurement of contact angles.

The finding that wettability, expressed as Cos for the contact angles, was in a linear relation to the amount of adsorbed BSA on the polystyrene surface shows that BSA adsorption may be quantitated by contact angle measurement without involvement of radioactive labelled BSA. Quantitation of antigen adsorption in important in all solid phase methods for determination of antibodies, e.g., TIA or enzyme-linked immunosorbent assay (ELISA) [7], and contact angle measurement may be a simple complement or alternative to the use of isotope-labelled antigens. It may however be mentioned that quantitation of antigen adsorption on polystyrene surfaces by contact angle measurement technique has only been studied with BSA.

That the contact angles of an antigen surface decreased when antibodies had been bound to the antigen surface does not agree with observations in

[8,9]. In [8,9] the contact angle measurement technique was used to study wettability of flat layers of bacteria (*Escherichia coli*) and an increase of the contact angles was observed when antibodies had been bound to the layers of bacteria. It may, however, be mentioned that the contact angle of the layers of bacteria were very low, (<20°) and that antibody binding to the layers of bacteria resulted in an increase of contact angle of only a few degrees.

Such low levels of contact angle make interpretation of results difficult since the roughness factor r is probably high at layers of bacteria leading to an underestimation of θ. The roughness factor r (defined as the ratio of the true area of the solid to the opponent area) has, according to [3], the following influence on the measured contact angle θ' in relation to the 'true' contact angle θ:

$$r = \frac{\cos \theta'}{\cos \theta}$$

As may be seen from the equation that the difference $(\theta - \theta')$ between the true and apparent contact angle increases with decreasing θ and increasing r. In order to reduce the difference $\theta - \theta'$, surfaces with r-values close to 1 should be used. Most probably, a flat polystyrene surface fulfill this requirement better than a layer of bacteria.

An interesting feature of the contact angle measurement technique for detection of antigen–antibody reactions is that the binding reaction is detected at the primary level. This is in contrast to most other methods used in serology, e.g., precipitation, agglutination, complement fixation or neutralization, which pre-suppose the existence of more or less complicated secondary manifestations of the binding reactions. This feature of the contact angle measurement technique comes to its full justification in detection of binding reactions of non-serological nature such as the binding between bacterial toxins or Sendai viruses to various specific gangliosides, supposed to be the natural tissue receptor for these substances. Such binding reactions are difficult to detect with other in vitro methods in which a secondary manifestation is used for visualization. However, detection of the above-mentioned binding reactions at the primary level with use of wettability determination (VCS technique) of adsorbed reagents on polystyrene surfaces has been shown to be a simple and theoretically interesting alternative [10,11]. The above-mentioned binding reactions also may be detected with the contact angle measurement technique (unpublished).

To summarize, the contact angle measurement technique is a useful technique in studies of quantitative aspects of antigen and antigen and antibody binding or other binding reactions on solid surfaces.

Acknowledgement

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