Technical Notes

The adsorption behaviour of two commercial IgG-preparations onto a polystyrene latex surface

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The adsorption behaviour of two commercial preparations of human IgG onto a polystyrene latex surface was studied. The adsorption isotherms obtained differed markedly; one preparation showed a plateau value of 0.4 $\mu g\ cm^{-2}$ which was reached at 0.1 $g\ l^{-1}$, whereas the other preparation showed no plateau value within the concentration range studied (0.1–7.0 $g\ l^{-1}$). Characterization by means of iso-electric focusing and HPLC also showed differences between the two preparations. No differences were observed when immuno-electrophoresis was carried out. These results stress the necessity for proper characterization of proteins used in adsorption studies.

Keywords: Polymers, surface properties, protein adsorption, immuno-globulin G, polystyrene latex, solution–depletion, protein characterization

Many studies concerning protein adsorption onto solid surfaces have been reported[1–14]. In only a few studies[1, 2, 8, 11, 13] was attention paid to the characterization of the protein used (Immuno-globulin G, IgG, for all references mentioned above).

The purpose of this study was to compare the adsorption behaviour of two IgG-preparations, obtained from different manufacturers, on to a polystyrene latex surface. Adsorbed amounts of protein were determined by means of solution-depletion measurements. Both IgG-preparations were characterized by HPLC, iso-electric focusing and immuno-electrophoresis.

MATERIALS AND METHODS

Human IgG obtained from Kabi AB, Stockholm, Sweden (KIgG; batch no. 78185) was prepared from Cohn fraction II + III. After precipitation with alcohol in the cold, chromatography on DEAE Sephadex was carried out, followed by freeze-drying. The total protein content was 95%, of which 98% was IgG[15].

Human IgG, obtained from the Centraal Laboratorium van de Bloedtransfusiedienst, Amsterdam, The Netherlands (CIgG) was prepared from Cohn fraction II + III. It was precipitated with alcohol in the cold and then freeze-dried[15]. The total protein content was 74% (as determined in our laboratory).

The proteins were characterized by HPLC (High Performance Liquid Chromatography) using a TSK-Gel SW 3000 column. Solutions (2.0 $g\ l^{-1}$) were made in elution buffer [Tris(hydroxymethyl)-aminomethane in 0.05 M H_2SO_4; pH 7.0] and eluted at a rate of 1.0 ml $min.^{-1}$. Iso-electric focusing was performed on both IgG-samples using the method described by Righetti and Drysdale[16]. Immuno-electrophoresis was carried out following the method of Grabar[17].

Polystyrene (PS) latex was obtained from Serva (Dow), [lot no. 41932; specific surface area 15 $m^2\ g^{-1}$; polystyrene content 10% (w/w); density 1.0 $g\ l^{-1}$; diameter latex particles 399 nm according to SEM]. Before use the latex was dialysed against PBS (Phosphate Buffered Saline; 0.01 M $NaH_2PO_4 \cdot 2H_2O$; 0.15 M NaCl; pH 7.35): polystyrene content after dialysis was 8% (w/w).

Samples for the adsorption experiments were prepared starting with protein solutions in PBS. To these solutions appropriate amounts of PS-latex were added. The samples (protein concentration 0.1–7.0 $g\ l^{-1}$) were then mixed and placed in a thermostatted water bath at 37°C. After an adsorption time of 3 h the latex particles were removed by filtration (0.2 μm red rim filter Schleicher and Schüll, type FP 030/3). Adsorbed amounts of protein were determined by measuring the (decrease of) protein concentration in the filtrate using u.v.-spectroscopy at $\lambda = 280$ nm (solution-depletion technique[18]).

RESULTS

Characterization

Figure 1 shows HPLC-chromatograms for both IgG-

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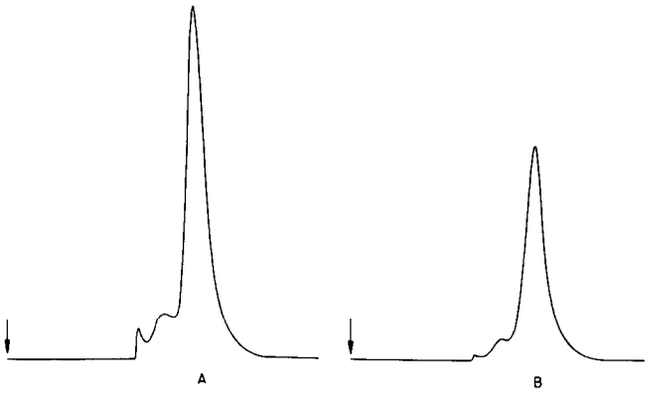

Figure 1 HPLC-chromatograms of KIgG (A) and CIgG (B) by refractive index detection at 20°C; 100 µl, 2.0 g l^{-1} injected at the point indicated by the arrow. Elution volumes of main fractions: (A) 6.83 ml; (B) 6.57 ml

preparations. It can be observed that KIgG contains more high-molecular weight components than CIgG.

Figure 2 shows the results of the iso-electric focusing experiments with KIgG and CIgG. CIgG contains protein components with iso-electric points (I.E.P.) in the low-pH range (5.2 < pH < 7.0), whereas KIgG does not contain any components with an I.E.P. < pH 7.0. Immuno-electrophoresis results do not show differences between the two preparations.

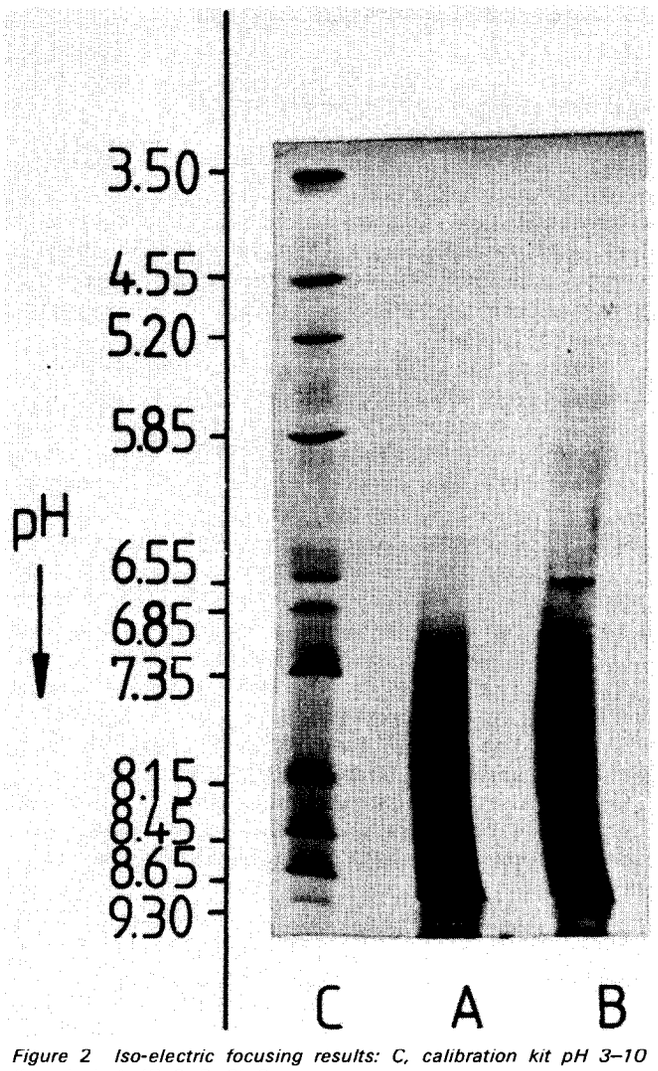

Figure 2 Iso-electric focusing results: C, calibration kit pH 3–10 (Hoechst); A, KIgG; B, CIgG

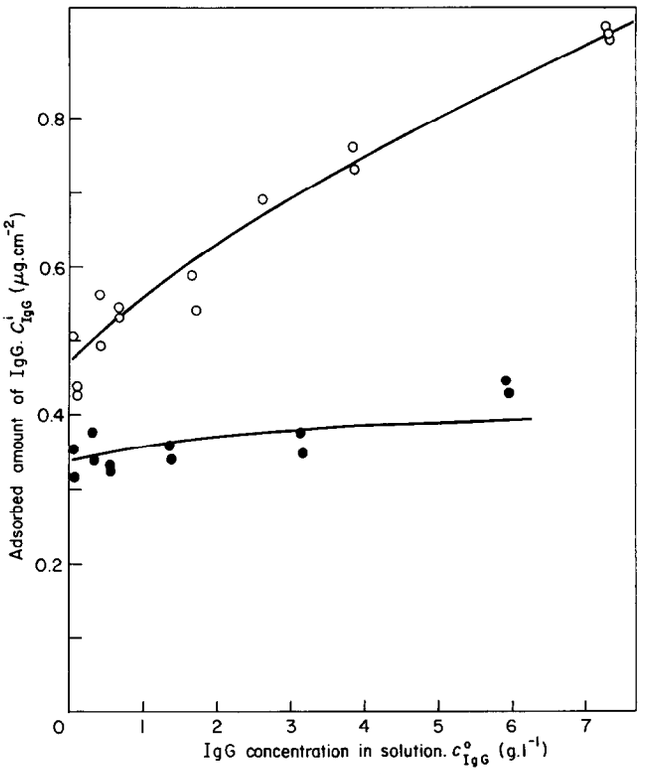

Figure 3 Adsorption isotherms for KIgG (○) and CIgG (●) on to polystyrene latex: adsorbed amount of IgG (c^i_{IgG}) versus IgG-concentration in solution (c^o_{IgG})

Adsorption experiments

Figure 3 shows the adsorption isotherms for KIgG and CIgG, respectively. Adsorption kinetics (data not incorporated in this paper) showed that an adsorption time of 3 h was sufficient for this system to reach equilibrium.

The adsorption isotherm for CIgG reaches a plateau value at low solution concentrations (0.4 µg cm^{-2} at 0.1 g l^{-1}), whereas the isotherm for KIgG does not reach a plateau at all within the concentration range studied. The adsorption isotherm appears to be typical for high-affinity adsorption in both samples.

DISCUSSION

IgG forms a rather heterogeneous group of proteins and therefore different preparation techniques in principle can yield different IgG-compositions. The results given above show that commercial preparations of IgG may differ, with respect to their composition, even when the immuno-electrophoresis patterns are identical. In general IgG components have iso-electric points at pH ≥ 5.2 but, unlike CIgG, KIgG does not contain components in the low-pH range (pH < 7.0). This is probably due to the ion-exchange chromatography procedure used in the preparation of KIgG.

Although both preparations show a high-affinity type adsorption isotherm, the CIgG-isotherm reaches a plateau value at low solution concentrations (0.1 g l^{-1}) whereas the KIgG-isotherm has not reached a plateau even at 7.0 g l^{-1}. The observed differences in adsorption behaviour can only be explained in terms of differences in protein composition between the two preparations.

Our results emphasize the necessity for protein adsorption experiments to be accompanied by a thorough characterization of the protein(s) used.

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