Letter to the editor

Cleaning procedure for protein G affinity columns

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Abstract

An efficient method for cleaning protein G affinity columns is described in which impurities are removed effectively with a combination of 4 mol/l urea and 0.1 mol/l sodium hydroxide. These cleaning conditions have only a minor deleterious effect on the binding capacity of the column and no effect on its selectivity for IgG. This cleaning procedure should prolong the life of costly affinity columns used in the purification of immunoglobulin G (IgG). © 2000 Published by Elsevier Science B.V. All rights reserved.

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Dear Editors,

Protein G affinity perfusion chromatography is a very important tool in the isolation of monoclonal antibodies from different tissues (Bjorck and Kronvall, 1984). Protein G can selectively bind to IgG from a much wider range of mammalian species than protein A (Akerstrom and Bjorek, 1986). However, the adsorbents are subject to contamination by lipids, proteins and nucleic acids from samples, such as serum, ascites and cell culture media. Sodium hydroxide is a particularly effective agent for the removal of these contaminants. Although the protein A polymer matrix is exceptionally stable and capable of withstanding treatment with 1 mol/l sodium hydroxide, the situation is different in the case of protein G (Fulton et al., 1991). This ligand is

denatured under these harsh chemical conditions, and hydrolysis of the peptide chain may occur (Anspach et al., 1995). This may reduce purification efficiency, lower sorbent capacity and provide sites for non-specific protein interactions due to the increased presence of denatured ligand and ligand leakage. For these reasons extremely basic conditions are not recommended by the manufacturer (PerSeptive Biosystems) for cleaning protein G affinity columns.

Three cleaning solutions were used in this study, i.e., 0.1 mol/l sodium hydroxide, 4 mol/l urea in 0.1 mol/l sodium hydroxide and 0.5 mol/l sodium hydroxide. The contaminated column was filled with cleaning solution and then maintained at room temperature for 60 min in the case of both 0.1 mol/l sodium hydroxide and 4 mol/l urea in 0.1 mol/l sodium hydroxide. In the case of 0.5 mol/l sodium hydroxide, the periods of exposure were 20, 10 or 5 min. When 0.1 mol/l sodium hydroxide was used it did not improve the chromatographic behaviour of

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IgG on the column, even after three cleaning operations. In contrast, 4 mol/l urea combined with 0.1 mol/l sodium hydroxide did restore the performance of the column, and the operating backpressure decreased to the original value after treatment. The efficiency of this procedure was such that there was no further improvement, even after 14 cleaning cycles with this reagent.

The dynamic capacity of the column was measured by frontal analysis. Fig. 1A shows that after cleaning an untreated protein G column with 4 mol/l

Fig. 1. (A) Capacity of protein G affinity perfusion column for IgG after cleaning with 4 mol/l urea in 0.1 mol/l NaOH. The capacity was measured by injection of 6 mg IgG in 2 ml loading buffer at a flow-rate of 0.2 ml/min for frontal analysis, followed by elution with elution buffer at a flow-rate of 1.0 ml/min. (B) Recovery of column capacity after cleaning. The measurement of capacity was conducted as described in (A). After cleaning, the column was stored at 5°C, and re-equilibrated with loading buffer. The capacity was then measured after different periods of storage.

urea in 0.1 mol/l sodium hydroxide, the capacity for IgG decreased and was 90 and 85% of the original on the next two operating occasions. But a further decline of capacity was not observed after each of the next 13 successive cleaning cycles. This may be due to variations in the stability of different protein G molecules linked to the matrix. Thus, after the first two cleaning cycles, protein G molecules characterized by unstable linkages were cleaved from the matrix. Consequently the capacity of the column was decreased by 15%; but, after that, the ligand was not affected by the cleaning, and the capacity remained constant.

The purity of IgG fractions collected from the affinity column was analyzed by SDS-PAGE. The IgG fractions were still of high purity after 15 cleaning cycles. The constant retention time and the high purity of the IgG fraction suggests that the affinity column retains the same selectivity and affinity for IgG as the original matrix.

The column efficiency gradually recovered with increasing storage times after cleaning, and remained constant after the fourth day. As shown in Fig. 1B, the immunosorbent capacities during the first 4 days were successively increased in comparison with that on the first day, then remained constant after the fourth day. This suggests that full recovery of the efficiency and capacity of the column requires a sufficient period of equilibration in appropriate buffer after cleaning. This may be due to partial unfolding of the secondary structure of protein G molecules in the cleaning solution. When the solution is replaced by loading buffer, the unfolded protein G will refold to the natural structure (Karplus and Sali, 1995).

After the exposure of the column to 0.5 mol/l sodium hydroxide for 5, 10 or 20 min, the chromatographic behaviour of the IgG was improved. However, the capacity of the column decreased by 10, 20 and 35%, respectively, compared to the original column. Therefore, such harsh conditions were not used for cleaning the affinity column in this study.

A POROS G/M (30×2.1 mm I.D.) column has been used for 2 years without significant loss of its separation efficiency and capacity following cleaning with 4 mol/l urea in 0.1 mol/l sodium hydroxide. This simple procedure may be applicable to several similar matrices used in the purification of many

different antibodies on both an analytical and a preparative scale.

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