Preparative and analytical chromatography of immunoglobulin G from whey using monolithic protein G affinity columns and stabilization of the isolates

Jernej Oberčkal[1,2], Marko Kete[2], Maja Zupančič Justin[2], Bojana Bogovič Matijašić[1]

[1]University of Ljubljana, Biotechnical faculty, Department of animal science, Institute od dairy science and probiotics
[2]Arhel Ltd.

INTRODUCTION

Immunoglobulin G (IgG) is the major immunoglobulin in bovine milk. It reaches concentrations of up to 0.7 g/L in mature milk and up to 32-212 g/L in colostrum (Gapper et al., 2017). In the process of casein coagulation, part of the IgG seems to precipitate or bind to caseins. IgG concentrations in whey are thus lower than in milk. Immunoglobulins in milk protect both the mammary gland and the calf from environmental pathogens and toxins when the calf's immune system is not yet developed. The antimicrobial activity of IgGs is utilized also in food supplements and animal feed. IgG molecules are subject to aggregation due to their delicate and branched 3D structure and are therefore unstable during prolonged storage or freezing. In commercial products, manufacturers add stabilizers (e.g., glycerol, NaCl, sucrose, and BSA) to products with declared IgG activity. The problem of aggregation is particularly prominent in the production of biologic drugs, which must be free of aggregates due to intravenous administration. Park et al. (2013) found that the addition of 4% mannitol and 2% sucrose at pH 5 prevented IgG aggregation and prolonged the stability of monoclonal IgG formulations.

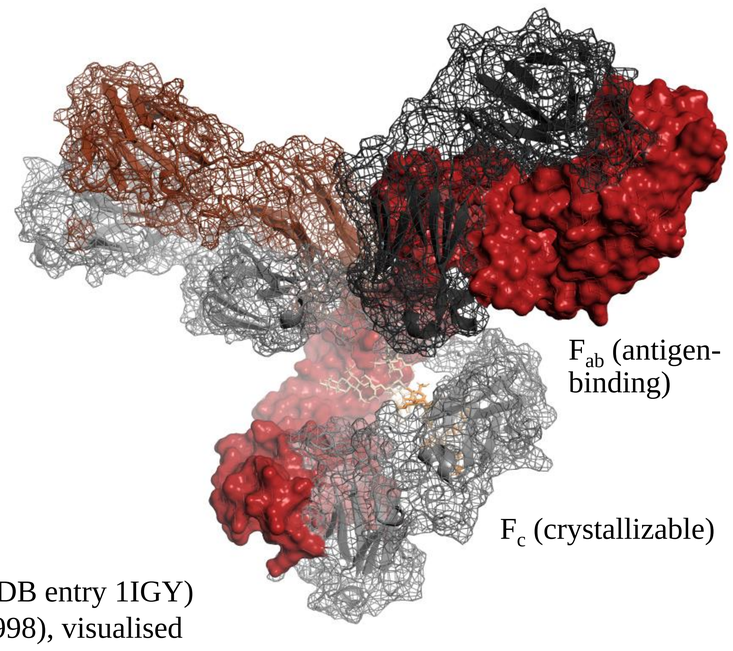

Figure 1:
Mouse IgG1 (PDB entry 1IGY) (Harris et al., 1998), visualised with Pymol.

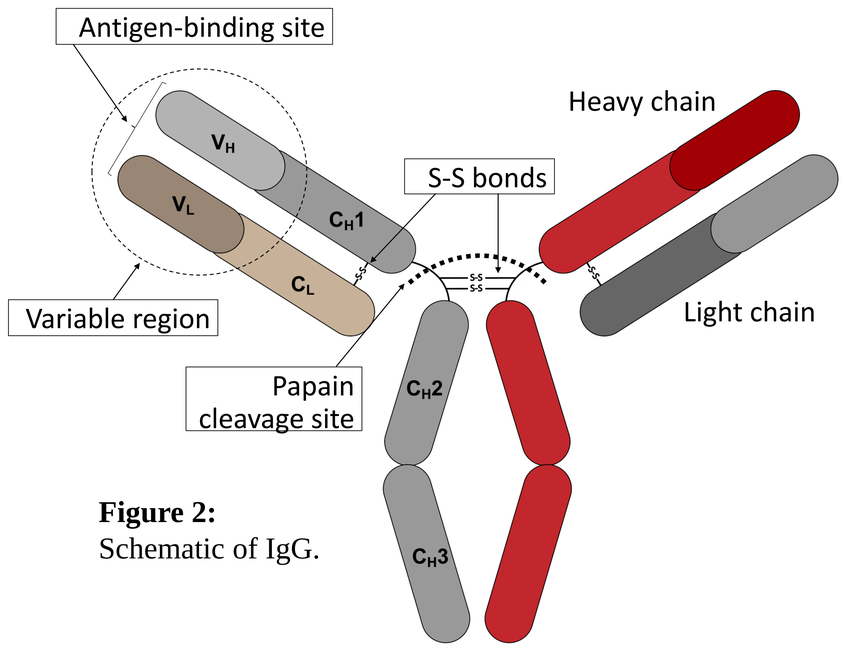

Figure 2:
Schematic of IgG.

METHODS

In several experiments we fractionated 100 mL – 200 mL of acid or sweet whey, from which lactoferrin was previously removed, with protein G affinity chromatography using a monolithic CIMmultus™-r protein G-1 column. After washing the column, we eluted the bound IgG with glycine at pH 2. We adjusted the pH of the collected fractions and added sodium phosphate and sodium chloride (PBS), sodium acetate or a solution of sodium chloride, mannitol and sucrose. We then stored these fractions under different conditions (lyophilised, liquid at 4° C or room temperature or subjecting them to several cycles of freezing and thawing) and then visually inspected the samples for presence of aggregates and measured the concentration of IgG.
We analysed the concentration of IgG in the whey and elution fractions with analytical protein G-affinity chromatography using the 0,1 mL CIMac™-r protein G-0,1 column. We developed the method to be selective for IgG in samples of whey. During scanning of the most abundant whey proteins, we found that lactoferrin also bound to the protein G column. We greatly reduced this binding by washing the column with 300 or 400 mM NaCl after injecting the sample. This NaCl wash did not affect the binding of IgG at the analytical level. At the preparative level, however, we found that addition of NaCl inhibited the release of IgG from the column, resulting in a much wider peak. But since our whey was without lactoferrin, addition of NaCl was not required.

RESULTS AND DISCUSSION

Figure 3 (A, B) depicts the SDS-PAGE analysis of the acid whey (A) and sweet whey (B) and the corresponding flow-through fractions (designated BT), elutions and the commercial IgG from Sigma-Aldrich. The analysis of elutions both from acid (FT) and sweet whey (FTS) shows that we isolated very pure fractions of IgG.
Figure 3 (C, D) shows the concentration of IgG on different days in fractions with or without added mannitol and with different storage methods. The measured IgG concentration was more dependent on the day of measurement than on the difference between the samples, indicating that the analytical method was not reliable. However, we were able to compare the concentrations of different samples on the same day of measurement. Similar IgG concentrations were determined in both types of whey: 0.089 g / L in acid and 0.085 g / L in sweet whey. No IgG was observed in the flow-through fraction when loading whey, therefore the column retained all of IgG (Fig. 3A). IgG breakthrough was achieved prior to application of 105 mL of FTS, presumably at a similar volume as in acid whey (at 80 mL acid whey per mL of column, our previous results) given that the two whey types had a similar IgG concentration. The column was saturated with IgG at 157 to 186 mL of FTS. When applying a larger volume, the concentration in the unbound fraction no longer increased and did not exceed the concentration in the applied FTS, as observed with some other proteins and other CIMmultus™ columns (our previous results). The addition of mannitol, sucrose and NaCl (and in the case of FTS isolation also the addition of acetate buffer only) stabilized milk IgG during lyophilization or freezing and thawing to such an extent that there was no clear difference in concentration between different sample treatments. Only glycine or the combination of glycine and PBS did not stabilize IgG during lyophilization or freezing and thawing: IgG partially precipitated.

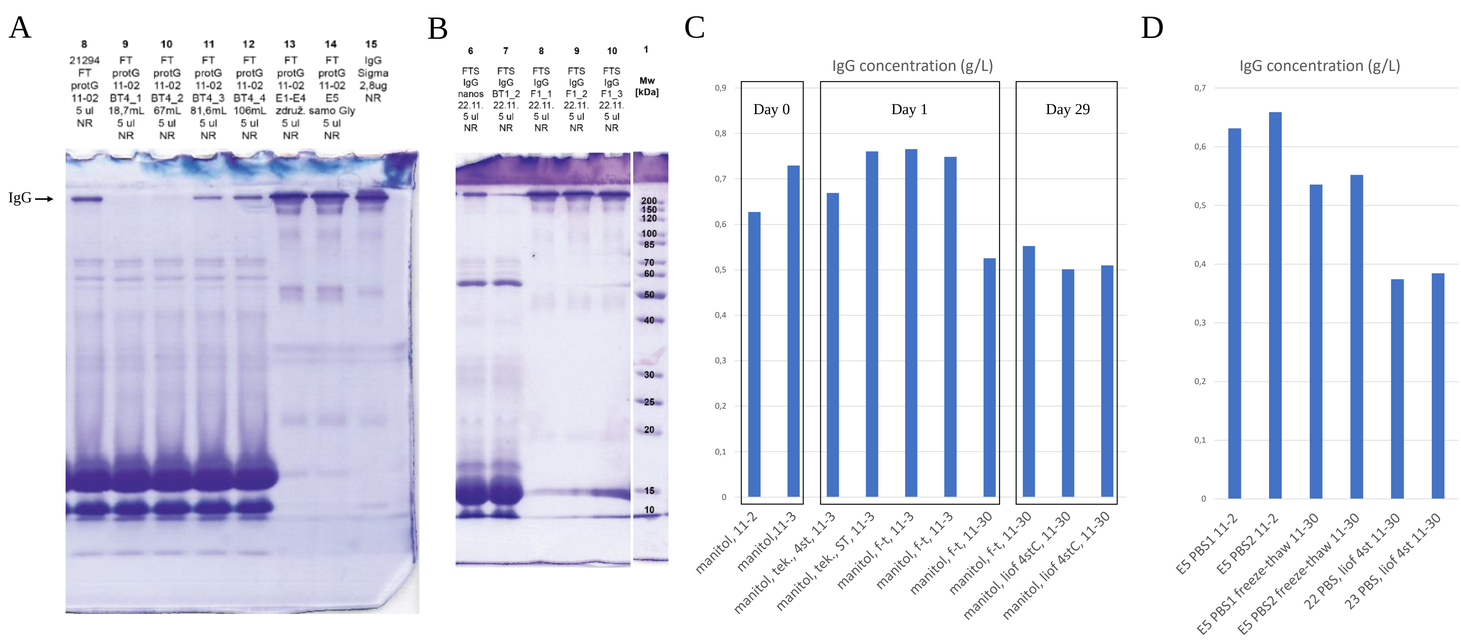

Figure 3:
A, B: Analysis of input whey (A–FT, B–FTS), unbound fractions, elutions, and commercial IgG (Sigma-Aldrich) by SDS-PAGE. C: IgG concentration in mannitol fractions. D: IgG concentration in mannitol-free fractions.

CONCLUSIONS

We successfully isolated very pure IgG from both types of depleted whey. The CIMmultus™-r protein G-1 column is less suitable for isolation of substances from sweet whey due to clogging (presumably by hydrophobic impurities). No such clogging was observed when isolating from acid whey. The addition of mannitol and sucrose or only acetate buffer protects milk IgG from aggregation and is therefore useful if we want to prepare a formulation with active IgG.

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