

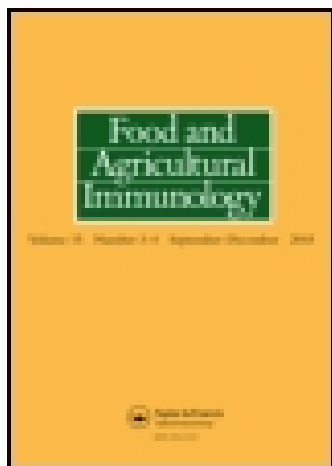
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Production of Monoclonal Antibodies for Detection of Antigens in Both Their Native and Denatured States

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Different immunization procedures were tested to find methods to enhance the proportion of monoclonal antibodies reacting equally well with native and denatured proteins for use in food analysis. Antibodies to soybean trypsin inhibitor with the desired characteristics were obtained by immunization with the denatured protein. Antibodies to ovomucoid were obtained by long-term immunization. The monoclonal antibodies to ovomucoid were of the IgM class.

Keywords: Food analyses, denatured components, monoclonal antibodies, immunization procedures

INTRODUCTION

Antibodies used for detection of specific ingredients in food should be able to detect these ingredients irrespective of the preceding treatments of the analyzed food. The common approach is to use antibodies towards components that are considered heat stable. However, heat stability of proteins is dependent on many parameters which vary in foods, implying that heat stability may be difficult to ensure. Therefore, antibodies recognizing a food component regardless of its state of denaturation would be very useful.

Although most antibodies recognize either the native or the denatured state of an antigen, the authors have tried to find immunization procedures that favour the production of antibodies reacting equally well with the denatured and the native form. Preliminary results for Kunitz soybean trypsin inhibitor (KSTI) and egg-white trypsin inhibitor ovomucoid (OM) are presented in this paper.

KSTI is a moderately stable non-glycosylated protein with two disulphide bridges in 181 residues (Koide & Ikenaka, 1973), whereas OM is heavily glycosylated (25%) and has a high disulphide density with nine disulphide bridges in 186 residues (Kato *et al.*, 1987).

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MATERIALS AND METHODS

KSTI and OM were purchased from Sigma Chemical Co. (St Louis, MO, USA; catalogue no. T-9003 and T-2011 respectively). Monoclonal antibodies (MAbs) were produced according to Bonde *et al.* (1991). KSTI used for immunization was either in the native form or denatured by heating to 95°C for 1 h. Denatured OM was reduced and alkylated with iodoacetamide according to Hirs (1967).

Mice were immunized intra-peritoneally with antigen emulsified in Freund's complete adjuvant and boosted 14–28 days later with antigen emulsified in Freund's incomplete adjuvant. Immunization with OM was continued over a period of 8 months with injections of native OM in Freund's incomplete adjuvant every month.

The resulting MAbs to the native and denatured antigens were tested in an enzyme-linked immunosorbent assay (ELISA), with antigens as solid phase and detection by peroxidase-coupled anti-mouse antibodies from Dako (Glostrup, Denmark). The relative values of the

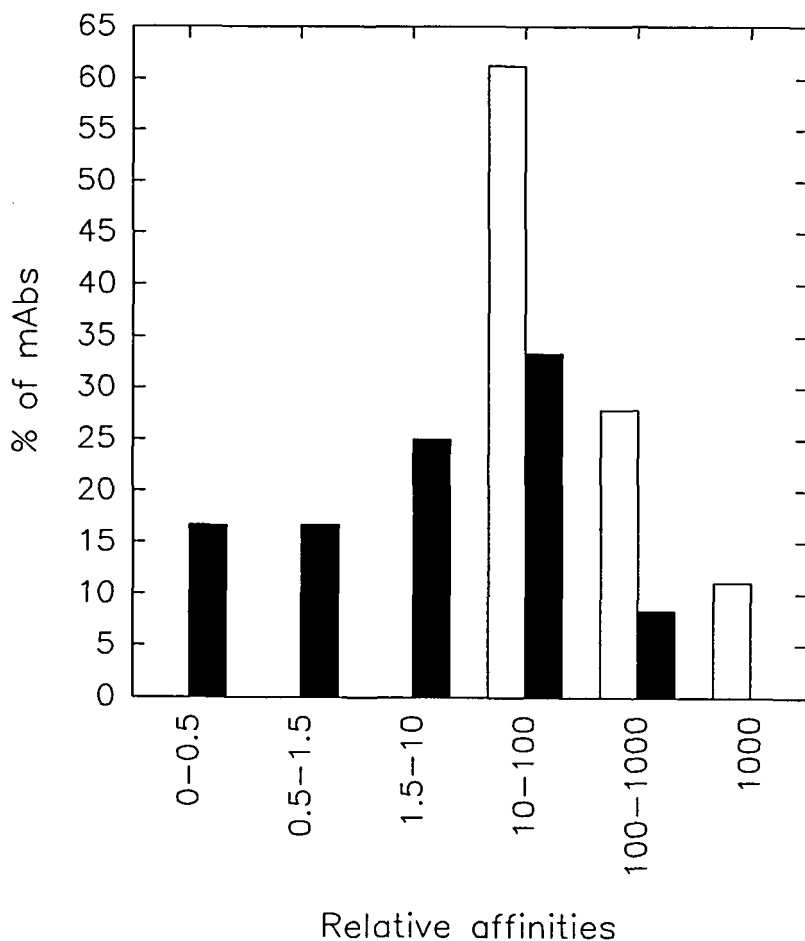


FIG. 1. Relative affinities towards the form of KSTI used for immunization versus the alternative form of KSTI (native or denatured). (□) MAbs from mice immunized with native KSTI ($n = 18$); and (■) MAbs from mice immunized with denatured KSTI ($n = 12$).

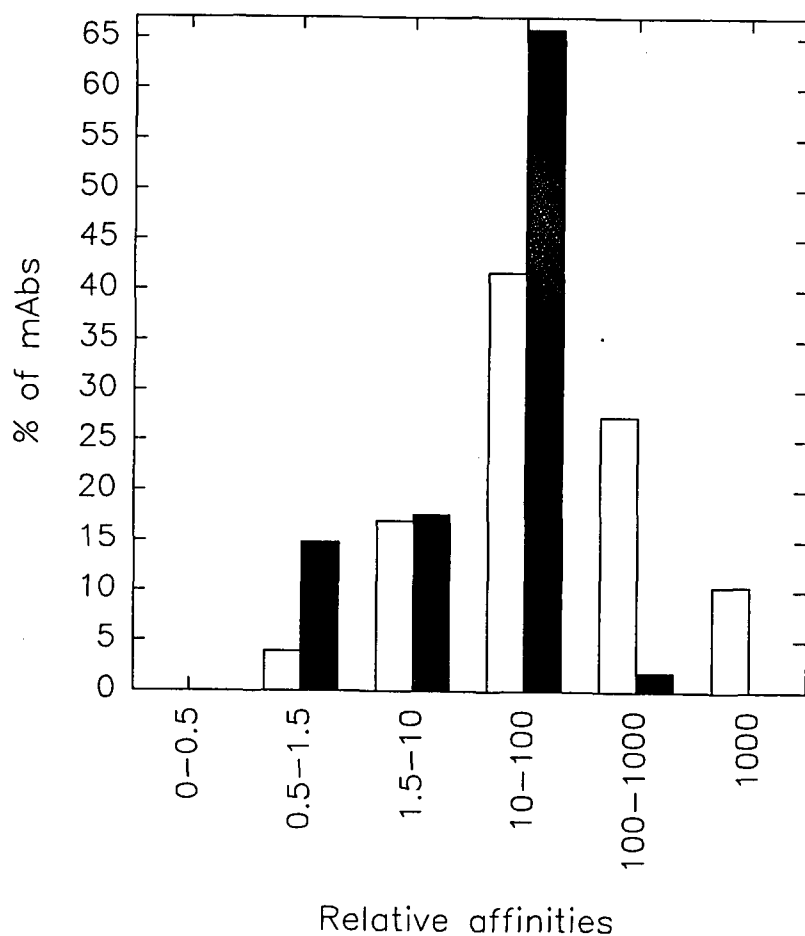


FIG. 2. Relative affinities towards native versus denatured OM. (□) MAb from mice immunized for 1 month ($n = 77$); and (■) MAb from mice immunized for 8 months ($n = 108$).

absorbances were taken to represent the relative binding of the two forms of the proteins. Selected MAb were further investigated by competitive inhibition ELISA.

RESULTS

MAbs towards KSTI were characterized by the affinities towards native and heated KSTI, the two forms of KSTI used for immunization. Figure 1 shows a comparison of the relative affinities of the MAb obtained to the form used for immunization versus the other form. One of the MAb (IgG₂) with equal affinity towards native and heated KSTI was further characterized and also found to react with KSTI denatured by Na₂SO₃ or *N*-acetyl cysteine.

MAbs towards OM were characterized by their relative affinities towards native and denatured OM. Figure 2 shows a comparison of the relative affinities obtained after short-term (1 month) and long-term (8 months) immunization with native OM.

Two MAb reacting with denatured OM were characterized; one from short-term and one from long-term immunization. The MAb from short-term immunization had a very low affinity; the other, MAb 3E5, obtained after 8 months' immunization, is being characterized

further. Apparently it binds to carbohydrate, as binding to native OM as well as denatured OM is inhibited by low molecular weight carbohydrate-containing peptides from OM.

DISCUSSION

The results for KSTI suggest that immunization with this antigen in the denatured state is preferable if the objective is to obtain antibodies reacting equally well with the native and the denatured antigen.

OM is more difficult to denature as only harsh conditions such as cleavage and alkylation prevent refolding. Therefore, antibodies reacting with native OM after immunization with denatured OM were not expected. However, rabbits immunized over 1 year with native OM developed antisera with a high binding capacity to native as well as denatured OM, and the hypothesis that this observation could be used in mice to enhance the probability of obtaining MABs with the desired characteristics was tested. As seen from Figure 2, it does to some extent.

Both of the investigated anti-OM MABs were of the IgM class, suggesting involvement of carbohydrate in the epitope. This was in accordance with the results obtained for the MAb 3E5. Although it may be tempting to raise carbohydrate-binding antibodies, several problems should be considered before systematic attempts are made to obtain such antibodies. One problem is that the same carbohydrate chain, or part of it, may be found in different glycoproteins. Therefore, attempts should be made to obtain antibodies recognizing a combination of carbohydrate and peptide chain, for example by immunization with low molecular weight carbohydrate-containing peptides coupled to carriers. Another problem is the possible modification of labile carbohydrate residues during food processing. This may be examined using carbohydrate-specific antibodies such as MAb 3E5.

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