ABSTRACT

The Characterization of Immobilized Papain and Pepsin on to a Chitosan Support and Their Use in the Fragmentation and Separation of Immunoglobulin G on a Synthetic Affinity Column

Miranda Faryal Williams

A number of supports, both synthetic and naturally occurring have been reported in the literature for the immobilization of enzymes as well as for use as affinity matrices for the purification of immunoglobulins and albumin. However, there are advantages and limitations associated with these supports. To date the degree of selectivity of the affinity gels towards the various classes of immunoglobulins or their fragments have not been fully determined.

The present study reports on the characterization of pepsin and papain covalently immobilized on to a chitosan support and the subsequent use of these immobilized enzymes to digest sheep IgG into its corresponding fragments F(ab)'$_2$, Fab and Fc. These fragments were then applied to an Avid AL ™, affinity column to effect separation.

The proteolytic enzymes pepsin and papain were first covalently immobilized on to a succinylated chitosan support through amide bond formation with carbodiimide which aided in the facile coupling of these enzymes. Optimal conditions of temperature and pH as well as loading capacity and storage stability were investigated for these two immobilized enzyme systems.

The results of these initial studies showed that there was essentially no shift in the pH optimum for the immobilized enzymes when compared to the free enzymes. The pH optimum for immobilized papain ranged between 6.5 - 7.0 and between 1.7 - 2.2 for immobilized pepsin. In addition, an increase in temperature optimum was observed for both immobilized preparations (immobilized papain ranged between 50- 70^o C, immobilized pepsin ranged between 45 – 50^o C) and hence offers a wider operational temperature working range than those of the corresponding free enzyme.

The results also showed that the loading at which maximum coupling efficiency is observed for immobilized pepsin and papain was approximately 30.6 and 27 mg/ g gel respectively. For both enzymes the coupling efficiency was found to be relatively low and to decrease as the amount of protein bound increased. In addition, storage stability studies

showed that immobilized papain retained at least 30% of its initial activity at 4^{o} C for 4 months, while immobilized pepsin retained 35% of its initial activity over three months.

Digestions of immunoglobulin G using both free and immobilized pepsin and papain at pH 4.0 and 6.5 respectively and incubation times up to 72 h were investigated. Products of IgG digestion were analysed by non – reducing PAGE electrophoresis. Immobilized pepsin was found to be effective in digesting IgG yielding the bivalent 84-97 kDa F(ab')$_2$ and 36kDa Fc fragment. Immobilized papain was effective in yielding both the bivalent F(ab')$_2$ and univalent Fab 45- 55 kDa and Fc fragment. However, the times taken to effect digestion were relatively long. Avid AL was found to bind to all three fragments F(ab')$_2$, Fab and Fc in addition to intact IgG.

Finally, a method is described for the affinity chromatographic purification of immunoglobulin M utilizing the affinity support Avid AL™. The results show that Avid AL is capable of binding small quantities of IgM under similar conditions used in the purification of IgG (0.1 M NaH_2PO_4, 1.5 M NaCl, pH 7.4).

Keywords: aza-arenophilic, chitosan, fragmentation, immobilization, immobilized enzymes, immunoglobulins, succinylated chitosan.