



***Recovery of Transforming  
Growth Factor- $\beta$ 2 from  
Whey Growth Factor  
Extract with  
Immunoaffinity  
Techniques***

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## Abstract

Transforming Growth Factor- $\beta$ 2 (TGF- $\beta$ 2) is a polypeptide with a range of biological activities upon mammalian cells. A great deal of research has indicated a number of possible therapeutic applications for TGF- $\beta$ 2 and as a consequence, it has considerable potential commercial value. A biologically active protein fraction, termed Whey Growth Factor Extract (WGFE), can be derived from cheese whey by using cation-exchange chromatography (Francis et al., 1995). This process has been patented and material is now available from a recently completed GMP pilot-scale facility. Many growth factors have been identified in WGFE and TGF- $\beta$ 2 is the most abundant of these. Its purification from WGFE has been demonstrated with size-exclusion gel filtration and reversed-phase HPLC (Rogers et al., 1996). However, this purification was time-consuming and labour-intensive, and therefore not economically feasible at commercial-scale. Immunoaffinity chromatography has potential for reducing purification costs and was considered as an alternative to the standard purification method.

This thesis describes attempts to develop an immunoaffinity process for the commercial-scale purification of TGF- $\beta$ 2 from WGFE. The standard purification method was used to partially purify TGF- $\beta$ 2. However, much lower specific TGF- $\beta$ 2 activities were observed for the final step of the purification than were reported by Rogers et al. (1996). Two separate immunisation strategies were employed to raise a titre against TGF- $\beta$ 2. One group of mice was immunised with partially purified TGF- $\beta$ 2. The second group was immunised with synthetic peptide that corresponds to amino acids 50-75 of TGF- $\beta$ 2. The second strategy successfully raised a specific titre and fusions generated two specific anti-TGF- $\beta$ 2 positive hybridomas. Antibody 5D4 stains TGF- $\beta$ 2 in western immunoblots and localises TGF- $\beta$ 2 in skin but neither of the antibodies inhibited the biological activity of TGF- $\beta$ 2.

Mathematical models of the immunoaffinity process were developed to identify the best antibody and configuration without the need for experiments that explore every possibility. Simple experiments determined the critical antibody properties to facilitate the modelling and rational design of the immunoaffinity system. Antibody was coupled to membrane and Sepharose but neither of these supports demonstrated specific TGF- $\beta$ 2 binding. However, both supports showed considerable non-specific TGF- $\beta$ 2 binding. Interestingly, Sepharose separated TGF- $\beta$ 2 from partially purified material in the absence of antibody. TGF- $\beta$ 2 was

purified with a combination of acid gel filtration, reversed phase HPLC and Sepharose column chromatography. However, the purification method is complex and offers only modest improvements over the method of Rogers et al. (1996). This work highlights some of the difficulties and pitfalls of immunoaffinity chromatography and it is anticipated that knowledge of these could help future workers to optimise this important technique.