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PCR-Cloning into Fusion Expression Vectors for Production of Bioactive Human Tumor Necrosis Factor aplha (rhTNF-α) and other Recombinant Proteins from GMOs

A.A. Kritis, I.S. Pappas, T. Brousali-Tsiftsoglou*, P. Dretaki and A.S. Tsiftsoglou

Laboratory of Pharmacology and Laboratory of Pharmaceutical Technology* Department of Pharmaceutical Sciences, Aristotle University, Thessaloniki, Macedonia, Greece

In the past few years there has been a great advancement in the field of Molecular Biology and Biotechnology and many new techniques have been invented and procedures evolved that allow the scientist to proceed in his research faster and in new fields with greater ease and efficiency.

One such technique that emerged out of the advancement in the field of Molecular Biology is the Polymerase Chain Reaction (PCR). Briefly, this technique allows the specific amplification of a target sequence of known DNA from quantities of less than 1pg to more than 500 μg. Further more new sequences, such as restriction sites can be introduced 5'or 3' of the amplified sequence facilitating thus the cloning procedure to a great extend. We employed this technique to amplify the cDNA encoding for the mature TNF-α protein. At the 3' end we introduced an Xba I restriction site that was used in the subsequent cloning procedure.

From this point on the scientist has a number of protein expression systems to chose from, thanks to the advancement of Biotechnology. There are eukaryotic or prokaryotic systems for protein expression and even animals can be used as live bioreactors. For the expression and purification of rhTNF-α we chose a prokaryotic protein expression and purification system that is cheap, efficient, produces the protein of our interest in high yields and provides means of facilitated purification of the protein produced. This system is the pMAL protein fusion and expression system. Briefly, our gene of interest, the amplified cDNA encoding for the mature TNF-α protein, is cloned in frame at the carboxyl-terminal of the gene encoding for the maltose binding protein (MBP) after a recognition sequence for a specific protease such as Factor Xa. The whole system is under the control of an inducible promoter. The construction is introduced into a bacterial host in our case TB1 and the bacteria are induced for protein production with IPTG. This way a fusion protein MBP-TNF-α is generated. This fusion protein retains the properties of both proteins and the maltose binding moiety of the molecule is used in the purification procedure. The induced bacterial culture is centrifuged, the cells are lysed and the protein extract is passed through an amylose (maltose polymer) column. MBP-TNF-α fusion is retained by the column and eluted with free maltose. Next the fusion protein will be digested with Factor Xa so that rhTNF-α will be released from the fusion protein. The subsequent steps deal with the purification of the rhTNF-α from the MBP and Factor Xa. We employed High Performance Liquid Chromatography (HPLC) using an anion exchange column. In our system rhTNF-α was retained by the column and eluted at about 150 mM NaCl. The purification procedure was reliable with high reproducibility and allowed us to purify rhTNF-α in high yields and to apparent homogeneity. Further more this expression and purification procedure allows rhTNF-α to retain its biological activity. We tested rhTNF-α produced by the described above procedure on WeHI-13Var cells and we found that 50% killing of the cells was effected at concentrations as low as $4x10^{-10}$ g/ml. The data presented here is valuable for formulating rhTNF-a as a potential biopharmaceutical product.