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Letter to the Editor

Comments on "Recovery of active anti TNF-α ScFv through matrix-assisted refolding of bacterial inclusion bodies using CIM monolithic support"

Recently two papers have appeared from our group on related, but distinct research topics that both concern anti tumor necrosis factor-alpha single chain variable fragment (anti TNF-α scFv) [1,2]. The purpose of this letter is to assist the reader by explaining and highlighting the differences in the results in these two reports. In the first of these two reports [1], the goals were to obtain soluble expression of anti TNF-α scFv, to purify soluble anti TNF-α scFv, and to study the biological activity of this agent by using an assay based on TNF alpha induced cytotoxicity inhibition in the human breast cancer cell line MCF-7. The goals of second study instead concerned the refolding and purification of anti TNF-α scFv inclusion bodies, which were again examined for their biological activity regarding cytotoxicity inhibition [2].

The methods used in the first report [1] involved the expression of anti TNF-α scFv in BL-21DE3 cells, followed by purification of the soluble fraction using convective interaction media (CIM) supported immobilized metal-ion affinity chromatography (IMAC) (CIM-IDA-Cu(II)) and ion exchange chromatography using DEAE (CIM-DEAE). In the first step of this purification, the protein was eluted by pH elution and in the second step a salt gradient was employed. In contrast to this, the methods used in the second report [2] involve the solubilization of inclusion bodies, followed by refolding on-column using CIM monoliths contained Ni(II), with eventual elution of the protein being obtained by adding imidazole to the mobile phase.

In both studies, biological activity was analyzed by competitive inhibition of TNF induced cytotoxicity in MCF-7 cells [1,2]. These measurements were carried out separately on the anti TNF-α scFv samples that were obtained in the two reports, but both assays used reagents that consisted of the same cells (MCF-7), positive controls (anti TNF-α monoclonal antibodies) and negative controls. Similar trends were noted in the two assays, but the IC_{50} values that were estimated for the two sets of samples were slightly different. In the case of Ref. [1], the IC_{50} was 8 μg for the soluble fraction of anti TNF-α scFv. In Ref. [2] the IC_{50} was 15 μg for solubilized and refolded anti TNF-α scFv inclusion bodies. These results indicated that both soluble anti TNF-α scFv [1] and solubilized and refolded anti TNF-α scFv inclusion bodies [2] obtained from *Escherichia coli* have comparatively good biological activity.

References

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