details by vibrational spectroscopy that it can represent a valuable contribution to understanding the role and behavior of AGP [4].

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MOLECULAR CLONING, E. COLI EXPRESSION AND PURIFICATION OF SCFV ANTIBODY FRAGMENTS OF DIAGNOSTIC/THERAPEUTIC INTEREST

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Fv fragments are the smallest antibody molecules that still retain the entire antigen-binding site. In single-chain Fv fragments (scFv), variable domains are joined by a flexible linker. Such scFv constructs, which generally retain full binding capacities towards the antigen, are the topic of very active research. First, they are of interest for structural studies because they usually yield crystals diffracting to higher resolution than the corresponding Fab fragments. Second, since they can be expressed in bacteria, they are also well suited for binding, mutagenesis and protein engineering studies [for review, see e.g.1].

In this work we describe molecular cloning, expression, purification and properties of two scFvs of potential diagnostic and immunotherapeutic use, scFv M75 and scFv TU?20.

Monoclonal antibody (mAb) M75 recognizes cell surface protein MN/CA IX strongly associated with several types of human carcinomas [2]. Radioactively labeled humanized scFv M75 could be used for tumor immunodetection and possibly for therapy. Monoclonal antibody TU-20 was raised against beta-III-tubulin, a specific neuronal marker in normal and neoplastic tissues. The antibody TU-20 fragments could thus be useful tools for probing beta-III-tubulin functions in neurons, as well as for immunohistochemical characterization of tumors of neuronal origin [3].

Coding sequences for light (V_L) and heavy (V_H) variable domains were obtained from total RNA, isolated from hybridoma cells, by RT-PCR using suitable pairs of primers. Single-chain Fv genes in the form V_L-linker-V_H-myc tag were then assembled and cloned into T7 promoter-driven expression plasmids. Bacterial strain *E. coli* BL21(DE3) was used for protein expression. The recombinant protein products accumulated in inclusion bodies as insoluble aggregates. To obtain refolded active proteins from inclusion bodies, several protocols were adapted to find optimal conditions for each scFv species.

Purification procedure comprising several conventional chromatography steps (ionex chromatography, gel filtration) yielded scFv proteins in amount and purity necessary for functional characterization. While scFv TU-20 in ELISA assay exhibits specificity and binding activity comparable to parental mAb TU-20, in case of scFv M75, ELISA assay was negative. The reasons for the lack of binding activity are under investigation.

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PROTEINS AND THEIR CRYSTALS

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Non-membrane proteins such as the pokeweed antiviral protein from Phytolacca acinosa (PAP-Saci) and the tryptophan (W)-repressor binding protein A (WrbA) and also membrane protein, the five-chlorophyll reaction center of photosystem II from Pisum sativum, have been crystallized in our laboratory.

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