Characterization of the 3H3 Antibody Fragment by Scattering Methods

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Antibodies feature highly specific binding and are therefore used for therapeutical purposes or for medical imaging. Besides the use of the whole antibodies the application of antibody fragments is widely spread and provides further advantages [1,2]. Antibody fragments can be produced more easily, show better pharmaco-kinetic profiles due to their size and can be used in drug targeting and as building blocks in biochemical engineering [3,4].

Single chain variable fragments (scFv) represent one type of antibody fragments. They are artificially created antibody fragments generated by fusion of the immunoglobulin (Ig) variable regions. ScFv are able to form dimers, so called diabodies, with two antigen binding sites [5]. In this work a 3H3 diabody expressed in P. pastoris should be used for crystallization after several steps of chromatographic purification. The molecular mass of the desired diabody is 53.9 kDa but the results of the size exclusion chromatography (SEC) showed products with a molecular mass of approximately 35 kDa. Up to now all attempts to crystallize this diabody for crystallographic investigations have failed and the presence of dimers could not be proven.

For a further clarification dynamic (DLS) and small-angle X-ray scattering (SAXS) were used which allow the investigation of the protein in solution and thus in native environment. Measurements of samples with different protein concentrations were performed. The comparison of the normalized scattering intensities showed no concentration related dependency. The radius of gyration and the molecular mass were evaluated using different approaches: absolute calibration, comparison with a standard, indirect-transformation method. The DLS was used for the determination of the hydrodynamic radius of the protein. In a further step rigid body refinement against the scattering was performed [6]. All together the results lead to the assumption that 3H3 actually is a diabody.

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