

ABSTRACT

Human carbonic anhydrases are metalloenzymes that are involved in many physiological processes in the body, but also play an important role in the pathogenesis of numerous diseases. Under regular conditions, expression of carbonic anhydrase IX (CAIX) is very limited, unlike that of other 14 human carbonic anhydrase isozymes. But in hypoxic tumors this enzyme is highly overexpressed on the cell surface. For this reason, this enzyme represents a good target for therapy and diagnosis of tumors and thus various anti-CAIX monoclonal antibodies and specific inhibitors are being developed.

In this work we investigated the possibility to use fluorescent polymer conjugate carrying a CAIX specific inhibitor for fluorescent labeling of tumor cells. Specific binding of polymer conjugate to different cell lines was investigated by flow cytometry and confocal microscopy. Binding properties of the polymer conjugate was compared to CAIX specific monoclonal antibody M75 and its single-chain fragment scFv M75.

Ability of the polymer conjugate to inhibit CAIX enzyme activity was also investigated. For these experiments, recombinant protein CAII was prepared and purified, which was also used for protein crystallization. Tests of inhibitory activities allowed to identify novel inhibitors CAIX with better inhibitory properties, which could be used for the preparation of more efficient polymer conjugates. Successful crystallization experiments and subsequent X-ray structure analyses provided structural information about the binding of selected inhibitors to the CAII active site. Based on these structures, it was possible to design a suitable position for connection to the copolymer for the future development of the second generation polymer conjugates.