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Detection of galectin-3 by novel peptidic photoprobes

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Abstract—Photoprobes were prepared with specificity for binding, labeling, and visualizing galectin-3 in a mixture of proteins. The probes were derived from a galectin-3 binding 15-mer peptide sequence in which a benzophenone photolabel was incorporated at the N-terminus and in another case as a phenyl alanine replacement in the middle of the sequence. Detection of galectin-3 was possible in *Escherichia coli* lysates that were spiked with various amounts of galectin-3.

The galectins are a group of medically relevant lectins that are raising a great deal of interest.[1,2] Among others, they play roles in processes involving the immune system and also in cancer. Particularly, galectin-3 has been implicated in cancer[3,4] and remarkably it acts via several mechanisms. It is linked to metastasis,[5] apoptosis,[6] and angiogenesis.[7] There are, however, also conflicting data regarding the role of the lectin in cancer.[3] One factor is its location, as galectin-3 can be located extracellularly, where its lectin abilities mediate metastasis. However, its anti-apoptotic properties are exerted mostly in the cytosol through protein–protein interactions and galectin-3 also acts in the nucleus.[6] Part of the confusion may be caused by the limitations of the used detection methods, often Western blotting,[8] thus arriving at incorrect conclusions.[3,9] An accurate, cheap, and easy method of detection of galectin-3 in complex biological samples can thus become an important tool in research but it also has the potential to become a tool for cancer diagnosis and prognosis.[8] Despite the fact that galectin-3 has well-described lectin properties, part of its biological activities derive from its abilities to bind other biomolecules such as RNA and proteins.[8] While we previously developed carbohydrate-based probes for galectin detection,[10] probes based on peptides are in principle equally valid. In fact, we previously reported on short pentapeptide sequences that bound galectins in the millimolar range.[11] Although this affinity is too low to prepare a successful probe, a recent report featured a 15-mer peptide with galectin-3 specificity that exhibited a K_d of 72 nM.[12] This peptide was shown to bind to the carbohydrate recognition domain (CRD) of the protein, did not bind to the related galectins 1 and 4, and was capable of blocking the galectin-3 interaction with a relevant disaccharide: the Thomsen–Friedenreich antigen. These features made the peptide an attractive candidate for the development of a new photoprobe. Photoprobes are increasingly used in proteomics research to capture enzymes for which no irreversible inhibitors exist or for proteins, such as lectins which bind but do not convert their substrate. Such probes can thus capture proteins as a function of their binding activity and were shown to function within a complex sample as was shown for kinases,[13,14] metalloproteases,[15,16] HMG-CoA reductase,[17] and aspartic proteases.[18] We here report on the synthesis of peptidic benzophenone photoprobes, based on the mentioned galectin-3 binding 15-mer peptide and their evaluation in galectin-3 detection experiments in the context of a cell lysate.

The benzophenone moiety was used as the photoreactive group that is to covalently capture the target protein,[19] since this group can easily be introduced into peptides as part of the available Fmoc-4-benzoyl-L-phenylalanine amino acid for solid phase synthesis. Furthermore, an alkyne moiety was introduced at the N-terminus, for the coupling of a fluorescent reporter molecule after the benzophenone moiety has captured the protein. This reporter molecule can be linked to the covalently cap-

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tured and thus labeled protein via 'click' chemistry and allows subsequent visualization in a gel.[20] Such a two-step protocol has advantages such as fewer artefacts due to non-specific effects of bulky labels in the crucial photoreaction step.

Two peptides **1** and **2** (Fig. 1) were synthesized on a TentaGel resin using solid phase Fmoc chemistry, cleaved from the resin, purified by preparative HPLC and analyzed by electrospray mass spectrometry.[21] Peptide **1** contains the benzophenone photoreactive group on the N-terminal part of the sequence while in peptide **2** a phenyl alanine residue in the middle of the sequence was replaced with the benzophenone-containing amino acid. In both cases 4-pentynoic acid was attached to the N-terminus.

To evaluate the probes, they were first exposed to galectin-3 (50 nM) alone at 18 μM probe concentration, irradiated at 366 nm for 30 min, and after introduction of the reporter molecule **3** by 'click' chemistry, visualized on a gel using a fluorescence scanner. The appropriate lanes of Figure 2 clearly show the band corresponding to the galectin at the correct molecular weight. Both probes were able to visualize galectin-3. Without light irradiation or in the absence of probe no fluorescence band was observed.

The next step was to evaluate the probes in the context of a complex protein mixture. To this end an *Escherichia coli* cell lysate was used which was spiked with various amounts of galectin-3. Following the protocol, a fluorescent scan was made of the gel (Fig. 3a). These images

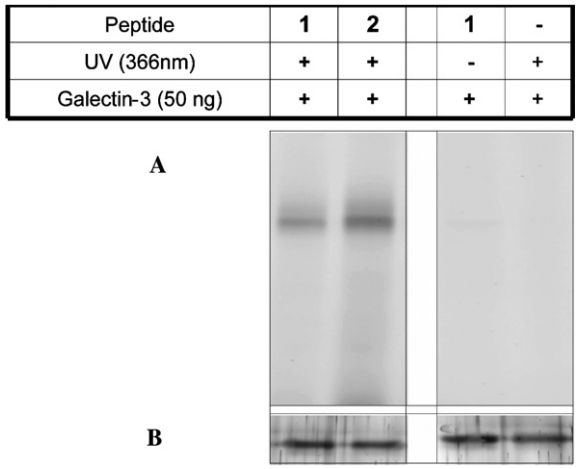

Figure 2. (A) Labeling of galectin-3 (50 ng) with peptides **1** and **2** (18 μM). Without UV irradiation or without adding peptide no fluorescent signal is detected. (B) Silver stain of the same gel as a control on the amount of galectin-3 present in each lane.

showed a clearly visible band of galectin-3 along with a few other proteins.[22] Probe **1** gave the clearest images but also with probe **2** detection was possible. The detection took place in a concentration-dependent manner since the staining intensity correlated to the concentration of galectin-3 that was used.

In summary, two new peptidic photoprobes were successfully applied in the detection of galectin-3, via a two-step protocol. Detection was demonstrated in a concentration-dependent fashion in a spiked bacterial

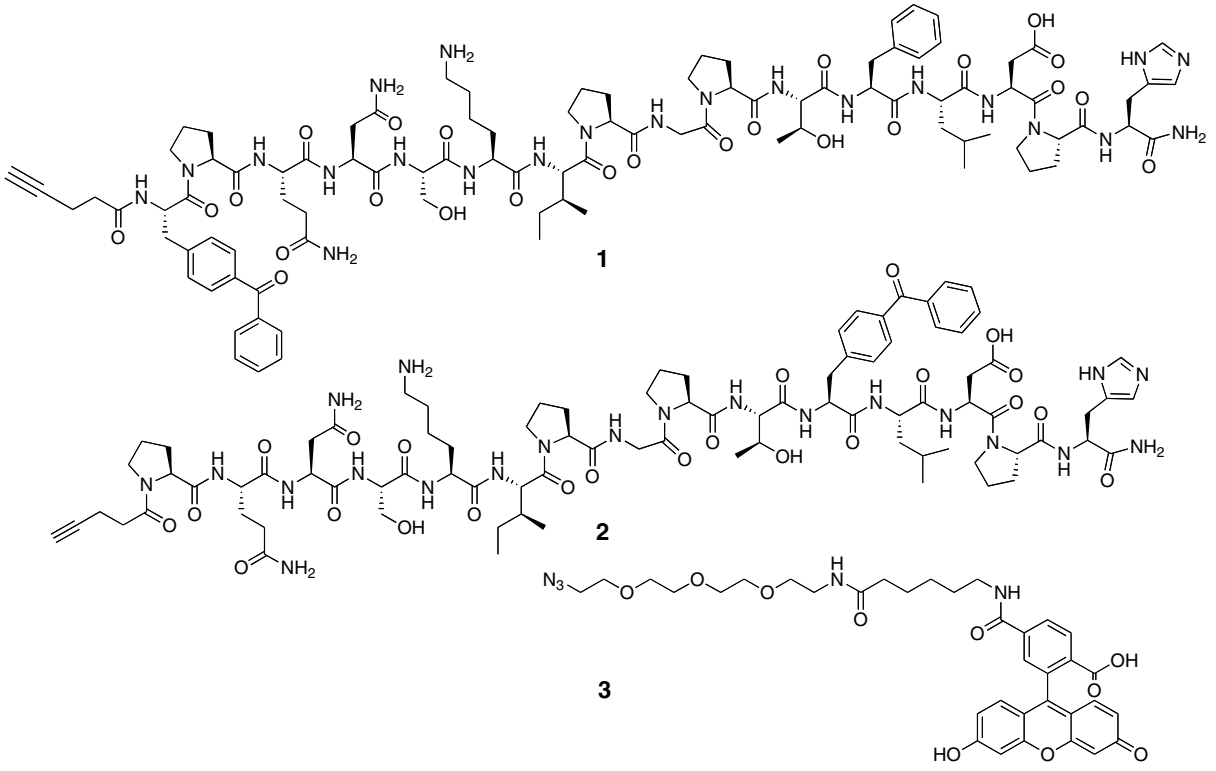

Figure 1. Structure of peptidic photoprobes **1** and **2** and reporter molecule **3**.

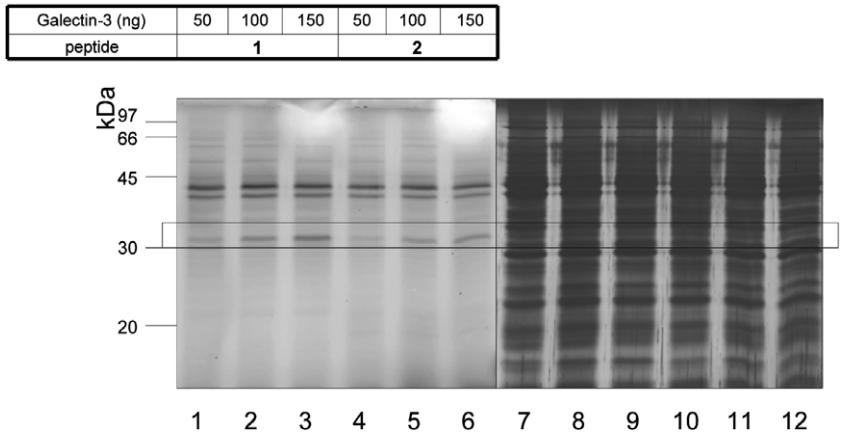

Figure 3. Labeling of different amounts of galectin-3 in the presence of 4 μg of a bacterial protein extract. The box indicates galectin-3. Peptide **1** is slightly more efficient in labeling galectin-3 than peptide **2** (probe concn 18 μM). Lanes 1–6: fluorescent image. Lanes 7–12: silver staining of the same gel, lanes 1–6, respectively.

lysate, thus representing an important step towards application as a research tool or even a tool for cancer diagnosis and prognosis.[23]

Acknowledgment

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22. The two most pronounced bands between 40 and 44 kDa were identified by mass spectrometry as phosphoglycerate kinase (lower band) and elongation factor EF-Tu (upper band). These are known to be present in high abundance in *E. coli*, according to Franzén, B.; Becker, S.; Mikkola, R.; Tidblad, K.; Tjernberg, A.; Birnbaum, S. *Electrophoresis* **1999**, *20*, 790; Karlin, S.; Mrázek, J.; Cambell, A.; Kaiser, D. *J. Bacteriol.* **2001**, *183*, 5025.
23. According to Iurisci, I.; Tinari, N.; Natoli, C.; Angelucci, D.; Cianchetti, E.; Iacobelli, S. *Clin. Cancer. Res.* **2000**, *6*, 1389, median galectin-3 levels of healthy individuals were 62 ng/mL, whereas for patients with metastatic gastrointestinal cancer this number was 320 ng/mL (with examples of up to 950 ng/mL). With commonly used enrichment steps these levels should be detectable with the peptidic probes.