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A Selective Activity-Based Probe for the Papain Family Cysteine Protease Dipeptidyl Peptidase I/Cathepsin C

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Dipeptidyl peptidase I (DPPI), also known as cathepsin C, is a lysosomal cysteine hydrolase expressed in most mammalian tissues. In myeloid cells and cytotoxic lymphocytes, it is found in secretory granules where it is solely responsible for NH_2-prodipeptide removal and catalytic activation of several leukocyte-derived serine proteases, including mast cell chymases (mMCP4 and mMCP5), neutrophil elastase (NE), cathepsin G, and cytotoxic lymphocyte-derived granzymes A and B.[1−3] Humans harboring loss of function mutations in the DPPI gene manifest several immune defense deficits implicated in pre-pubertal aggressive peridontitis,[4] Haim−Monk[5] and Papillon−Lefevre syndrome.[6] Other studies have indicated a significant role for DPPI in thrombin regulation, fibroenctin turnover,[7] angiogenesis,[8] acute experimental arthritis,[3] cytotoxic lymphocyte-mediated apoptosis, and host immune defense.[1] Therefore, DPPI represents a tractable target for therapeutic intervention in a diverse array of human disease states.

The study of protease function requires tools that allow enzyme activity to be monitored in the context of complex biological environments. Such functional studies of protease regulation have been greatly facilitated by activity-based probes (ABPs), small molecules that covalently label the active site of an enzyme through an activity-dependent chemical reaction.[9] The choice of a reactive functional group or "warhead" coupled with the structure of the primary binding scaffold dictates probe selectivity.[10−13] An example of a general probe that targets clan CA cysteine proteases is DCG-04[12b] (Figure 1). This and other class-specific probes, however, require biochemical separation methods, such as electrophoresis or liquid chromatography, in order to resolve individual activities within a complex proteome.

While substantial progress has been made in activity-based protein profiling using an ever increasing variety of ABPs, relatively few probes have been described that target a single distinct enzyme target.[14] Such highly selective ABPs are extremely valuable for monitoring specific protease activities without the need for separation methods. These probes have particular value as tools for direct imaging of protease activity in live cells or whole organisms. In this communication, we report a novel ABP that can be used to selectively label DPPI in complex proteomes as well as in intact cells. This probe provides an important new tool for the study of DPPI in a number of human pathologies.

The distinctive amino dipeptidase activity of DPPI makes it unique within the family of CA clan papain family proteases. We therefore reasoned that development of probes that contain a free α-amino dipeptide should prove highly selective for this target. To minimize disturbance of the dipeptide recognition element, we needed to place the tagging moiety in the prime side binding region of the probe. These structural requirements pose a number of restrictions on the type of warhead that can be applied in the design of the ABP. The reactive epoxysuccinate used in a number of papain

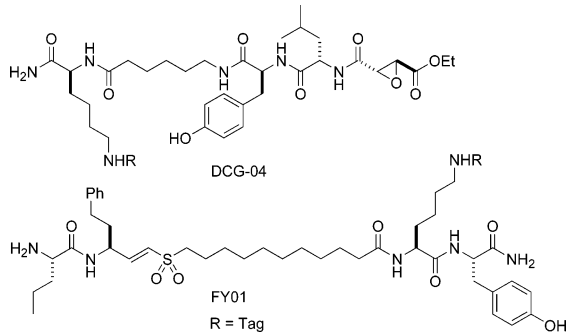

Figure 1. The general papain activity-based probe DCG-04 and the selective DPPI probe FY01.

family specific probes cannot be used because the warhead is attached to the N-terminus of the peptide scaffold. The more versatile acyloxymethyl ketones,[13] which allow the incorporation of dipeptide free amines, are not suitable since their prime site element acts as a leaving group upon covalent binding to the target enzyme. For these reasons, we turned our attention to the vinyl sulfone moiety.[15] This reactive group has been characterized in the past as a covalent inhibitor of cysteine proteases, and recent synthetic advances allow attachment of small molecule fragments on both sides of this scaffold by construction of the vinyl sulfone moiety using Horner−Emmons chemistry on solid support.[16]

On the basis of the reported kinetic data and stability of several vinyl sulfone inhibitors,[17] we decided to use the norvaline−homophenylalanine dipeptide free amine as the most optimal recognition sequence for use in vivo. The resulting probe FY01 (Figure 1) was synthesized on solid support and isolated in 9% yield after HPLC purification. An extended alkyl spacer was chosen because the use of smaller, aromatic moieties in this region increased potency of the probe toward cathepsin B (data not shown). We also chose to include a lysine−tyrosine tagging element to allow introduction of both fluorophores and an ^{125}I radioisotope tag.

To test selectivity, initial experiments with BODIPY-TMR tagged DCG-04[12d] and FY01 were conducted in a homogenate of rat liver, which contains a number of previously characterized cathepsin proteases.[12c] The DCG-04 probe (Figure 2, left panel) labels all four of the main rat liver cathepsins at a concentration of 10 μM. To confirm the selectivity of labeling, the general papain family protease inhibitor JPM-OEt[12a,18] was used to pretreat extracts prior to probe labeling. Treatment of the homogenates with FY01 (right panel) results in selective labeling of DPPI at a much lower concentration than observed for DCG-04. This increased sensitivity of FY01 allows labeling at a concentration where virtually no background is detected.

To verify the identity of the protease labeled by FY01, spleen tissue lysates from wild-type and DPPI-deficient mice[1] were used

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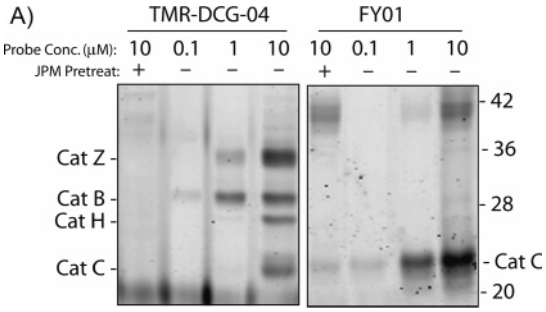

Figure 2. FY01 is a selective label of cathepsin C. The general probe DCG-04 labels multiple cathepsin activities in rat liver homogenates (left panel), whereas FY01 is selective for DPPI (right panel). Labeling of all specific protease targets can be blocked by pretreatment with 50 μM of the papain family protease inhibitor JPM-OEt (JPM pretreat).

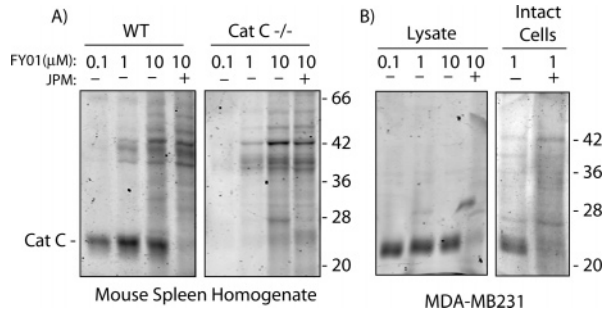

Figure 3. (A) FY01 labeling of total spleen extracts from wild-type and DPPI-deficient mice. Note the labeling of DPPI in wild-type spleen tissue that is completely absent in the corresponding DPPI-deficient tissues. Selective labeling of DPPI is observed at the lowest probe concentration, while some nonselective labeling of proteins occurs at high probe concentrations. (B) FY01 specifically labels DPPI in intact breast cancer cells. Lysates of the breast cancer cell-line MDA-MB231 were treated with increasing concentrations of FY01 as indicated (left panel). Live MDA-MB-231 cells were incubated with 1 μM FY01 after pretreatment with JPM-OEt or vehicle control (right panel). Specific labeling of DPPI is observed in both extracts and intact cells and can be blocked by pretreatment with JPM-OEt.

for similar profiling experiments (Figure 3A). Intense labeling of an approximate 23 kD band in the wild-type tissues is absent in the knock-out tissues, confirming the reactivity of FY01 toward DPPI. Although higher concentrations of probe gave rise to background labeling of a number of higher molecular weight proteins, the potency of FY01 allows its use at a concentration where selective labeling is observed.

To determine if FY01 was capable of specific labeling of DPPI in live cells, we treated cultures of the human breast cancer cell-line MDA-MB-231. Initial labeling of cell lysates at several concentrations gave clean labeling of the 23 kD DPPI band (Figure 3B, left). Incubation of intact cells with FY01 showed specific labeling of the same 23 kD band. This result confirms that the probe is freely cell permeable and provides highly selective labeling of the desired target protease in live cells.

In conclusion, the data presented here show that the novel probe FY01 is a selective reagent for DPPI and can efficiently label its target in an activity-dependent manner in both crude tissue extracts and intact cells. Therefore, this ABP seems to be an ideal tool to study DPPI in vivo. Imaging applications are the subject of future investigations and will be reported in due course.

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Supporting Information Available: Synthetic details on the preparation of FY01 and other experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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