ELSEVIER

monitor

MOLECULES

The use of combinatorial chemistry libraries for the discovery of biologically active substances

Selective substrates of proteinase 3 produced through the use of combinatorial chemistry

Human neutrophil elastase, cathepsin G and proteinase 3 are synthesised and stored in the azurophilic granules of polymorphonuclear neutrophils. These members of the serine protease family are involved in several physiological processes, such as degradation of the components of the extracellular matrix (e.g. elastin and collagen) [1] and control of cytokine activity (e.g. tumour necrosis factor-α [TNF-α]) [2]. Recent work has revealed that only proteinase 3 is involved in the regulation of cell proliferation [3]. Thus, the 30-kDa proteinase 3 is an excellent target for drug discovery. Owing to high sequence identity with human neutrophil elastase and nearly identical primary specificity with human neutrophil elastase [1], it is, however, currently difficult to discriminate between the effects of those two proteinases. Published efforts to date that have attempted to provide some discrimination have relied on the use of fluorogenic substrates to investigate the prime site of these enzymes, where the main structural difference between proteinase 3 and elastase is located [4]. On the basis of crystal structures of both enzymes, it is clear that the proteinase 3 substrate binding site is much more polar than in the case of elastase. The main differences between proteinase 3 and human neutrophil elastase occur in the active site, with the former preferring a combination of charged/polar residues in the first three prime positions and the latter preferring small aliphatic nonpolar amino acid residues in this region of the protease [5]. To advance knowledge of how one might better discriminate between these proteinases, recent work has disclosed efforts on the synthesis and kinetic evaluation of a tripeptide library with general formula ABZ-X_3-X_2-X_1-ANB-NH_2 that was designed to select fluorogenic substrates of proteinase 3. In this general formula, ABZ is an amino benzoic acid, ANB-NH_2 is an amide of 5-amino-2-nitrobenzoic acid, X_2 and X_3 are the set of all proteinogenic amino acids except Cys, and X_1 can be the amino acids Ala, Abu, Val, Nva, Ser, Thr, Ile, Leu and Nle [6]. In this work, the ABZ and ANB-NH_2 at the N- and C-termini of peptides synthesised served as donor and acceptor, respectively, and such substituents allow the fluorescence resonance energy transfer (FRET) within the peptides synthesised. The process of deconvolution of this library against proteinase 3 and human neutrophil elastase then revealed a sequence that is selectively and efficiently hydrolysed by proteinase 3. Thus, to determine the influence of modification of C-terminal chromophore, peptides with ANB and *para*-nitroanilide were synthesised and their substrate proteinase 3 and human neutrophil elastase activity was evaluated [6]. After solid-phase synthesis of library members, followed by deconvolution, the isolated peptides were assayed for the ability to inhibit enzymatic hydrolysis in the presence of either proteinase 3 or human neutrophil elastase. Among peptides present in the library, the most active fluorogenic substrates of proteinase 3 were selected and a determination of proteinase 3 substrate specificity and human neutrophil elastase substrate activity of selected library members was then carried out. One of the peptides with the highest specificity constant for proteinase 3 ($k_{cat}/K_M = 189 \times 10^3\ M^{-1}\ s^{-1}$) was ABZ-Tyr-Tyr-Abu-ANB-NH_2. This compound, therefore, represents the first example of a short peptide that undergoes selective proteolysis by proteinase 3 and displays no significant hydrolysis in the presence of human neutrophil elastase. This work warrants further investigation to broaden the SAR and understanding in this series, and moves towards compounds with more drug-like properties commensurate with oral delivery.

Assessing inhibitory potency of galectin ligands identified from combinatorial (glyco)peptide libraries using surface plasmon resonance spectroscopy

The lectin family of galectins are implicated in causing undesired biological interactions *in vivo* [7]. Owing to the involvement of galectins in intercellular interactions, such as angiogenesis and tumour spread and invasion, human galectins 1 and 3 represent attractive targets for drug design. Interactions mediated by the lectin–glycan recognition, therefore, have attracted interest for inhibitor development. Recently, library screening approaches to detect sugar-based inhibitors have been undertaken [8]. Galectins themselves are involved in carbohydrate–protein interactions as well as protein–protein interactions, thus (glyco)peptide ligands may prove versatile targets for the development of synthetic inhibitors. Glycopeptides may be useful targets at modulating galectin binding because the carbohydrate moiety provides the specificity of the interaction, whereas the peptide backbone may actively participate in binding by, for example, hydrogen bonding or hydrophobic interactions. Glycopeptides can be generated in a library format via a combinatorial approach, and several glycopeptide libraries have been generated via this strategy to date. This work has allowed the identification of effective mimics [9]. Recent work has described the systematic examination of the capacity of human galectins 1 and 3-binding (glyco)peptides to interfere with lectin–glycan interaction

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2009.02.004

by surface plasmon resonance [10]. Several solid-phase libraries were synthesised on PEGA1900 resin (0.2 mmol/g loading, 300–500 μM) and Wang resin (0.68 mmol/g loading, 200–400 mesh, pre-functionalised with a Rink amide linker; NovaBiochem, Läufelfingen, Switzerland).

Compounds were synthesised by the split-and-mix methodology and screened at room temperature. The beads (approximately 25 mg, approximately 10,500 in number) were incubated with the fluorescently labelled galectin (at a concentration of 1.5 μM). The most fluorescent beads were manually collected and analysed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). To define the inhibitory potency of a selection of re-synthesised (glyco)peptides systematically, a surface plasmon resonance-based inhibition assay with immobilised asialofetuin was used. For active mixtures, re-synthesis as singletons followed and the singletons were then re-screened. From this effort, several active compounds were obtained. One of the most active glycopeptides was (iPIF(Lac-)TRR) which possessed an IC_{50} of 25 μM for interference with galectin binding. Here, P is the amino acid Pro, I is Ile, F is Phe, T is Thr, R is Arg. Lac is the sugar moiety Lac(β1-*O*)-coupled to Thr. This work is of interest because the compounds synthesised via this library chemistry have revealed beneficial effects of presenting the carbohydrate ligand in distinct hexapeptide contexts. Further work in this area is warranted, in particular structural analysis of these complexes may disclose peptide–galectin contacts beyond the central lactose moiety.

1 Rao, N.V. *et al.* (1991) Characterization of proteinase-3 (PR-3), a neutrophil serine proteinase: structural and functional properties. *J. Biol. Chem.* 266, 9540–9548

2 Bedard, M. *et al.* (1993) Release of interleukin-8, interleukin-6, and colony-stimulating factors by upper airway epithelial cells: implications for cystic fibrosis. *Am. J. Respir. Cell Mol. Biol.* 9, 455–462

3 Desmedt, C. *et al.* (2006) Impact of cyclins E, neutrophil elastase, and proteinase 3 expression levels on clinical outcome in primary breast cancer patients. *Int. J. Cancer* 119, 2539–2545

4 Korkmaz, B. *et al.* (2004) Design and use of highly specific substrates of neutrophil elastase and proteinase 3. *Am. J. Respir. Cell Mol. Biol.* 30, 801–807

5 Brubaker, M.J. *et al.* (1992) Human neutrophil proteinase 3: mapping of the substrate binding site using peptidylthiobenzyl esters. *Biochem. Biophys. Res. Commun.* 188, 1318–1324

6 Wysocka, M. *et al.* (2008) Design of selective substrates of proteinase 3 using combinatorial chemistry methods. *Anal. Biochem.* 378, 208–215

7 Gabius, H.-J. (2006) Cell surface glycans: the why and how of their functionality as biochemical signals in lectin-mediated information transfer. *Crit. Rev. Immunol.* 26, 43–79

8 André, S. *et al.* (2006) Glycosyldisulfides from dynamic combinatorial libraries as *O*-glycoside mimetics for plant and endogenous lectins: their reactivities in solid-phase and cell assays and conformational analysis by molecular dynamics simulations. *Bioorg. Med. Chem.* 14, 6314–6326

9 Maljaars, C.E.P. *et al.* (2006) Identification and affinity determination of lectin ligands from combinatorial *O*-, *S*-, and *N*-glycopeptide libraries. *J. Comb. Chem.* 8, 812–819

10 Maljaars, C.E.P. *et al.* (2008) Assessing the inhibitory potency of galectin ligands identified from combinatorial (glyco)peptide libraries using surface plasmon resonance spectroscopy. *Anal. Biochem.* 378, 190–196

Paul Edwards
mepauledwards@fsmail.net