European Journal of Medicinal Chemistry 117 (2016) 187–196

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

A structural insight into the P1–S1 binding mode of diaminoethylphosphonic and phosphinic acids, selective inhibitors of alanine aminopeptidases

Ewelina Węglarz-Tomczak [a], Łukasz Berlicki [a], Małgorzata Pawełczak [b], Bogusław Nocek [c], Andrzej Joachimiak [c], Artur Mucha [a, *]

[a] Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland
[b] Institute of Chemistry, University of Opole, Oleska 48, 45-052 Opole, Poland
[c] Midwest Center for Structural Genomics and Structural Biology Center, Biosciences Division, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois 60439, USA

ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form
4 April 2016
Accepted 6 April 2016
Available online 9 April 2016

Keywords:
Metalloaminopeptidases
Aminopeptidase N
Neisseria meningitidis
Phosphonic and phosphinic acids
APN-inhibitor complex structures
S1 binding mode

ABSTRACT

N′-substituted 1,2-diaminoethylphosphonic acids and 1,2-diaminoethylphosphinic dipeptides were explored to unveil the structural context of the unexpected selectivity of these inhibitors of M1 alanine aminopeptidases (APNs) versus M17 leucine aminopeptidase (LAP). The diaminophosphonic acids were obtained via aziridines in an improved synthetic procedure that was further expanded for the phosphinic pseudodipeptide system. The inhibitory activity, measured for three M1 and one M17 metalloaminopeptidases of different sources (bacterial, human and porcine), revealed several potent compounds (e.g., $K_i = 65$ nM of **1u** for *Hs*APN). Two structures of an M1 representative (APN from *Neisseria meningitidis*) in complex with *N*-benzyl-1,2-diaminoethylphosphonic acid and *N*-cyclohexyl-1,2-diaminoethylphosphonic acid were determined by the X-ray crystallography. The analysis of these structures and the models of the phosphonic acid complexes of the human ortholog provided an insight into the role of the additional amino group and the hydrophobic substituents of the ligands within the S1 active site region.

1. Introduction

A detailed substrate fingerprint study [1] has recently revealed a broad substrate tolerance of the S1 pocket of the M1 alanine metalloaminopeptidase from *Neisseria meningitidis* (*Nm*APN) – a gram-negative diplococcus bacterium that is considered the major causative agent of meningitis and other meningococcal diseases [2–4]. Fluorogenic substrates, derived from unnatural α-amino acids that comprise bulky hydrophobic P1 side-chain residues bearing a fragment of a basic character, were ranked among the most favored ligands. A similar hydrophobic and base-oriented P1 specificity profile was observed for mammalian (human and porcine) [5] and protozoan (*Plasmodium falciparum* [6] and *Eimeria tenella* [7]) alanine aminopeptidases, the abundantly spread metallohydrolases of multiple functions and medical implications [8–10]. The privileged features of the amino acid substrates can be readily translated into the structure of potential inhibitors. The α-aminoalkylphosphonic acids provide an opportunity to install the preferred P1 substituents on the N–C–P scaffold and are commonly recognized as transition state analogue inhibitors of zinc metalloaminopeptidases [11]. However, the insertion of an additional heteroatom-based group into the substituent structure is a separate and not trivial task, in particular to be performed in a parallel manner. One such convenient modification is aziridinephosphonate ring opening to yield N′-substituted 1,2-diaminoethylphosphonic acids, which was originally proposed to provide inhibitors of metalloaminopeptidases from the porcine kidney [12]. The compounds contain an extra β-amino group that modifies the character of the P1 substituent to basic. Indeed, several compounds were found to be good inhibitors of mono-zinc alanyl aminopeptidase and discriminate versus two zinc atom-containing leucine aminopeptidase (LAP), for which they exhibited poor or no inhibition [12]. This was a quite unique observation, as the structural fragment $H_2N–C–PO_2$ typically provides much more effective

---

[*] Corresponding author.
E-mail address: artur.mucha@pwr.edu.pl (A. Mucha).

http://dx.doi.org/10.1016/j.ejmech.2016.04.018
0223-5234/

complexation systems for the two zinc ions in LAP than for the single one in APNs [11,13]. Apparently, the additional β-amino group does not allow convenient P1–S1 side-chain docking (hydrophobic residues are strongly preferred) and distorts the overall binding mode to this particular aminopeptidase. The precise reasons for the good affinity to the porcine APN remain elusive. For *Nm*APN, we recently postulated that an additional heteroatom group located in the proximity of the side chain is able to enhance interactions with the glutamate-rich anionic site that is formed by Glu117, Glu260 and Glu316 and typically dedicated to the binding the N-terminus of peptidic substrates (Fig. 1, panel **A**) [1].

As N′-substituted 1,2-diaminoethylphosphonic acids (**1**) are also promising lead compounds to obtain specific and potent inhibitors of alanyl aminopeptidase by the P1′ elongation to phosphinic dipeptides (**2**, Fig. 1, panel **B**), we have decided to revisit the issue. An extensive series of novel compounds was obtained in an improved synthetic approach. Their inhibitory activity was tested towards four enzymes: *Nm*APN, human (*Hs*APN) and porcine APN (*Ss*APN), and LAP. Finally, the crystal structures of two inhibitor complexes (*Nm*APN-**1h**, and *Nm*APN-**1n**) were determined by X-ray crystallography and compared against modeled *Nm*APN-inhibitor and *Hs*APN-inhibitor complexes, providing structural insight of the ligand–protein interactions and inhibition modes. The potential of the P1′ extension was evaluated for selected N′ substituents.

2. Results and discussion

2.1. Synthesis of 1,2-diaminoethylphosphonic acids

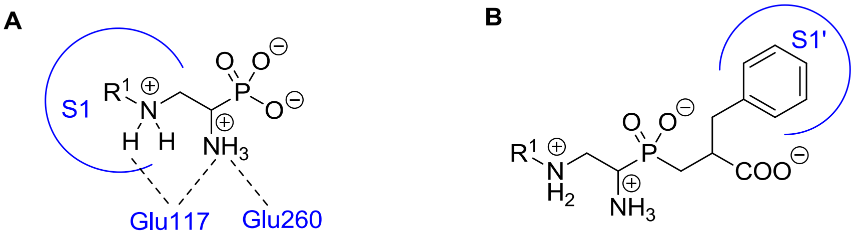
The α,β-unsaturated system of dialkyl vinylphosphonates **3** (Scheme 1) is the usual substrate for the synthesis of 1,2-diaminoethyl derivatives **1** in a pathway via aziridinephosphonates. In the classical Gabriel-Cromwell method, bromine is readily added to the unsaturated bond to produce 1,2-dibromophosphonate esters **4** [14]. Then, consecutive eliminations of HBr with gaseous and liquid ammonia yield the heterocyclic system **5** in the historical literature method (Scheme 1, pathway **A**) [14]. Nucleophilic substitution with a primary or secondary amine enables the aziridine ring opening [15]. However, the approach is characterized by at least two shortcomings. First, ammonia is the reagent of choice to obtain the non-substituted aziridine, but the elimination is capricious and not pure [14,16]. Second, for not fully clear reasons, esters of aziridinephosphonate **5** are very resistant to the nucleophilic attack of an amine and ring opening. As a consequence, they demand hydrolysis to free acids **6** prior to substitution [15,16]. These complications were associated with problematic purification, as a careful ion-exchange chromatography was applied after each of those steps.

N-Tosylaziridines are considered much more susceptible to nucleophilic substitution than their non-modified counterparts. Accordingly, our first alternative approach involved the verification of *N*-tosylaziridinephosphonates **7** as convenient intermediates (Scheme 1, pathway **B**). Indeed, we managed to open a tosylated phosphonate diester with benzylamine in a simple and clean manner. However, obtaining such a material (**7**) by nitrene-mediated aziridination appears to be far more problematic. Contrary to the literature data, different versions of both metal and non-metal assisted oxidative additions of Chloramine T (*N*-chlorotosylamide sodium salt) to the double bond of **3** are ineffective [17–19]. Extensive optimization of the reaction conditions with other tosyl precursors and hypervalent iodobenzene-based reagents [20–22] finally led to the desired products under copper(I) catalysis [21], but in a very poor yield (<10% after column chromatography). Although the subsequent substitution to compounds **8** looked promising, we decided to abandon the approach.

In such circumstances, the elimination option was reconsidered, but ammonia was replaced by Et_3N (the first stage) and subsequently by a benzylamine derivative (the second step) [23]. The use of benzyl or α-methylbenzylamine gave N-substituted aziridinephosphones **9** in a reasonable yield (Scheme 1, pathway **C**). The benzylamine derivatives were chosen as they could be readily hydrogenated to free aziridine **5**. Diphenylmethylamine (benzhydrylamine) did not work to the same extent, apparently because of the decreased nucleophilicity and steric hindrance of the amino group. Products **9** were easily purified by flash chromatography on silica. The subsequent catalytic hydrogenation was performed in standard conditions prior to the ring opening with selected amines. The two-step procedure appeared more practical to obtain **5** than the direct use of ammonia.

An excess of the reacting amine, harsh reaction conditions and the use of a water medium in place of an organic solvent ensured the substitution without the prerequisite hydrolysis of the ester groups. As a matter of fact, both transformations proceeded simultaneously, and the 1,2-diaminoethylphosphonate products were in the form of ammonium salts of phosphonate monoesters **10** and preparatively separated after HPLC chromatography to afford **11**. Apparently, the monohydrolysis occurring in basic aqueous conditions sufficiently increased the susceptibility of the aziridine to substitution for it to be opened without requiring exhaustive hydrolysis. Different primary and secondary structurally diversified (linear, branched, cyclic and benzyl, including heteroatom-substituted, listed in Scheme 1 and Table 1) amines reacted readily. In the cases of difunctional amines, the high excess of the nucleophilic component ensured mono-substitution, including evident regioselectivity. For example, the aromatic amino function of 4-aminobenzylamine remained non-modified, as evidenced in products **11r**.

Acidic conditions, applied to remove the remaining alkyl ester



1, R^1 = alkyl, cycloalkyl, arylalkyl

2

Fig. 1. Structure of N′-substituted 1,2-diaminoethylphosphonic acids (**1**) and 1,2-diaminoethylphosphinic pseudodipeptides (**2**) and the presumed role of the amino groups in binding to *Nm*APN.