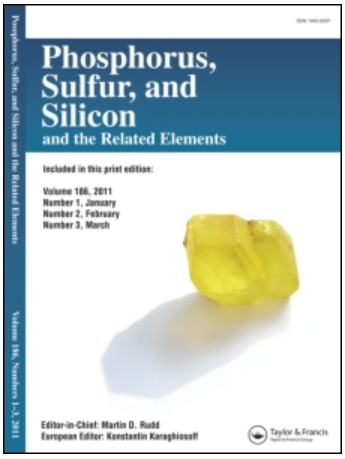


Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

X-Ray Crystallographic Analyses of Human α-Thrombin Complexed to Peptidyl Aminophosphonates: Evidence of a Binding Mechanism

Donovan Green; Emmanuel Skordalakes; Michael F. Scully; Christopher Goodwin; Sandrine Merette; Andrew Burd; Vijay V. Kakkar; John Deadman

To cite this Article Green, Donovan , Skordalakes, Emmanuel , Scully, Michael F. , Goodwin, Christopher , Merette, Sandrine , Burd, Andrew , Kakkar, Vijay V. and Deadman, John(1999) 'X-Ray Crystallographic Analyses of Human α-Thrombin Complexed to Peptidyl Aminophosphonates: Evidence of a Binding Mechanism', Phosphorus, Sulfur, and Silicon and the Related Elements, 144: 1, 545 — 548
To link to this Article: DOI: 10.1080/10426509908546302
URL: http://dx.doi.org/10.1080/10426509908546302

Phosphorus, Sulfur and Silicon, 1999, Vol. 144-146, pp. 545-548
Reprints available directly from the publisher
Photocopying permitted by license only

X-Ray Crystallographic Analyses of Human α-Thrombin Complexed to Peptidyl Aminophosphonates: Evidence of a Binding Mechanism

DONOVAN GREEN, EMMANUEL SKORDALAKES, MICHAEL F. SCULLY, CHRISTOPHER GOODWIN, SANDRINE MERETTE, ANDREW BURD, VIJAY V. KAKKAR and JOHN DEADMAN

Thrombosis Research Institute, Emmanuel Kaye Building, Manresa Road, Chelsea, London SW3 6LR, U.K.

We sought to study *O,O*-Diphenyl dipeptidylaminophosphonates and their interaction with the coagulation protease, human α-thrombin. The 'fibrinogen-like' recognition sequence D-Phe-Pro-Arg, is a template of high affinity for thrombin. This was modified by replacing the 'P1' Arg by a neutral side chain, and the unnatural amino acid diphenylalanine was used in place of the 'P3' Phe. The tripeptides of general formula D-Dpa-Pro-NHCHR^{P}(OPh)$_2$ are highly selective for thrombin amongst other serine proteases; and, more importantly for the design of an efficacious anti-thrombotic agent, show particularly low activity toward other coagulation serine proteases of the cascade. However the kinetics of thrombin inhibition were seen to be dependent on the compounds' structure. To explain this we have studied compounds (1), where R is pentyl and compound (2) where R is (3-methoxy)propyl, for which the kinetics of inhibition were analysed as competitive (2 μM) and slow, tight-binding (final K_i 20nM) respectively. Analysis of the X-Ray crystal structure of these compounds complexed to human α-thrombin, unusually shows no covalent bond formed between the phosphorus nucleus and the serine 195 in the catalytic site of the enzyme. These observations are at variance to the proposed mechanism of action of other phosphorus based serine protease inhibitors.

Keywords: thrombin; dipeptidylaminophosphonates; anti-thrombotic agents

INTRODUCTION

When blood comes into contact with the wall of a chronically or acutely damaged blood vessel, the 'trypsin-like' multifunctional serine protease thrombin (the penultimate enzyme in the coagulation cascade), catalyses the conversion of fibrinogen to fibrin via specific proteolysis of peptide bonds involving the carboxyl group of arginine in the substrate. Here Arg 16 (Aα-chain) and Arg 14 (Bβ-chain) are attacked to liberate fibrin and fibrinopeptides A and B respectively[1]. The formation of fibrin stimulates the conversion of factor XIII to factor XIIIa (by

thrombin)[2], to cross link and stabilise the fibrin polymer, which matures to a haemostatic plug[3]. In addition to exerting a positive feedback on its own production by activating coagulation factors V and VIII, thrombin also activates platelets and endothelial cells: to cause morphological change, aggregation and the synthesis and release of thromboxane A2, platelet activating factor (PAF) and lysosomal enzymes. The tendency toward blood clot formation as a consequence of thrombin generation, is a dominant feature of disorders such as ischaemic heart disease, stroke, peripheral vascular disease, deep venous thrombosis and pulmonary embolism. These conditions can severly cripple or kill an individual[4]. The therapeutic control of thrombin is therefore still a major pharmaceutical target since current drug therapies to ameliorate these conditions are clinically inconsistent and often elicit debilitating side effects.

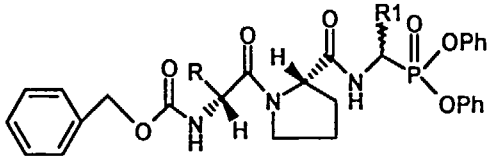
DISCUSSION

There are numerous examples in the literature of antithrombotic agents that have been modelled on the fibrinogen recognition sequence, 'Phe-Pro-Arg', where the amide bond (P1-P1') of the substrate fibrinogen has been replaced by a number of reactive functionalities (such as chloromethylketones, trifluoromethylketones, β-diketones, aldehydes, boronic acid esters and sulphonamides), that are capable of interacting with the catalytic machinery of the enzyme[5]. To that end peptidyl (α-aminoalkyl) phosphonate esters, where the scissile peptide bond of the substrate is replaced by the α-aminoalkyl phosphonate moiety have earlier been reported as potent inhibitors of various serine proteases[6].

A range of *O,O*-diphenyldipeptidylaminophosphonates of general formula Z-D-Aa-Pro-NHCHR^{P}(OPh)$_2$ were synthesised in our laboratory and that of others. A general route to these compounds involves synthesis of the phosphonate via 'one-pot' condensation of benzyl carbamate, aldehyde and triphenyl phosphite.[7] Inhibitors where R is (3-guanidino)-propyl and Aa is D-Phe, were found to be competitive, reversible inhibitors of thrombin[8], with K_i in the μM range. This contrasts with the boronic acid series for which, H-D-Phe-Pro-BoroArg[9] is one of the most potent of thrombin inhibitors known (final K_i <40pM). Further Z-(4-AmPhGly)^{P}(OPh)$_2$, where R is the arginine analogue amidinophenyl,[10] is a potent trypsin inhibitor, (k_{obs} 2000$M^{-1}s^{-1}$), but was found to have lower activity against human thrombin (k_{obs} 80$M^{-1}s^{-1}$). It is generally accepted that the mechanism of inhibition of serine proteases by diphenylpeptidylphosphonates normally involves nucleophilic substitution on the phosphorus atom by the γ oxygen atom of the catalytic Ser 195 of the protease. The substitution proceeds through a penta-coordinate phosphorus transition state to give a stable tetrahedral monophenoxyphosphonyl derivative.[10] A recent X-ray structure of Z-(4-AmPhGly)^{P}(OPh)$_2$ bound to bovine trypsin is consistent with this mechanism

and shows that the second phenoxy group is hydrolysed during an ageing process[11,12]. The greater leaving group propensity in the diphenyl analogues,[13,14] over the comparative stability of the dialkoxy series indicates that the phosphonylation event is more likely to occur in the former case, and therefore favour their therapeutic utility.

In our recent study we found that compound (1) had a similar, μM range, potency to the P1 arginine compounds. Compound (2) with the neutral 'P1' methoxypropyl side chain (shown below) was extremely selective for the enzyme with a K_i (after 1h pre-incubation) of 20nM, which is similar to the compound where Aa is H-Dpa studied earlier.[15] Kinetic analysis showed that the compound (2) as the *O,O*-diphenyl ester inhibitors displayed two-stage, slow-tight binding inhibition,[16] in contrast to the competitive inhibition that was shown by a series of *O,O*-dialkyldipeptidyl-aminophosphonates.[17]



Z-D-Dpa-Pro-NHPGly^P(OPh)$_2$; R = Ph_2CH; R1 = pentyl (Compound 1)

Z-D-Dpa-Pro-NHMpGly^P(OPh)$_2$; R = Ph_2CH; R1 = 3-methoxypropyl (Compound 2)

Compound (1) was crystallised with thrombin in the presence of the hirugen peptide [as ref 18. The Hirugen peptide binds to the anion binding exosite of thrombin and promotes crystallisation, without a marked influence on the catalytic rates achieved at the active site]. Analysis of the structure showed no electron density for the P1 group, indicating that no covalent interaction occurred with the Ser-195 of thrombin.

When the X-ray crystal structure of the adduct formed Mpg^P(OPh)$_2$ compound (2) and human α–thrombin (obtained at 1.4Å) was analysed, the mode of interaction was totally unexpected, on the basis of what had earlier been observed in other complexes. No covalent interaction with the serine is observed, and the diphenyl esters are both intact. This is facilitated by insertion of one phenoxy group into the specificity pocket of thrombin. On the basis of these findings, structure activity studies of tripeptidyl phosphonates with human thrombin[19] must now be revised.

ACKNOWLEDGEMENTS

We are grateful for the use of the Daresbury facility for the analysis of the complex of Z-Dpa-Pro-NHMpg^{P}(OPh)$_2$. We also thank Professor Harry Hudson for helpful discussions. This work was supported by the Thrombosis Research Trust.

References

[1] K. Bailey, Bettleheim, L. Lorand, and W.R. Middlebrook, *Nature,* **167,** 233–234, (1951); L. Lorand, *Nature,* **167,** 992–993, (1951); L. Lorand, *Biochem. J.,* **52,** 200–203, (1952); L. Lorand and W.R. Middlebrook, *Biochem. J.,* **52,** 196–199, (1952).

[2] L. Lorand, *Thromb. Diath. Haemorrh., Suppl.,* **7,** 238–248, (1961); L. Lorand and K. Konishi, *Arch. Biochem. Biophys.,* **105,** 58–67, (1964).

[3] J. Bruner-Lorand, T.R.E. Pilkington and L. Lorand, *Nature,* **210,** 1273–1274, (1966); W.W. Roberts, L. Lorand, L.F. Mockros, *Biotechnology,* **10,** 29–42, (1973); L.F. Mockros, W.W. Roberts, L. Lorand, *Biophys. Chem.,* **2,** 164–169, (1974); L. Shen and L. Lorand, *J. Clin. Invest.,* **71,** 1336–1341, (1983).

[4] C.J.L. Murray and A.D. Lopez, *Science,* **274,** 740–743, (1996).

[5] W.C. Ripka and G.P. Vlasuk, *Ann. Rep. Med. Chem.,* **62,** 71–89, (1997).

[6] J. Oleksyszyn and J.C. Powers, *Biochemistry* (and references therein), **30,** 485–493, (1991).

[8] C.J. Wang, T.L. Taylor, A.J. Mical, S. Spitz and T.M. Reilly, *Tett. Letts.,* **33,** 7667–7670, (1992).

[7] J. Oleksyszyn, L. Subotkowska, P. Mastalerz, *Synthesis,* 985–986, (1979).

[9] C.A. Kettner, L. Mersinger, R. Knabb, *J.Biol.Chem.,* **265,** 18289–18297, (1990).

[10] J. Oleksyszyn, B. Boduszek, C. Kam and J.C. Powers, *J. Med. Chem.,* **37,** 226–231, (1994).

[11] Q. Zhao, I.M. Kovach, A. Bencsura and A. Papathanassiu, *Biochemistry,* **33,** 8128–8138, (1994).

[12] J.A. Bertrand, J. Oleksyszyn, C. Kam, B. Boduszek, S. Presnell, R.R. Plaskon, F.L. Suddath, J.C. Powers and L. Williams, *Biochemistry,* **35,** 3147–3155, (1996).

[13] J. Fastrez, L. Jespers, D. Lison, M. Renard, E. Sonveaux, *Tett. Letts.,* **30,** 6861–6864, (1989).

[14] B. Boduszek, J. Oleksyszyn, C. Kam, J. Selzler, R.E. Smith, J.C Powers, *J.Med. Chem.,* **37,** 3969–3976, (1994).

[15] L. Cheng, C.A. Goodwin, M.F. Scully, V.V. Kakkar and G. Claeson, *Tett. Letts.,* **32,** 7333–7336, (1991); C. Tapparelli, R. Metternich, C. Ehrhardt, M. Zurini, G. Claeson, M.F. Scully, S.R. Stone, *J.Biol. Chem.,* **268,** 4734–4741, (1993).

[16] J. Deadman, G. Claeson, M.F. Scully, *J. Enzyme Inhibition,* **9,** 29–41, (1995).

[17] D. Green, G. Patel, S. Elgendy, J.A. Baban, E. Skordalakes, W. Husman, C.A. Goodwin, M.F. Scully, V.V. Kakkar and J. Deadman, *Phosphorus, Sulphur and Silicon,* **109–110,** 533–536, (1996).

[18] E. Skordalakes, R. Tyrell, S. Elgendy, C.A. Goodwin, D. Green, G. Dodson, M.F. Scully, J.H. Freyssinet, V.V. Kakkar, and J. Deadman, *J.Am.Chem.Soc.,* **119,** 9935–9936, (1997).

[19] H.L. Jiang, K.X. Chen, Y. Tang, J.Z. Chen, Q.M. Wang and R.Y. Ji, *Acta Sin. Pharmacol.,* **18,** 36–44, (1997).