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Characterization and isolation of L-to-D-amino-acid-residue isomerase from platypus venom

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Summary. Platypus venom contains an isomerase that reversibly interconverts the second amino-acid residue in some peptides between the L-form and the D-form. The enzyme acts on the natriuretic peptides OvCNPa and OvCNPb, and on the defensin-like peptides DLP-2 and DLP-4, but it does not act on DLP-1. While the isomerization of DLP-2 to DLP-4 is inhibited by the amino-peptidase inhibitor amastatin, it is not affected by the leucine amino-peptidase inhibitor bestatin. The enzyme, that is only present in minute quantities in an extract of the venom gland, is thermally stable up to 55 °C, and it was found by anion-exchange chromatography to be acidic. Isolation of the isomerase was carried out by combined ion-exchange chromatography and reverse-phase high performance liquid chromatography (HPLC).

Keywords: DLP – Peptide isomerase – Platypus venom peptides

Introduction

The male Australian platypus, *Ornithorhynchus anatinus*, is a unique mammal that bears venomous spurs on its hind limbs (Calaby, 1968). The toxic spurs are used to establish territories and to ward-off potential enemies, including dogs and humans. Platypus envenomation is peculiar in that the excruciating pain it causes is not easily relieved by common treatments (Fenner et al., 1992).

Platypus venom is a complex mixture of non-protein and protein components (see Fig. 1) whose roles in the venom are not fully understood. For example, a group of polypeptides of ~5 kDa referred to as defensin-like peptides or DLPs (Torres et al., 1999, 2000; Torres and Kuchel, 2004) adopt the β-defensin fold structure, however, they do not bear anti-microbial or sodium-channel inhibiting properties. Natriuretic peptides, OvCNPa and OvCNPb, which are the most physiologically active components discovered so far (de Plater, 1998; de Plater et al., 1995, 1998a, b; Torres et al., 2002a), cause relaxation of rat uterine muscle, mast cell histamine release, and oedema.

We discovered that OvCNPb and DLP-2 in platypus venom are unusual in that each peptide contains a D-amino acid residue at position 2 (Torres et al., 2002b, 2005). Subsequently, and of potentially more general importance for mammalian cell biology, we also discovered that an L-to-D-amino-acid-residue isomerase(s), also referred to as peptidylaminoacyl-L/-D-isomerase, is present in extracts of the platypus venom gland (Torres et al., 2006). This isomerase of ~50–60 kDa is responsible for the posttranslational creation of OvCNPb and DLP-2 from the all L-form-containing OvCNPa and DLP-4, respectively.

Although uncommon in higher organisms, peptides containing D-amino-acids have been found in frogs (Montecucchi et al., 1981; Richter et al., 1987; Kreil, 1997), spiders (Heck et al., 1994; Shikata et al., 1995), crustaceans (Soyez et al., 1994, 2000; Yasuda et al., 1994), and molluscs (Kamatani et al., 1989; Ohta et al., 1991; Jimenez et al., 1996; Jacobsen et al., 1998). These unusual peptides are more stable than their all-L precursors as they are less susceptible to protease degradation. In some instances they are more active than their all-L counterparts (Kamatani et al., 1989; Heck et al., 1994; Kreil, 1997).

The discovery of D-amino-acid-containing polypeptides and their isomerase in the platypus suggests the possibility

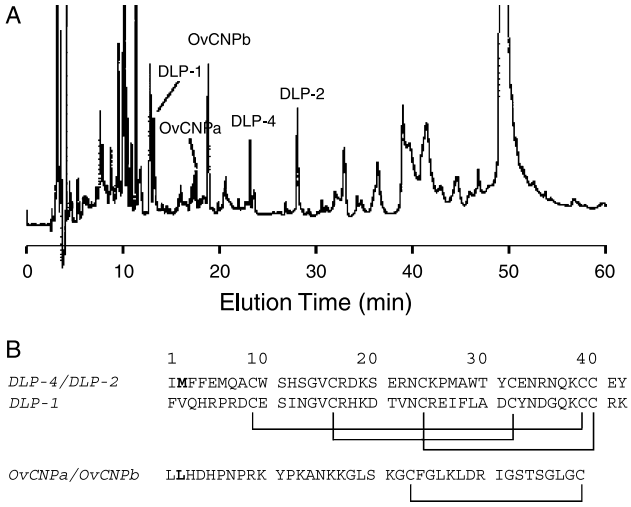

Fig. 1. RP-HPLC of platypus venom gland extract and primary structures of selected polypetides. **A** RP-HPLC of 50 µl of the homogenised gland was loaded onto a Synergi 4µ Hydro RP (250 × 4.6 mm, Phenomenex) column in water containing 0.1% (v/v) TFA. The components were eluted using a flow rate of 1.0 ml min^{-1} and linear gradients of 5–20% buffer B (acetonitrile containing 0.1% (v/v) TFA) over 5 min followed by 20–45% buffer B over 40 min at room temperature. **B** Primary structures of DLP-2/DLP-4, DLP-1 and OvCNPa/OvCNPb. The second amino-acid residues shown in bold are in the L-form in DLP4, and OvCNPa but are in the D-form in DLP-2 and OvCNPb. The disulfide-bonding patterns are indicated by the lines below the sequences

that such peptides and their respective isomerase are present in higher animals, including humans. A funnel web spider isomerase that acts near the C-terminus was identified about a decade ago (Heck et al., 1994; Shikata et al., 1995) and, more recently, a 52 kDa isomerase that acts on the second amino-acid from the N-terminus of selected peptides has been characterized from a frog (Jilek et al., 2005). The spider isomerase has significant amino-acid-residue sequence similarity with serine proteases, while the frog isomerase has some similarity to the N-terminal H-domain of human IgG-Fc binding protein. Thus it seemed very relevant to perform a similar study of the platypus isomerase and to determine whether this enzyme is similar to the frog isomerase, or to any other mammalian protein. In the present study, we characterized further the platypus isomerase and investigated several chromatographic procedures to isolate the enzyme using DLP-2 as a substrate for an HPLC-based enzyme assay.

Materials and methods

Venom-gland extracts

Venom glands were obtained in Tasmania from adult male platypuses that had been accidentally killed by motor vehicles. The glands were dissected out, finely sliced, and then placed in a container with 50 ml of cold 1% (w/v) trehalose solution. Homogenization was done with a Bamix cutter employing short bursts to ensure that the temperature remained low. The sample was centrifuged for 5–10 min at 4500 × *g* at 5 °C; the decanted liquid was then filtered through a 0.2 µm Sartorius Minisart filter (Göttingen, Germany). In the isomerase assay, the venom was fractionated by centrifugal ultrafiltration as described previously (Torres et al., 2005, 2006) to obtain the isomerase-active fraction with nominal MW >30 k.

DLPs and Bestatin

Polypeptides tested in isomerase assays were synthetic DLP-1 and DLP-2. These were produced manually using 2-(1-H-Benzotriazol-1-yl-)1,1,3,3 tetramethyluronium hexafluorophosphate (HBTU) activation of Boc-amino acids with in-situ neutralization chemistry, as previously described (Schnolzer et al., 1992; Torres et al., 2002b). A bestatin (Sigma) solution was prepared by dissolving 0.5 mg in 1 ml 50 mM PBS (phosphate buffered saline) to yield a final concentration of 1.45 mM.

L-to-D-amino-acid-residue isomerase assay

The incubation mixture was typically prepared by combining 20 µl of fractionated venom gland extract, 20 µl of 50 mM PBS, 10 µl of peptide (~0.3 mM) and 6 µl of 40 mM EDTA in a 200 µl HPLC sample tube. In the bestatin inhibition experiment, the mixture contained 25 µl of >30 kDa fractionated venom gland extract, 20 µl of DLP-2 (~0.3 mM), 5 µl of 40 mM EDTA, and 20 µl of bestatin (1.45 mM). Incubation was carried out for ~24 h in a water bath at 37 °C. Sampling of 5 µl–10 µl volumes for RP-HPLC analysis was carried-out to monitor the extent of isomer conversion during the time course. A Phenomenex Synergi 4µ Hydro-RP analytical column (250 mm × 4.60 mm) was used; the flow rate was 1 ml min^{-1}, and components were detected at light wavelengths of 215 nm or 280 nm. The solvent system consisted of 0.1% (v/v) trifluoroacetic acid in water (Buffer A) and 0.1% (v/v) trifluoroacetic acid in acetonitrile (Buffer B). Typically for RP-HPLC the solvent gradient for the assay was 5–32% B for 2 min, 32–40% B for 7 min, 40–60% B for 0.5 min, 60% B for 0.5 min, and 60–5% B for 1 min.

Chromatography

Anion-exchange fractionation of components was performed using a Pharmacia Mono Q HR 5/5 column with a solvent system consisting of 50 mM Bis-Tris (solvent A) and 50 mM Bis-Tris/0.5 M NaCl (solvent B) at pH 7.4. Samples were applied at a flow rate of 1 ml min^{-1} and the fractions were detected at a light wavelength of 280 nm. A typical solvent gradient profile was 0–25% B for 10 min, 25–100% B for 1 min, hold at 100% B for 2 min, and then 100–0% B for 2 min. Cation-exchange chromatography was performed using a Pharmacia Mono S HR 5/5 column. Buffers used to stabilize the pH in both starting and eluting solvents were MES (for pH values between 4.3 and 7.4) and Tris (pH 7.4). The eluting solvent (solvent B) also contained 0.5 M NaCl. In the combined cation- and anion-exchange chromatography performed at pH 7.4, 20 mM Tris was used as the buffer in both the starting and eluting solvents.

For RP-HPLC of the whole gland extract, a Phenomenex Jupiter C4 5µ 300 column (250 mm × 4.60 mm) and a Phenomenex Synergi 4µ Hydro-RP analytical column (250 mm × 4.60 mm) were used. Solvent systems and flow rate were identical to those used in the isomerase assay (see above).

Results

DLP-1 as a substrate for the isomerase

The test for possible conversion of DLP-1 by the enzyme was done in an incubation mixture that initially contained DLP-2 as well; this was done to determine if there was active enzyme in the mixture. As shown in Fig. 2A, an

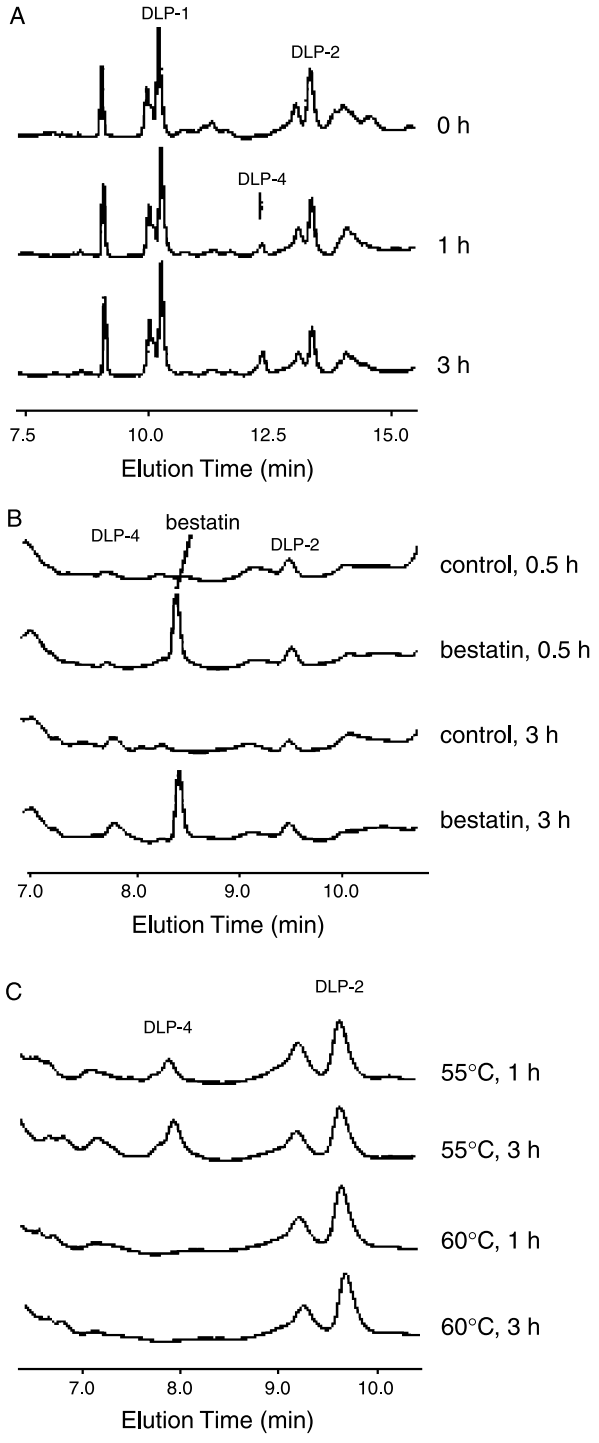

Fig. 2. Characterization of the platypus venom isomerase. **A** Incubation of DLP-1 and DLP-2 with a >30 kDa fraction of venom gland extract, at 37 °C. The enzymic reaction was monitored at 0 h, 1 h, and 3 h. **B** Effect of bestatin on the isomerization of DLP-2 to DLP-4, at 37 °C. RP-HPLC of the incubation mixture at 0.5 h and 3 h at 37 °C. A control experiment with no bestatin was performed to assess accurately the extent of inhibition by bestatin. **C** Temperature stability of the platypus isomerase. RP-HPLC of samples incubated at the two temperatures (55 °C and 60 °C) after 1 h and 3 h of incubation. The >30 kDa venom gland fractions were exposed to 55 °C and 60 °C for 5 min prior to incubation with DLP-2, at 37 °C

RP-HPLC peak corresponding to DLP-4 appeared after 1 h, and its intensity increased up to 3 h. The DLP-2 peak, on the other hand, decreased with incubation time. This outcome was consistent with the conversion of DLP-2 to DLP-4. The DLP-1 peak did not decrease with incubation time and no other peak (besides the DLP-4 peak) emerged. Thus DLP-1 was not converted by the isomerase.

Bestatin inhibition

An inhibition study was conducted simultaneously with a control experiment for which 50 mM PBS was added instead of bestatin solution. This analysis was carried-out to determine accurately the extent of the bestatin effect on the DLP-2/DLP-4 conversion. Figure 2B shows the RP-HPLC traces of the control and bestatin-treated mixtures after 0.5 h and 3 h of incubation at 37 °C. The DLP-4 peak was present in both cases and its ratio with respect to the DLP-2 peak increased with incubation time. These results indicate that isomerase-catalysed conversion occurred even in the presence of bestatin. The peak intensities of DLP-4 and DLP-2 in the control HPLC chromatograms and from the bestatin-treated experiments were very similar, indicating that bestatin at ~0.4 mM had no detectable effect on the isomerase activity.

Temperature stability

The temperature stability of the enzyme was tested by subjecting the >30 kDa venom fraction to various temperatures higher than 37 °C for 5 min and then preparing the incubation mixture with DLP-2 as substrate. By this means, it was observed that the isomerase was stable up to 55 °C. Figure 2C shows the conversion of DLP-2 to DLP-4 of the >30 kDa fraction at exposure temperatures of 55 °C and 60 °C. The data clearly illustrated that the enzyme was still active in the fraction after subjecting it to 55 °C for 5 min; however it lost its activity when exposed to 60 °C for 5 min, implying denaturation of the enzyme at this temperature.

Ion-exchange chromatography

Anion-exchange chromatography of gland extracts gave fractions for which the active component eluted late in the experiment, whereby the salt concentration was high, being at ~0.25 M NaCl (see Fig. 3A). This suggests that the isomerase is acidic under these conditions, as it bound tightly to the Mono Q anion exchange column. As shown in Fig. 3B, SDS PAGE had an active late-eluting fraction that contained other high molecular size components between 50 and 110 kDa. A 57 kDa band was also present

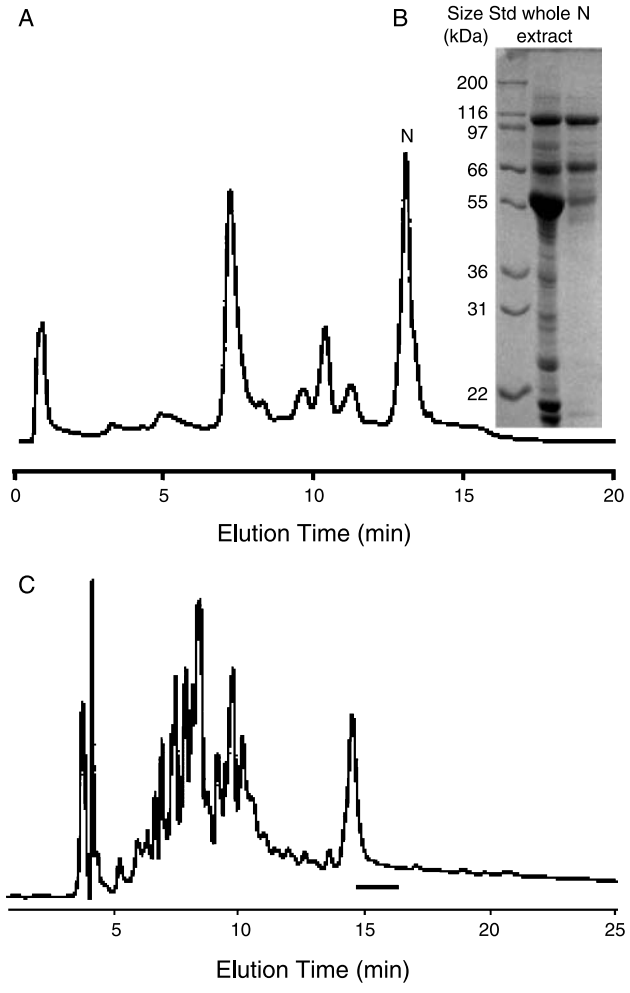

Fig. 3. Anion and RP-HPLC chromatograms of the whole venom-gland extract. **A** Anion-exchange chromatogram of 100 µl gland extract sample using a Mono Q 'strong' anion exchange column. The fraction corresponding to the peak labelled N had isomerase activity. **B** SDS-PAGE of the whole extract and anion fraction N. **C** RP-HPLC trace of venom-gland extract using a shallow acetonitrile gradient. The active fraction is indicated by a horizontal line below the trace

in the active fraction but was less intense than that obtained in size-exclusion chromatography; this suggests that the isomerase did not correspond to the particular major 57 kDa component in the size-exclusion fractionation (Torres et al., 2006). Our attempts to purify the active component using shallower gradients in anion-exchange chromatography were unsuccessful as evidenced by there being only a single broad peak that had isomerase activity.

Cation-exchange chromatography was also performed using a Mono S column. Prior to gradient elution at pH 7.4, the isomerase, plus some other components, were eluted from the column, while a substantial amount of 110 kDa and other proteins were adsorbed. This meant that cation-exchange chromatography could also be used to fractionate the venom and that the two ion-exchange methods (cation- and anion-) could be usefully combined.

To increase the efficiency of separation and/or prevent substantial loss of material, the two columns were connected in series; the outlet of the Mono S column was connected to the inlet of the Mono Q column and 20 mM Tris, pH 7.4, was used as the buffer. This gave good peak separation in the chromatograms because at the start of the chromatography, when no eluting salt was used, the isomerase passed unimpeded through the Mono S column but was adsorbed on the Mono Q column. The Mono S, which adsorbed many unwanted proteins, was then removed from the set-up prior to the introduction of a salt gradient to elute the isomerase from the Mono Q column.

RP-HPLC

Fractions obtained by RP-HPLC were tested for isomerase activity after removal of acetonitrile from the sample. In a typical experiment, using a uniform linear elution gradient, the fraction that contained the prominent peak corresponding to 57 kDa had isomerase activity. However, an RP-HPLC experiment using a shallower elution gradient produced a chromatogram (Fig. 3C) in which the active component did not correspond to the largest peak but rather to a very small one as a shoulder on the right of the large peak. This confirmed that the isomerase in the venom was only present in small quantities and that it was not the major 57 kDa component.

Combined ion-exchange chromatography plus RP-HPLC

The fact that RP-HPLC could be used to isolate the isomerase meant that it could be combined with ion-exchange methods (cation and anion) described earlier to improve the purification of the enzyme. The resulting RP-HPLC chromatogram and SDS-PAGE gel of the fractions are given in Fig. 4A and B. The most active component was fraction 6, but some activity was also detected in fraction 5. Thus, in SDS PAGE, the isomerase was expected to appear as a band corresponding to a size of 50–60 kDa that is only present in fractions 5 and 6. In addition, the isomerase protein-band was expected to be more intense in fraction 6 than in fraction 5.

Comparison of the lanes in the SDS PAGE gel in Fig. 4B shows a protein band that satisfies the above conditions. Clearly, an intense band at ~50 kDa appeared in the fraction-6 lane, and this particular band was also present in the

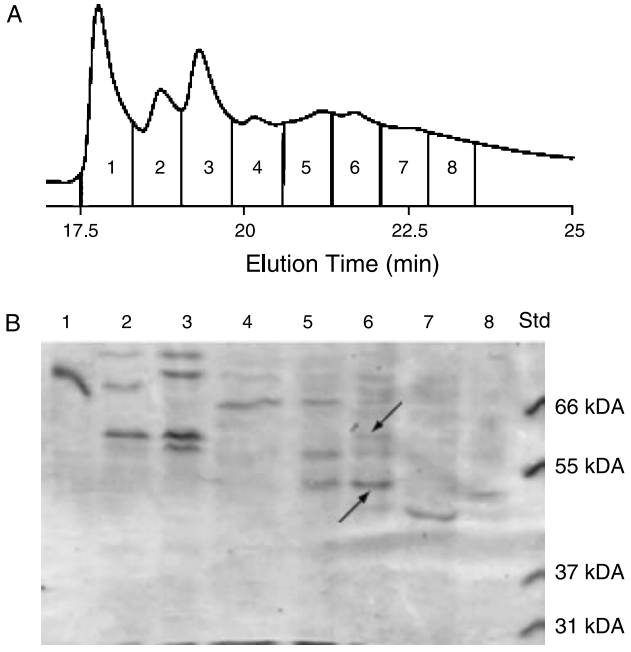

Fig. 4. Combined ion-exchange and RP-HPLC of the whole venom-gland extract. **A** RP-HPLC of the isomerase-active fraction collected by combined cation- and anion-exchange chromatography of 1 ml of venom gland extract. While fractions 5 and 6 of the RP-HPLC analysis had isomerase activity, fraction 6 was more active. **B** SDS-PAGE of the RP-HPLC fractions in **A**. Peptide bands with molecular size ~50 kDa and ~60 kDa are indicated by arrows

fraction-5 lane, but with less intensity. A band at ~57 kDa was also present in both lanes 5 and 6 but this was more intense in the fraction-5 lane. Another protein suspected to be isomerase-active showed a faint band at ~60 kDa in the fraction-6 lane; this band however was absent from fraction 5. It is not possible at this stage to rule-out completely this protein as the isomerase because it might still have been present in a very small amount in fraction 5 but was below the limit of detection on the SDS PAGE experiments.

Discussion

The substrates for the platypus venom isomerase that have been identified so far include DLP-2/DLP-4, DLP-2A/DLP-4A (first seven amino-acid residues of the polypeptide linked to Ser–Arg–Ser–Arg–Ser), and OvCNPa/OvCNPb (Torres et al., 2005, 2006). While the enzyme interconverts these molecules, it was shown in the present study that it does not act on DLP-1. This begins to define the specificity of the enzyme, suggesting the importance of the hydrophobic nature of the N-terminal residues of the peptide substrates. We showed previously that amastatin inhibits the conversion of DLP-2 to DLP-4 (Torres et al., 2006); however in the present study we showed that bestatin did not affect the rate of this conversion. The first residue in bestatin is aromatic, similar to that in DLP-1 (Phe) while the first residue in amastatin is aliphatic, similar to those in DLP-2/DLP-4 (Ile) and OvCNPb/OvCNPa (Leu). This information will be important in developing an understanding of the binding site of the enzyme and its mechanism of substrate binding and catalysis.

The fact that the platypus venom isomerase was stable even when exposed to temperatures up to 55 °C for 5 min indicates a relatively robust structural fold. This property of the enzyme may be exploited in future methods of separating it from other components in a mixture of proteins. Various chromatographic techniques have now been employed to isolate the isomerase and it is clear that the platypus gland extracts have contained several protein components, so this necessitated the use of two or more chromatographic modalities for the isolation. Fortunately, an RP-HPLC fraction was found to be isomerase active and this enabled us to use the higher resolution of this technique for isolation of the enzyme. The fraction obtained by RP-HPLC was considerably less active than fractions obtained from either size-exclusion or ion-exchange, and its activity was recovered only after the acetonitrile used in the method was removed. The combined ion-exchange and RP-HPLC provided much better separation because this utilizes both the charge and hydrophobicity properties of proteins. We have also investigated other chromatographic modalities such as hydroxylapatite (HAP) and hydrophobic interaction (HIC) columns but their performance in separation were inferior. The limited amount of platypus gland extract available and the extent of loss of isomerase activity after each chromatographic step made it unfeasible to append these techniques to separate further the isomerase from other proteins.

We are currently sequencing candidate bands isolated from active fractions on the SDS-PAGE gel and will shortly begin constructing a cDNA library of the platypus venom gland. This should allow the expression of putative isomerase genes and test their isomerase activities. Further characterization of the isomerase has been hindered by the paucity of details of monotreme proteins in public protein databases. Many of the proteins that have been isolated from the venom are unique and do not share obvious homology with known proteins in other species. (de Plater et al., 1995; de Plater, 1998; Torres et al., 1999). It is possible that the platypus venom isomerase is homologous with the isomerase from frog skin (Jilek et al., 2005) since they both act on the second amino-acid residue of peptides; but it may also be unique amongst mammals since the platypus itself is phylogenetically unique.

If the isomerase is more widely distributed in higher mammals it would constitute a further means of post-translational modification of proteins in these organisms. Such changes to proteins could lead to alternative folding pathways for many proteins and in principle alter their degradation pathways as well. Studies in other mammals will be greatly facilitated by a knowledge of the primary, secondary and higher-order structures, its gene sequence, its range of substrate specificity and the development of rapid assays of the isomerase, based on this knowledge. The present work indicates that we are poised for interesting findings in these more general investigations.

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References

Calaby JH (1968) The platypus (*Ornithorhynchus anatinus*) and its venomous characteristics. In: Bucherl W, Buckley EE, Delofeu V (eds) Venomous animals and their venoms. Academic Press, New York, pp 15–29

de Plater G (1998) Fractionation, primary structural characterisation and biological activities of polypeptides from the venom of the platypus (*Ornithorhynchus anatinus*). Ph.D. Thesis. Australian National University, Canberra, Australia

de Plater GM, Martin RL, Milburn PJ (1995) A pharmacological and biochemical investigation of the venom from the platypus (*Ornithorhynchus anatinus*). Toxicon 33: 157–169

de Plater GM, Martin RL, Milburn PJ (1998a) A C-type natriuretic peptide from the venom of the platypus (*Ornithorhynchus anatinus*): structure and pharmacology. Comp Biochem Physiol Part C Toxicol Pharmacol 120: 99–110

de Plater GM, Martin RL, Milburn PJ (1998b) The natriuretic peptide (ovCNP-39) from platypus (*Ornithorhynchus anatinus*) venom relaxes the isolated rat uterus and promotes oedema and mast cell histamine release. Toxicon 36: 847–857

Fenner PJ, Williamson JA, Myers D (1992) Platypus envenomation – a painful learning-experience. Med J Aust 157: 829–832

Heck SD, Siok CJ, Krapcho KJ, Kelbaugh PR, Thadeio PF, Welch MJ, Williams RD, Ganong AH, Kelly ME, Lanzetti AJ (1994) Functional consequences of posttranslational isomerization of Ser46 in a calcium channel toxin. Science 266: 1065–1068

Jacobsen R, Jimenez EC, Grilley M, Watkins M, Hillyard D, Cruz LJ, Olivera BM (1998) The contryphans, a D-tryptophan-containing family of Conus peptides: interconversion between conformers. J Pept Res 51: 173–179

Jilek A, Mollay C, Tippelt C, Grassi J, Mignogna G, Mullegger J, Sander V, Fehrer C, Barra D, Kreil G (2005) Biosynthesis of a D-amino acid in peptide linkage by an enzyme from frog skin secretions. Proc Natl Acad Sci USA 102: 4235–4239

Jimenez EC, Olivera BM, Gray WR, Cruz LJ (1996) Contryphan is a D-tryptophan-containing Conus peptide. J Biol Chem 271: 28002–28005

Kamatani Y, Minakata H, Kenny PT, Iwashita T, Watanabe K, Funase K, Sun XP, Yongsiri A, Kim KH, Novales-Li P (1989) Achatin-I, an endogenous neuroexcitatory tetrapeptide from *Achatina fulica* Ferussac containing a D-amino acid residue. Biochem Biophys Res Commun 160: 1015–1020

Kreil G (1997) D-amino acids in animal peptides. Annu Rev Biochem 66: 337–345

Montecucchi PC, de Castiglione R, Piani S, Gozzini L, Erspamer V (1981) Amino acid composition and sequence of dermorphin, a novel opiate-like peptide from the skin of *Phyllomedusa sauvagei*. Int J Pept Prot Res 17: 275–283

Ohta N, Kubota I, Takao T, Shimonishi Y, Yasudakamatani Y, Minakata H, Nomoto K, Muneoka Y, Kobayashi M (1991) Fulicin, a novel neuropeptide containing a D-amino-acid residue isolated from the ganglia of *Achatina fulica*. Biochem Biophys Res Commun 178: 486–493

Richter K, Egger R, Kreil G (1987) D-Alanine in the frog-skin peptide dermorphin is derived from L-alanine in the precursor. Science 238: 200–202

Schnolzer M, Alewood P, Jones A, Alewood D, Kent SB (1992) In situ neutralization in Boc-chemistry solid phase peptide synthesis. Rapid, high yield assembly of difficult sequences. Int J Pept Prot Res 40: 180–193

Shikata Y, Watanabe T, Teramoto T, Inoue A, Kawakami Y, Nishizawa Y, Katayama K, Kuwada M (1995) Isolation and characterization of a peptide isomerase from funnel web spider venom. J Biol Chem 270: 16719–16723

Soyez D, Toullec JY, Ollivaux C, Geraud G (2000) L to D amino acid isomerization in a peptide hormone is a late post-translational event occurring in specialized neurosecretory cells. J Biol Chem 275: 37870–37875

Soyez D, Van Herp F, Rossier J, Le Caer JP, Tensen CP, Lafont R (1994) Evidence for a conformational polymorphism of invertebrate neurohormones. D-amino acid residue in crustacean hyperglycemic peptides. J Biol Chem 269: 18295–18298

Torres AM, Alewood D, Alewood PF, Gallagher CH, Kuchel PW (2002a) Conformations of platypus venom C-type natriuretic peptide in aqueous solution and sodium dodecyl sufate. Toxicon 40: 711–719

Torres AM, de Plater GM, Doverskog M, Birinyi-Strachan LC, Nicholson GM, Gallagher CH, Kuchel PW (2000) Defensin-like peptide-2 from platypus venom: member of a class of peptides with a distinct structural fold. Biochem J 348: 649–656

Torres AM, Kuchel PW (2004) The beta-defensin-fold family of polypeptides. Toxicon 44: 581–588

Torres AM, Menz I, Alewood PF, Bansal P, Lahnstein J, Gallagher CH, Kuchel PW (2002b) D-Amino acid residue in the C-type natriuretic peptide from the venom of the mammal, *Ornithorhynchus anatinus*, the Australian platypus. FEBS Lett 524: 172–176

Torres AM, Tsampazi C, Geraghty DP, Bansal PS, Alewood PF, Kuchel PW (2005) D-amino-acid residue in a defensin-like peptide from platypus venom: effect on structure and chromatographic properties. Biochem J 391: 215–220

Torres AM, Tsampazi M, Tsampazi C, Kennet EC, Belov K, Geraghty DP, Bansal PS, Alewood PF, Kuchel PW (2006) Mammalian L-to-D-amino-acid-residue isomerase from platypus venom. FEBS Lett 580: 1587–1591

Torres AM, Wang X, Fletcher JI, Alewood D, Alewood PF, Smith R, Simpson RJ, Nicholson GM, Sutherland SK, Gallagher CH (1999) Solution structure of a defensin-like peptide from platypus venom. Biochem J 341: 785–794

Yasuda A, Yasuda Y, Fujita T, Naya Y (1994) Characterization of crustacean hyperglycemic hormone from the crayfish (*Procambarus clarkii*): multiplicity of molecular forms by stereoinversion and diverse functions. Gen Comp Endocrinol 95: 387–398

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