Pulchellalactam: A CD45 Protein Tyrosine Phosphatase Inhibitor from the Marine Fungus *Corollospora pulchella*

KHISAL A. ALVI*, ALLEN CASEY and BIPIN G. NAIR

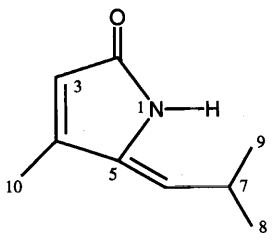
Natural Products Discovery Services, MDS Panlabs, 11804 North Creek Parkway South, Bothell, WA 98011, U.S.A.

(Received for publication December 8, 1997)

CD45 protein tyrosine phosphatase is a large-molecular mass transmembrane glycoprotein expressed on all hematopoietic cells except erythrocytes.[1] It is a member of the receptor-like transmembrane protein tyrosine phosphatase[2] (PTPase) family. Expression of CD45 has been shown to be important for activation of both B and T cells *via* their antigen-specific receptors[3,4] and has generated considerable interest in the study of lymphocyte activation as well as a possible target for drug intervention in various auto-immune and/or inflammatory diseases.

As a part of our effort to find enzyme inhibitors from microbial sources, we identified a fungal extract which exhibited very potent activity in our CD45 assay. Bioassay directed fractionation of the crude extract yielded a novel pulchellalactam (**1**) as the active component of this extract. Here we describe the isolation, structure determination and biological characteristics of **1**.

The recombinant baculovirus was constructed to encode the entire cytoplasmic domain of the human CD45 tyrosine phosphatase protein (CD45-IPD), consisting of two tandem PTPase domains. The recombinant protein was produced in a baculovirus expression system using Sf9 insect cells. Furthermore, the vector was designed to produce a fusion protein containing a polyhistidine sequence and to facilitate purification *via* a single step purification method using a nickel-sepharose affinity column. CD45 tyrosine phosphatase activity was assayed by monitoring residual phosphorylation of the substrate poly(glu-Na,Tyr, 4:1) bound to an ELISA plate as described earlier.[5] Briefly, successive layering of biotinylated mouse anti-phosphotyrosine antibodies and streptavidin-linked β-galactosidase conjugate allowed fluorescent detection using fluorescein-di-β-galactopyranoside which was hydrolyzed to fluorescein and detected by a Cytofluor 300 fluorescence plate reader (Millipore). Protein tyrosine phosphatase 1B (PTP1B) was purchased from Upstate Biotechnology and the activity was also measured employing the same procedure described for CD45 phosphatase activity.



Pulchellalactam (**1**)

Corollospora pulchella (ATCC 62554) was isolated from a sample of driftwood from Peleliu. It is a marine Ascomycete of the family Halosphaeriaceae. The genus is characterized by having ascospores with hyaline, ribbon like appendages all around the middle and at each apex, developing by the peeling off of the exospore; most species have a long spine-like appendage at each end.[6]

C. pulchella was maintained at 23°C on yeast malt extract agar with artificial seawater (yeast extract 0.4%, malt extract 1%, glucose 0.4%, agar 2%; prepared in 50% Instant Ocean artificial seawater) and trace elements with 0.5% trace element solution (ferrous sulfate heptahydrate 0.1%, manganese sulfate 0.1%, cupric chloride dihydrate 0.0025%, calcium chloride 0.0132%, boric acid 0.0056%, ammonium molybdate tetrahydrate 0.0019%, zinc sulfate 0.02%; prepared in 0.6 N HCl).

A 1.0 ml aliquot of spore and mycelia homogenate was inoculated into a 250 ml Erlenmeyer flask containing 25 ml of seed medium (glucose 2%, Pharmamedia 1.5%, ammonium sulfate 0.3%, zinc sulfate 0.003%, calcium carbonate 0.4%, yeast extract 0.5%; prepared in 50% Instant Ocean artificial seawater). The seed culture was shaken on a rotary shaker at 250 rpm at 28°C for 2 days. A 1.0 ml aliquot of seed culture was transferred to a 250 ml Erlenmeyer flask containing 30 ml of yeast malt extract (without agar) at pH 7.8, with artificial seawater and trace elements. The culture was shaken on a rotary shaker at 250 rpm at 28°C for 6 days. The process was replicated to produce three liters of broth.

Fermentation broth (3 liter) was extracted with ethyl

Table 1. ^{1}H and ^{13}C NMR chemical shifts of pulchellalactam (**1**).

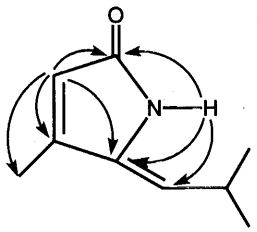
Carbon No.	^{13}C chemical shifts ppm	^{1}H chemical shifts ppm
1		9.41 (bs)
2	173.1	
3	119.7	5.94 (bs)
4	150.2	
5	137.0	
6	125.5	5.40 (d, J=9.87 Hz)
7	25.9	2.26 (dq, J=9.90, 6.65 Hz)
8	23.0	1.10 (d, J=6.67 Hz)
9	22.9	1.10 (d, J=6.67 Hz)
10	12.9	2.11 (d, J=1.16 Hz)

acetate (4.5 liters). The extract was dried over Na_2SO_4 and concentrated *in vacuo* to dryness to yield a brown material (525 mg). The extract was first fractionated *via* our standard dual mode HSCCC (high speed countercurrent chromatography) protocol[7] that concentrated the activity in fractions 72~75. Further purification of the pooled HSCCC fraction was achieved by reversed-phase HPLC which yielded pulchellalactam.

Pulchellalactam was isolated as an oily material. The UV spectrum showed a maximum at 275.9 nm, and the molecular formula was determined to be $C_9H_{13}NO$ by HREI-MS analysis (*m/z* 151.1012 calcd for $C_9H_{13}NO$, found 151.1004). ^{13}C and ^{1}H NMR spectra ($CDCl_3$) showed 9 carbon and 12 proton signals, respectively (Table 1). The DEPT spectra indicated the presence of three methines, three methyl groups and three quaternary carbons. Only 12 protons were accounted for from the ^{13}C NMR data suggesting the presence of one exchangeable proton in the molecule. Four down field ^{13}C NMR signals between δ 119.7~150.2 were suggestive of one substituted diene system in the molecule. An additional unsaturation was denoted by a ^{13}C NMR signal at δ 173.1 indicative of a α, β unsaturated lactam carbonyl carbon. One degree of unsaturation remained which was assigned to one ring.

The connectivity of proton and carbon atoms was confirmed by the HMQC spectrum. The ^{1}H-^{1}H COSY spectrum revealed two separate proton spin systems: H-3/H-10 and H-6/H-7/H-8/H-9. ^{1}H-^{13}C long range couplings observed in the HMBC spectrum are shown in Fig. 1. For example, cross peaks were observed between H-3 to C-2, C-4, C-5 and C-10 and from H-6 to C-4, C-5, C-7. The most important correlations were

Fig. 1. Significant HMBC correlations for pulchellalactam.



observed between the NH and C-5 and C-2 which allowed us to place the NH group between these carbon atoms. Thus, the gross structure of pulchellalactam was established as shown.

Pulchellalactam exhibited a dose-dependent inhibition of CD45 activity with an IC_{50} of 124 μg/ml. This was comparable with the inhibition of CD45 activity observed in the presence of a known tyrosine phosphatase inhibitor, sodium orthovanadate[8] which inhibited the activity with an IC_{50} of 91.9 μg/ml. The inhibition was specific for CD45 as another protein tyrosine phosphatase, PTP1B,[9] was not inhibited by pulchellalactam (data not shown).

References

1) Trowbridge, I. S.; H. L. Ostergaard & P. Johnson: CD45: A leukocyte-specific member of the protein tyrosine phosphatase family. Biochim. Biophys. Acta 1095: 46~56, 1991
2) Fischer, E. H.; H. Charbonneau & N. K. Tonks: Protein tyrosine phosphatases: A diverse family of intracellular and transmembrane enzymes. Science 253: 401~406, 1991
3) Koretzky, G. A.; J. Picus, T. Schultz & A. Weiss: Tyrosine phosphatase CD45 is required for T-cell antigen receptor and CD2-mediated activation of a protein tyrosine kinase and interleukin 2 production. Proc. Natl. Acad. Sci. 88:2037~2041, 1991
4) Weaver, C. T.; J. T. Pingel, J. O. Nelson & M. L. Thomas: CD8+T-cell clones deficient in the expression of the CD45 protein tyrosine phosphatase have impaired responses to T-cell receptor stimuli. Mol. Cell. Biol. 11: 4415~4422, 1991
5) Alvi, K. A.; B. Nair, C. Gallo & D. Baker: Screening of microbial extracts for tyrosine kinase inhibitors. J. Antibiot. 50: 264~265, 1997
6) Kohlmeyer, J. & B. Volkmann-Kohlmeyer: Illustrated key to the filamentous higher marine fungi. Botania Marina 34:.1~61, 1991
7) Alvi, K. A.; J. Peterson & B. Hofmann: Rapid identification of elaiophylin and geldanamycin in streptomyces fermentation broths using CPC coupled

with a photodiode array detector and LC-MS methodologies. J. Ind. Microbiol. 15: 80～84, 1995
8) GORDON, J.: Use of vanadate as protein-phosphotyrosine phosphatase inhibitor. *In* Methods. Enzymol. *Ed.*, HUNTER, T. *et al.* 201, pp. 477～482, Academic Press San Diego California, 1991
9) TROWBRIDGE, I. S.: CD45: A prototype for transmembrane protein tyrosine phosphatases. J. Biol. Chem. 266: 23517～23520, 1991