Communication to the Editor

CHLOROPEPTINS I AND II, NOVEL INHIBITORS AGAINST gp120-CD4 BINDING FROM *Streptomyces* sp.

Sir:

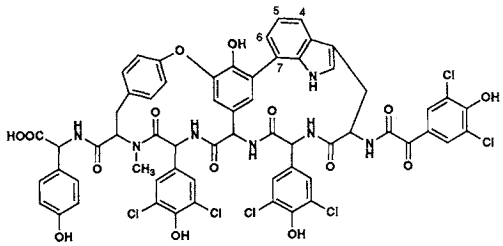
Recently, we reported the first gp120-CD4 binding inhibitors of microbial origin, isochromophilones I and II, produced by *Penicillium* sp.[1]. Now, we report additional two gp120-CD4 binding inhibitors, chloropeptins I(**1**) and II(**2**) (Fig. 1), produced by a soil actinomycete, *Streptomyces* sp. WK-3419. The major component **1** is a new compound, while the other one **2** was identified with complestatin[2] which has been reported to inhibit the hemolysis of erythrocytes sensitized by the complement system[3]. This communication deals with the fermentation, isolation, characterization and biological activities of chloropeptins I and II.

During the screening and fermentation as well as the isolation of active principles, the inhibitory activities against gp120-CD4 binding were monitored by enzyme-linked immunosorvent assay (ELISA) using recombinant soluble CD4 and recombinant gp120 as described in a previous report[1].

The production and isolation of chloropeptins I and II were carried out as follows. A roopful of mycelia from a slant of *Streptomyces* sp. WK-3419 was transferred into a 500-ml Erlenmeyer flask containing 100 ml of a seed medium consisting of glucose 0.1%, starch 2.4%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5%, and $CaCO_3$ 0.4% (adjusted to pH 7.0 before sterilization). The flask was incubated at 27°C on a rotary shaker at 200 rpm for 4 days to give a seed culture. The seed culture (600 ml) was transferred to a 50-liter jar fermentor containing a production medium (30 liters) consisting of starch 2.4%, glucose 0.1%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5%, $CaCO_3$ 0.4%, trace metal solution 1% and allophane, non-crystalline aluminosilicic cray 0.3% (pH 7.0). Fermentation was carried out at 27°C for 6 days. The cultured broth (60 liters) was centrifuged and the mycelium cake was extracted with 20 liters of 70% aqueous acetone. The extract was concentrated to a small volume (*ca.* 1.0 liter) under reduced pressure, and then extracted twice with 1.0 liter of ethyl acetate at pH 2. The organic solvent layer was concentrated to dryness and was chromatographed on a silica gel column using chloroform - methanol - water (40 : 16 : 3 and 15 : 10 : 2), methanol and methanol - water - acetic acid (5 : 4 : 1) as developing solvent systems. The active fractions were collected and concentrated to dryness (360 mg). The active substances, **1** and **2**, were obtained as yellow powders (111 mg and 67 mg, respectively) by HPLC using Shiseido Capcell pak C_{18}-SG column.

The physico-chemical properties of **1** were as follows: mp., >300°C; $[\alpha]_D^{26}$, −18.7° (c=0.16, DMSO); UV λ_{max}^{MeOH} nm (ε), 214 (64,600), 239(sh), 285(sh), 291(14,600), and 304(sh). The molecular formula was determined to be $C_{61}H_{45}N_7O_{15}Cl_6$ by HRFAB-MS (m/z 1325.1093 (M^+), calcd for 1325.1105), which is the same as that of complestatin. The physico-chemical properties of **2** coincided with those of complestatin[2], indicating that **2** is identical with complestatin. The IR and ^{13}C and ^1H NMR spectra of **1** were similar to those of **2**. However, in the ^1H NMR of **1**, three aromatic proton signals at δ 7.22, 6.94 and 7.08 were assigned to H-4, H-5 and H-6, respectively, on the indole moiety; while the corresponding aromatic proton signals for **2** at δ 7.44, 6.84 and 7.25 have been assigned to H-4, H-5 and H-7, respectively. This

Fig. 1. Structures of chloropeptins I (**1**) and II (complestatin, **2**).



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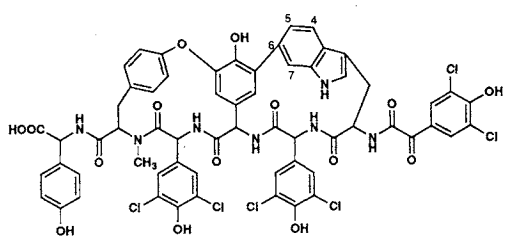

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Fig. 2. ^{1}H NMR assignment of the indole moieties of chloropeptins I and II (complestatin).

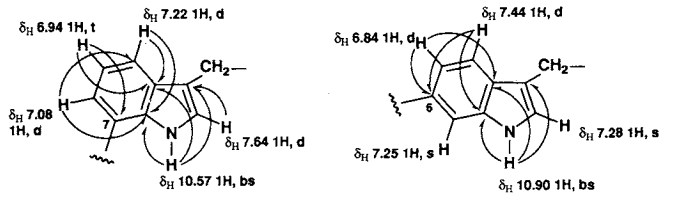

Table 1. Inhibition of HIV replication by chloropeptin I in the viral core protein level.

Sample	Viral core protein p24 synthesized (ng/ml)		
	Day 2	Day 3	Day 4
None	0	97.3	129.6
Chloropeptin I (7.5 μM)	0	0	7.3

indicates that C-7 of the indole ring is conjugated to an adjacent phenol group in **1** (Fig. 2). From the above data, it is concluded that **1** is a new compound and the structure was determined as shown in Fig. 1. **1** was named chloropeptin I as peptide structure having many chlorine atoms.

The inhibitory activities against gp120-CD4 binding were assayed by ELISA method described previously[1]. **1** and **2** inhibited gp120-CD4 binding with IC_{50} of 2.0 and 3.3 μM, respectively. They exhibits no antimicrobial activity against various bacteria and fungi at 1.0 mg/ml and no cytotoxycity at 20 μM for B-16 melanoma.

Anti-HIV activity was assayed as described previously[1]. As shown in Table 1, **1** significantly inhibited HIV replication in peripheral human lymphocytes at 7.5 μM. It exhibited no effect on cell proliferation in lymphocytes at the same concentration.

As mentioned above, **1** and **2** were found to inhibit the binding between gp120 and CD4, and to exhibit selective anti-HIV activities. MOMOTA *et. al.*[4] reported that complestatin (**2**) inhibited HIV-1-induced cytopathicity and syncytium formation in $CD4^+$ T-lymphocyte. The above results suggest that the inhibition of gp120-CD4 binding by **1** and **2** may block of HIV entry into lymphocytes, therefore the anti-HIV activities were observed.

The structure elucidation of **1** will be reported elsewhere.

Acknowledgments

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