COMMUNICATIONS TO THE EDITOR

Siamycins I and II, New Anti-HIV Peptides: I. Fermentation, Isolation, Biological Activity and Initial Characterization

Sir:

In a systematic search for microbial products active in the syncytia inhibition assay (SIA)[1], two streptomycetes strains AA6532 (ATCC 55290) and AA3891 (ATCC 55286) were found to produce new antiviral antibiotics, named siamycins I and II, respectively. They are peptide group antibiotics structurally related to each other, and both show good inhibitory activity against HIV and HSV viruses. This paper describes the fermentation, isolation, physico-chemical properties and biological activities of siamycins I and II.

Streptomyces sp. AA3891 and AA6532 were isolated from soil samples collected in Madhya Pradesh, India and Tokyo, Japan, respectively. Siamycin I was produced by the strain AA6532 in 500-ml Erlenmeyer flasks using medium composed of lactose 2%, dextrin (Nichiden Kagaku Co.) 2%, glucose 0.5%, corn steep liquor (Oji Corn Starch Co.) 1%, rice bran 1.5%, K_2HPO_4 0.05% and $CoCl_2 \cdot 6H_2O$ 0.0002%, pH 7.0. The flasks were shaken on a rotary shaker (200 rpm) at 28°C for 88 hours. Antibiotic production in the fermentation broth was estimated by the SIA. The medium for siamycin II production by the strain AA3891 consisted of soluble starch 2%, glucose 1%, Pharmamedia (Traders Protein Co.) 1%, Brewer's yeast extract (Asahi Breweries, Ltd.) 0.3%, NZ-amine (Humko Sheffield Chemical) 0.3%, Allophane (Shinagawa Brick Co.) 0.5% and $CaCO_3$ 0.3%, pH 7.0. Incubation was carried out for 112 hours at 28°C and 200 rpm.

The whole broth from the strain AA6532 (9.8 liters) was extracted with *n*-butanol (5 liters), and the extract was concentrated *in vacuo* to a residue (13.9 g). The solid was applied onto a column of Diaion HP-20 (40 mm i.d. × 300 mm). The column was washed with 30% aqueous methanol (600 ml) and then eluted with 80% aqueous methanol (800 ml). The eluate was monitored by the SIA, and evaporation of the active eluate yielded a crude sample of siamycin I (1.44 g). This material was chromatographed on a reversed-phase silica gel column (YMC-gel, ODS A60, 40 mm i.d. × 450 mm) and eluted with acetonitrile - 0.15% KH_2PO_4, pH 3.5 (4 : 6). Active fractions in the SIA were pooled, concentrated and extracted with *n*-butanol. Evaporation of the extract afforded a semi-pure solid (703 mg). The solid was further purified by Sephadex LH-20 column chromatography (40 mm i.d. × 600 mm) and eluted with methanol to give pure siamycin I (454 mg). Pure siamycin II (65 mg) was obtained in the same manner from the harvested broth of the strain AA3891 (8.5 liters).

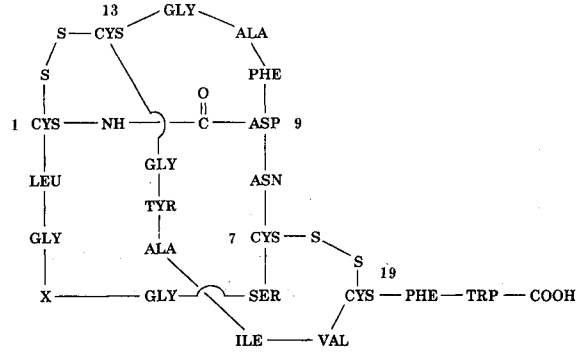
Siamycins I and II were isolated as white amorphous powders. Both antibiotics are soluble in dimethylsulfoxide and methanol, slightly soluble in alkaline water but practically insoluble in ethyl acetate, chloroform and water. They gave a positive reaction to sulfuric acid but were negative to ninhydrin and Sakaguchi tests. The physico-chemical properties of siamycins I and II are summarized in Table 1. Siamycins are peptide group antibiotics based on their IR spectra. Amino acid analysis revealed that both compounds contained the same residues with the exception that one of two valines in saimycin I is replaced by isoleucine in siamycin II. This finding was supported by their ionspray mass spectral results. Chemical structures and conformations of siamycins I and II (Fig. 1) were established by extensive

Table 1. Physico-chemical properties of siamycins I and II.

	Siamycin I	Siamycin II
Appearance	White powder	White powder
M.P. (dec.)	255°C	255°C
$[\alpha]_D^{27}$ (MeOH)	-91° (*c* 0.5)	-89° (*c* 0.25)
UV λ_{max} nm (MeOH)	281 nm (ϵ 6,900)	280 nm (ϵ 6,500)
Molecular formula	$C_{97}H_{131}N_{23}O_{26}S_4$	$C_{98}H_{133}N_{23}O_{26}S_4$
Elemental analysis Found:	C: 51.34, H: 5.85, N: 13.74, S: 5.78	NT
Ionspray-MS (*m/z*)	2164 $(M+H)^+$	2178 $(M+H)^+$
IR ν_{max} (KBr) cm^{-1}	3300, 2960, 1660, 1515, 1230	3300, 2960, 1659, 1515, 1230
TLC[a] Rf	0.51	0.56
HPLC[b] Rt (minutes)	13.1	18.0
Consist of amino acid (mole)	Asp(1), Asn (1),Ser(1), Gly(4), Ala(2), Tyr(1), Val(2), Ile(1), Leu(1), Phe(2), Trp(1), Cys(4)	Asp(1), Asn(1), Ser(1), Gly(4), Ala(2), Tyr(1), Val(1), Ile(2), Leu(1), Phe(2), Trp(1), Cys(4)

[a]: Merck Kiesel gel 60F_{254}; nBuOH-AcOH-H_2O (4:1:1).
[b]: YMC gel A301-3, C_{18}, 3 μm (4.6 mm I.D.x100 mm); CH_3CN-0.15% KH_2PO_4 (4:6, pH 3.5); 1 ml/minute; detection, UV 254 nm.

Fig. 1. Structures of siamycins I and II.



Siamycin I X = VAL
Siamycin II X = ILE

Table 2. Antibacterial spectra of siamycins I and II.

Test organism	MIC (μg/ml)	
	Siamycin I	Siamycin II
Staphylococcus aureus FDA209P JC-1	3.1	3.1
S. aureus smith	6.3	6.3
S. aureus A15036 (MRSA)	3.1	3.1
Micrococcus luteus ATCC 9341	1.6	1.6
Bacillus subtilis ATCC 6633	1.6	1.6
Escherichia coli Juhl	>100	>100
E. coli K-12	>100	>100
E. coli NIHJ JC-2	>100	>100
Klebsiella pneumoniae ATCC 10031	>100	>100
Citrobacter freundii GN 7391	>100	>100
Salmonella typhi 901	>100	>100
Pseudomonas aeruginosa A9843A	>100	>100

Medium : Nutrient agar (Difco), pH 7.0
Inoculum size : 10^5 cells/ml
Incubation conditions : 32°C, 18 hours

Table 3. Anti-HIV activity of siamycins I and II in CEM-SS cells.

Compound	ID_{50} (μg/ml)	TD_{50} (μg/ml)
Siamycin I	7	>500
Siamycin II	9	>500
2',3'-Dideoxyinosine	2.3	>500

Table 4. Anti-HSV activity of siamycins I and II, and acyclovir.

Compound	ID_{50} (μg/ml)	TD_{50} (μg/ml)
Siamycin I	48	>100
Siamycin II	27	>100
Acyclovir	0.28	>100

2D NMR experiments[2,3]. Siamycins are structurally quite similar to RP71955[4], with slight differences in the amino acid sequences.

Siamycins showed antibacterial activity against Gram-positive bacteria (Table 2). Anti-HIV activity was evaluated by using the XTT assay method[5] (Table 3). Both antibiotics exhibited potent inhibitory activity against the HIV LAV_{BRU} strain, and cytotoxicities of siamycins I and II in CEM-SS cells were low. Anti-HSV activity was examined by using the KOS strain[6] (Table 4). Siamycins showed weaker anti-HSV activities than acyclovir.

Acknowledgments

The authors wish to express their thanks to the members of the bioassay, fermentation and isolation groups in Tokyo Research Center for their contributions.

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(Received December 26, 1994)

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