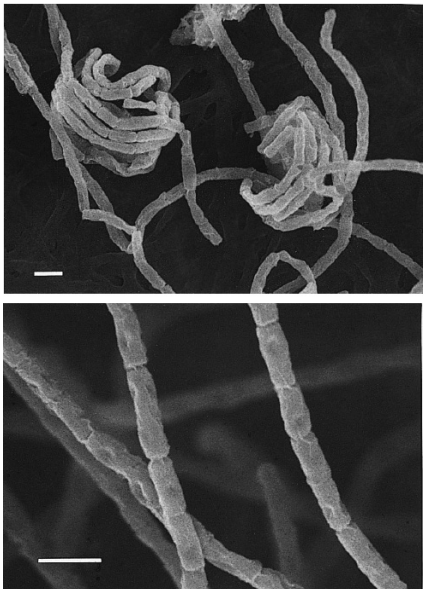
Actinohivin

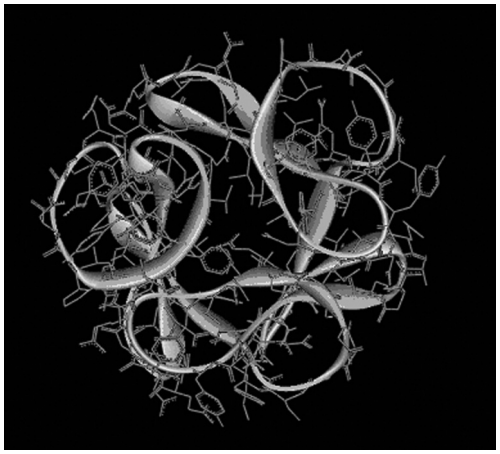
1. Discovery, producing organism and structure[1-4,6)]

Actinohivin (AH was isolated from a cultured broth of an actinomycete strain using a syncytium formation assay system established by our group and identified to be an anti-HIV protein.[3)] The producing organism was recognized as a new genus and named *Longispora albida* K97-0003^{T}.[6)] AH consists of a 114-amino-acid chain that exhibits internal sequence triplication.[2)] (Segments 1-3,; 1-38, 39-76 and 77-114, respectively).[2)] The gene encoding AH has been cloned and a production system of recombinant AH in *Escherichia coli* has been established.[1)]

1–38	ASVTIR**N**AQ**TGR**L**LDSNY**N**GN**VYTLP**A**NGG**N**YQ**RW**TGP**
39–76	GDGTVR**N**AQ**TGR**C**LDSNY**D**GA**VYTLP**C**NGG**S**YQ**KW**LFY**
77–114	SNGYIQ**N**VE**TGR**V**LDSNY**N**GN**VYTLP**A**NGG**N**YQ**KW**YTG**



Longispora albida K97-0003^{T}



Proposed 3D modeling of actinohivin
The modeling protocol was executed by the automated computer program FAMS (K. Ogata and H. Umeyama, *J. Mol. Graph. Model.* **18**, 258–272, 305–306 (2000)).

2. Physical data[2)]

White powder. 114 amino acids; mol wt 12,524. Isoelectric point 8.3.

3. Biological activity[2, 5-9)]

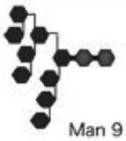
AH potently inhibits the T-cell line (T) and macrophage (M)-tropic syncytium formation in co-incubation of HeLa/T-env/Tat and HeLa/CD4/Lac-Z cells and of HeLa/M-env/Tat and HOS/CD4/CCR5/Lac-Z cells. AH is a potent inhibitor against the infection of T- and M-tropic HIV-1 strains, including strains resistant to drugs inhibiting reverse transcriptase and protease, and HIV-2 strains.

4. Mechanism of action[7-15)]

AH binds to gp120 from both the T- and M tropic HIV-1 in a concentration dependent manner. AH does not bind to non-glycosylated gp120, gp120 treated with α(1-2) mannosidase and cells with CD4 and co-receptors. Therefore, sugar chains of gp120, especially Manα(1-2)Man terminal units of high-mannose type sugar chains, play a crucial role in AH binding to gp120. It was also found that AH has low affinity to a Manα(1-2)Man, a high-mannose type sugar chain or glycoproteins with low density of high-mannose type sugar chains. Conversely, it binds strongly only to glycoproteins such as gp120 having many high-mannose type sugar chains. Cyanovirin-N, a 101 amino acid anti-HIV lectin from a cyanobacterium, has high affinity not only to a glycoprotein with single high-mannose type sugar chain but also to a high-mannose type sugar chain.

Deletion of one or two segment (s) among three segments of AH caused severe decrease of anti-syncytium formation activity. AH requires cooperative binding of three segments to high-mannose type sugar chains of gp120 for exhibiting high activity. Consequently, AH exhibits a high affinity by the so-called 'cluster effect' of lectin only when three high-mannose type sugar chains bind to three segments of AH. Thus, AH binds only to glycoproteins having many high-mannose type sugar chains such as gp120 to show much higher specificity than cyanovirin-N.

Comparison between AH and cyanovirin-N in affinity to sugar chains and glycoproteins[7)]

	 Manα(1-2) Man	 Man 9	 RNase B	 gp120
AH	[a] 5.8×10^{-4}	[a] 3.9×10^{-4}	——	[c] 3.4×10^{-8}
Cyanovirin-N	[b] 1.5×10^{-6}	[b] 6.7×10^{-9}	[c] 3.0×10^{-8}	[c] 1.7×10^{-8}

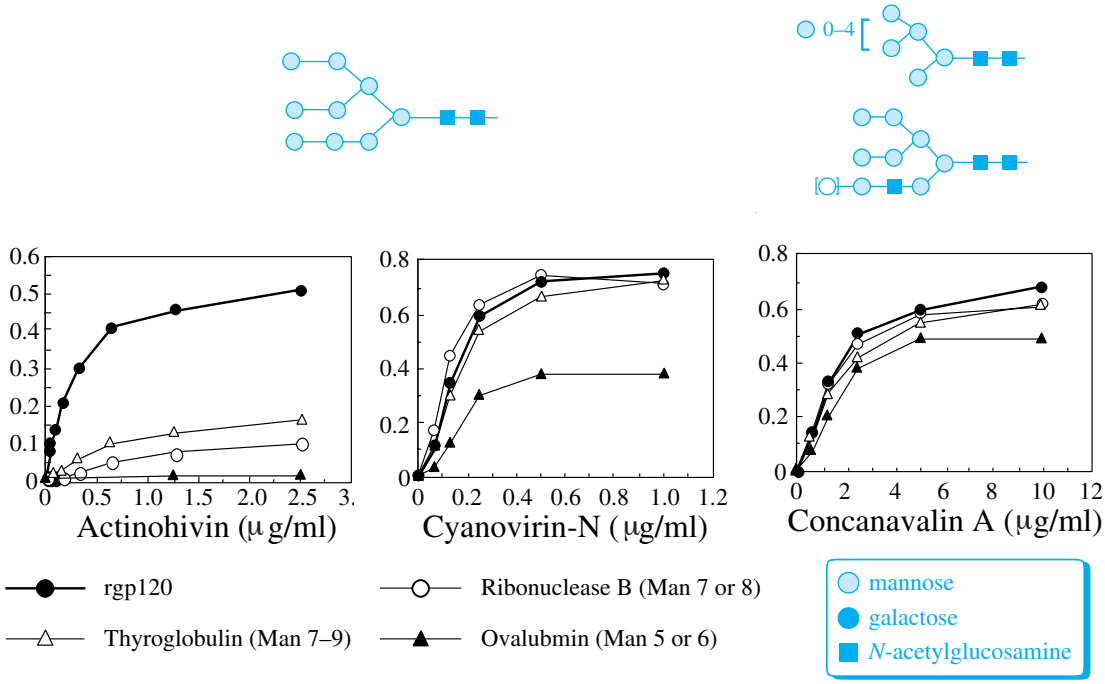
a: determined by frontal affinity chromatography b: colorimetry c: biosensor IAsys

(KD: M)

5. Mutants with improved activities[8)]

A mutant of AH, His-Seg 1 trimer, of which segments 2 and 3 are replaced with segment 1, exhibited 2-fold high syncytium formation inhibitory activity. His-TEV-AH dimer/RTB-L containing two AH molecules shows about 20-fold high anti-syncytium formation activity compared with that of His-TEV-AH. Furthermore, His-TEV-AH dimer/RTB-L had 2-20-fold anti-HIV activity against various primary isolates, including strains resistant to inhibitors of reverse transcriptase and protease.

Binding affinities of actinohivin, cyanovirin-N and concanavalin A to known high-mannose type glycoproteins[5)]



Syncytium formation inhibiting activities of AH and AH dimers[8)]

Protein	Structure	IC_{50} (nM)
His-TEV-AH	His tag, TEV*1, Segment 1, Segment 2, Segment 3	127
Recombinant AH	Segment 1, Segment 2, Segment 3	113
His-TEV-AH dimer /RTB-L	-Q-K-W-L-P-T-N-N-T-Q-P-F-V-T-T-I-R-N-A- (RTB*2)	7
AH dimer/RTB-L		14

*1 TEV : TEV protease recognition sequence
*2 RTB : residues 132-143 of ricin B chain

Anti-retroviral activities of actinohivin by MAGI assay[5)]

Virus	IC_{50} (μM)
T-tropic HIV-1	
IIIB	0.002
NL4-3	0.016
O18A (primary isolate)	0.11
M-tropic HIV-1	
JR-CFS	0.038
HIV-2	
ROD	0.014
EHO	0.004

Anti-HIV activities of AH and His-TEV-AH dimer/RTB-L against clinical isolates[8)]

		IC_{50} (nM)	
HIV strain	resistance	AH	His-TEV-AH dimer/RTB-L
307	sensitive	620	35
36	sensitive	>10,000	4
Bal	sensitive	34	4
214	sensitive	44	6
251	RTI/PI	23	7
NL4-3	sensitive	34	2
182	NNRTI/PI	30	9
242	RTI/NNRTI/PI	140	76
158	RTI/NNRTI/PI	10	2

RTI: reverse transcriptase inhibitor NNRTI: non-nucleotide RTI PI: protease inhibitor

6. References

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