Metal-Chelating Inhibitors of a Zinc Finger Protein HIV-EP1. Remarkable Potentiation of Inhibitory Activity by Introduction of SH Groups

Masami Otsuka, Mikako Fujita, and Yukio Sugiura

HIV-EP1 is a C_2H_2 type zinc finger protein which binds to DNA kB site present in the long terminal repeat of HIV provirus. Previously we have reported zinc chelators having histidine-pyridine-histidine skeleton and were successful to inhibit the DNA binding of HIV-EP1 by removing zinc from the zinc finger domain. Aiming at the potentiation of the inhibitory activity, we synthesized novel chelators comprising pyridine and aminoalkylthiol. These showed marked inhibitory activity on the DNA binding of HIV-EP1. In particular, one of them having bis(2-mercaptoethyl)amino side chain showed inhibitory activity (IC_{50}, ~4μM), 10 times stronger compared with the strongest inhibitor that we reported previously.

Key words: HIV-EP1 / Zinc finger / Transcription factor / DNA-binding

Zinc finger proteins constitute a major group of transcription factor and play important roles in the gene expression at the terminus of cellular signal transduction. Our interest has been focused on a C_2H_2 type zinc finger protein HIV-EP1 which binds to DNA kB site (5'-GGGACTTTCC-3') present in the long terminal repeat of HIV provirus to activate the HIV-1 gene expression. Inhibition of HIV-EP1 would lead to the interference of the replication of AIDS virus. In the previous ICR Annual Report, we described a new strategy for the inhibition of zinc finger proteins, i. e. removal of zinc from the finger domain by use of chelator. Thus, heterocyclic ligands comprising a dimethylaminopyridine and histidine units such as 1 exhibited remarkable zinc-binding capability and showed marked inhibitory effect on the DNA binding activity of HIV-EP1.

We now intended to replace the imidazole in the inhibitor **1** by mercapto group since mercapto group is contained in all known zinc finger proteins as a key ligating residue. It was considered that replacement of the imidazole moiety of our previous synthetic chelators by a mercapto group would alter the fundament of the metal binding characteristics and hence we prepared novel chelators **2** and **3** and some related sulfur-containing ligands.

Compounds **2** and **3** were found to be easily autoxidized under basic condition, resulting in the formation of disulfides whose main constituents were those assignable to **11** and **12**.

BIOORGANIC CHEMISTRY —Bioactive Chemistry—

Scope of research

The major goal of our laboratory is to elucidate the molecular basis of the activity of various bioactive substances by biochemical, physicochemical, and synthetic approaches. These include studies on the mechanism of sequence-specific DNA cleavage by antitumor or carcinogenic molecules, probing the DNA fine structure by various chemicals, studies on the DNA recognition of zinc-finger proteins, construction of artificial restriction enzyme, and model study on the cooperative mechanism of DNA binding by dimeric peptides. Also studied are the design and synthesis of functional molecules that effectively regulate the intracellular signal transduction or that applicable to fluorescence detection of DNA.

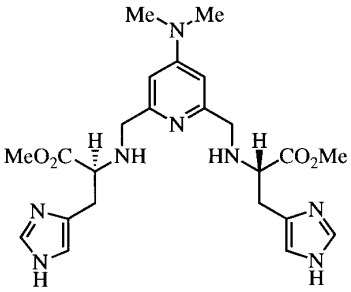
Prof
SUGIURA, Yukio
(D Pharm Sci)

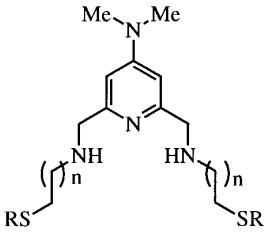
Assoc Prof
OTSUKA, Masami
(D Pharm Sci)

Instr
MORII, Takashi
(D Eng)

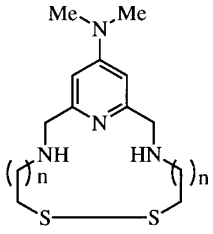
Assoc Instr
OKUNO,Yasushi

StudentsNAGAOKA, Makoto (DC)
AIZAWA, Yasunori (DC)
INOUE, Teruhiko (DC)
KUBOTA, Naoki (MC)
SAWADA, Mamoru (MC)
YASOSHIMA, Kenichi (MC)
ARAKI, Michihiro (MC)
KAMIUCHI, Tatsuya (MC)
SASAKI, Daisuke (MC)
YOKONO, Masanori (MC)
SAEGUSA, Nana (UG)
SUZUKI, Kazuo (UG)
HARA, Yuji (UG)
MATSUSHITA, Keizo (RS)





2 R = H, n = 1
3 R = H, n = 2
4 R = *t*-Bu, n = 1
5 R = *t*-Bu, n = 2
6 R = Trt, n = 1
7 R = Me, n = 1
8 R = Me, n = 2
9 R = S-Nps, n = 1
10 R = S-Nps, n = 2



11 n = 1
12 n = 2

We found disulfides **9**—**12** to be stable in air, easy to handle, and quantitatively reducible by dithiothreitol to generate either **2** or **3** in *situ*. Therefore, we employed **9**—**12** as practical equivalents for **2** and **3** in the biochemical experiments described below.

The inhibitory effects of imidazole compound **1**, mercapto compounds **2**, **3**, and alkylthio compounds **4**—**8** on the DNA binding of HIV-EP1 were studied by electrophoretic mobility shift assay. Compound **2** or **3** generated from **9** or **10** was indistinguishable from that obtained from **11** or **12** in terms of the inhibitory activity. Mercapto compounds **2** and **3** exhibited remarkable inhibitory effect much stronger than that of imidazole compound **1**. The most potent was compound **2**, which inhibited DNA binding of HIV-EP1 almost completely at 30 μM concentration, whereas 300 μM of **1** was required for the effective inhibition. IC_{50} of **2** was ~4 μM. Thus, inhibitory activity of compound **2** was shown to be 10 times stronger than that of **1**. *tert*-Butylthio, trirylthio, and methylthio analogues **4**—**8**, and other thiols, e. g. 2-aminoethanethiol, glutathione, and dithiothreitol, showed markedly lowered inhibitory effect at 30 μM concentration. It should be noted that inhibitory effect of 2-aminoethanethiol was small but significant because this constitutes the side chain of the inhibitor **2**, demonstrating the effect of assembling the 2-aminoethanethiol units on a pyridine ring in potentiating the inhibitory activity.

As previously reported, compound **1** was shown to abstract zinc from the zinc finger site of HIV-EP1 because the DNA-HIV-EP1 binding was restored by the addition of zinc before or after the inhibition reaction. In contrast, when zinc was introduced after the DNA binding inhibition reaction with **2** or **3**, virtually no or limited recovery of HIV-EP1-DNA complex was observed. This mechanism of the inhibition, seemingly distinct from that of our previous inhibitors, could be a new clue to the specificity issue to distinguish zinc finger proteins, which is our next subgoal.

References

1. Otsuka M, Fujita M, Sugiura Y, Ishii S, Aoki T, Yamamoto T, Inoue J, *J. Med. Chem.*, **37**, 4267-4269 (1994).
2. Otsuka M, Fujita M, Aoki T, Ishii S, Sugiura Y, Yamamoto T, Inoue J, *J. Med. Chem.*, **38**, 3264-3270 (1995).
3. Fujita M, Otsuka M, Sugiura Y, *J. Med. Chem.*, **39**, 503-507 (1996).