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INFORMATION

Hokkaido University confered the degree of Doctor of Philosophy (Ph. D) in Veterinary Medicine on March 25, 1997 to 25 recipients and June 30, 1998 to 3 recipients.

The titles of their theses and other information are as follows:

Studies on the inhibitory activity of 5'-end modified guanine-rich oligodeoxynucleotides against human immunodeficiency virus type 1 infection in vitro

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A number of in vitro anti-HIV agents have been reported and inhibitors of HIV reverse transcriptase and protease were proven to be effective in HIV-infected patients. However, drug therapy for acquired immunodeficiency syndrome (AIDS) currently approved for use is limited to above two classes of drugs. Drug-resistant variants eventually and serious side effects appear after treatment with current HIV drugs. Therefore, the current trend in chemotherapy for AIDS is desired to use different agents in combination, since appropriate agent combinations could make good synergistic efficacy against HIV, minimize potential side-effects and delay the emergence of such variants. In order to obtain a good combination partner which possesses novel mechanism of action, I began my investigation to apply so called antisence approach. Among the various dimethoxytrityl(DmTr)-modified oligodeoxynucleotides(ODNs) tested for anti-HIV-1 activity, SA-1042 (5'-DmTr-TGGGAGGTGGGTCTG-3') was evaluated potent in inhibiting cytopathic effect of HIV-1 in MT-4 cells. The study of mode of action indicated that SA-1042 prevented the virus adsorption and fusion steps of HIV replication by binding to gp120, but it did not act as an antisense molecule. Unlikely known adsorption and fusion inhibitors, dextran sulfate and soluble CD4, susceptibility of SA-1042 was active to both fresh HIV-1 clinical isolates and laboratory strains with syncytium-inducing (SI) phenotype and non-SI phenotype in human peripheral blood mononuclear cells, respectively.

The study identifying the minimum sequence of SA-1042 required for achieving potent activity revealed that a 5'-modified 6-mer (SA-1080: 5'-DmTr-TGGGAG-3') was the best sequence for full activity. Optimization of 5'-end substituents of the 6-mer ODN was also performed. A dibenzyloxybenzyl(DBB) group was found to be the best of all tested substituent with respect to both the potent anti-HIV activity and cytotoxicity.

Based on the biophysical and the computer-aided study, three-dimensional graphics model of 6-mer ODNs (R-95288) bearing a DBB group at 5'-end and a 2-hydroxyethylphosphate group at 3'-end was made. It consisted of a parallel stranded G-quadruplex folded into an intermolecular four G-tetrad and one A-tetrad, being similar to ISIS5320 with anti-HIV activity in the reported G-quadruplex compounds. However, the role of the 5'-end-substituent for antiviral

action is unique compared to other G-quadruplex compounds : that is, 5'-end modification of G-rich ODNs enhanced stability, ability of forming a hyperstructure and capability of binding to gp120, resulting in interference with the binding of virus to receptors. Thus, I concluded R-95288 as a unique G-quadruplex agent with potent anti-HIV-1 activity.

The results in this thesis demonstrate that the short modified ODN compound exhibit potent anti-HIV-1 activity in vitro by specific interaction with the V3 region and CD4 binding site region of HIV-1 gp120. Inhibitors of virus adsorption or entry to host cells are attractive combination candidates. Taken together, 5'-modified G-rich ODN may provide a new class of HIV chemotherapy for HIV infectious disease, therefore I will expect it as new combination partner in chemotherapy for AIDS.

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BIOCHEMICAL STUDIES ON BOVINE FERRITIN

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Ferritin (Ft), an iron-storage protein, is an intracellular protein with a molecular weight of 480 kDa, but also present in serum. Serum Ft level reflects the amount of iron stored in the body, and increases in inflammatory and malignant diseases. The fetal serum Ft level gradually increases as gestation progresses. The aims of this study are to clarify the molecular structure of bovine Ft and to assess the pathophysiological significance of the serum Ft. The results are summarized as follows :

1) To clarify what causes the reversed mobility of bovine Ft H(eart)- and L(iver)-subunits (H : 18 kDa, L : 21 kDa) compared to other mammalian Ft subunits by SDS-PAGE, the cDNA clones for bovine Ft H- and L-subunits were isolated from a bovine spleen cDNA library and sequenced. The bovine H and L chains were composed of 180 and 174 amino acid residues with calculated molecular weights of 20,920 and 19,856, respectively. The amino acid sequence of bovine H and L chains shows high homologies with those of the corresponding chains of the other mammalian Fts. These results suggest that the much slower mobility of bovine L chain compared to other mammalian L chains on SDS-PAGE may result from significant differences in the binding affinity of SDS to these L chains.

2) To access clinical significance of measurement of serum Ft in bovine serum, a highly sensitive ELISA for bovine serum Ft was developed using avidin-biotin complex technique. Serum Ft levels of cows with leukemia, inflammatory diseases, and theileriasis were significantly higher than those of nonpregnant cows. These results suggest that bovine serum Ft may be inflammation and malignant markers and that high Ft levels of theileriasis caused by *Theileria sergenti* result from the activation of reticuloendothelial system.

3) To elucidate physiological roles of serum Ft in bovine fetal circulation, the levels of Ft and its iron in fetal bovine sera were estimated. In 13