Study on the inhibition of protein production by L-cysteine derivatives of nucleic acid bases

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ABSTRACT
Isopoly (S-carboxymethyl-L-cysteine) derivatives of nucleic acids bases were prepared as antisense compounds. In previous studies, we investigated the properties of these compounds in vitro, and revealed that these compounds in vivo regulated the cell death presumably due to the inhibition of protein production. In this study, western and northern blots were carried out in order to reveal the mechanism of this inhibition for N-methyl-D-aspartate receptor in neuroblastoma x glioma hybrid NG108-15 cell line. In addition, we investigated the resistance of these compounds against cell extract and the metabolism. In conclusion, we proved that these compounds inhibited the protein production by antisense mechanism.

INTRODUCTION
Antisense compounds are paid more attention after the genome project. So many kinds of antisense are studied and most of it has a similar structure to DNA. On the other hand, there are other types of antisense like PNA. These compounds are synthetic polymer having nucleic acid bases. Nevertheless there are many kinds of antisense, there is a serious problem of a lower permeability. Some kinds of carrier have been studied to increase permeability.

Oligomers of isopoly (S-carboxymethyl-L-cysteine) derivatives of nucleic acids bases (CAS, Fig. 1) were prepared as antisense compounds, that were designed in our laboratory [1-7]. The structure of it is different from DNA. That is to say, it has peptide bond of L-cysteine instead of phosphodiester bond. Peptide bond was introduced into it for the purpose of stability against nucleases. The synthesis of CAS is very simple, so it is possible to synthesize CAS oligomers of many different length and sequences in useful quantities. These oligomers were found to form a stable complex with oligomers of DNA or RNA in vitro and inhibit the cell death in vivo. but we could not reveal the mechanism of that inhibition. In this study, we investigate the effect of the cysteine derivatives in order to reveal the mechanism as antisense compounds. The target of the antisense compounds is zeta 1 subunit of N-methyl-D-aspartate receptor (NMDAR) in neuroblastoma x glioma hybrid NG108-15 cell line. Development of NG108-15 cell line was induced by all trans retionic acid. Expression of NMDAR was detected by immunoperoxidase staining against NMDAR.[8]
RESULTS AND DISCUSSION

Cysteine monomers of nucleic acid bases were prepared by coupling of aminoethyl bases with S-carboxymethyl-L-cysteine, which was prepared by the reaction of L-cysteine hydrochloride monohydrate and ethyl bromoacetate (Scheme 1). The oligomers were prepared by solid phase synthesis using p-nitrobenzophenone oxime resin prepared from Bio Bease SX-1 (200 ~400 mesh). The first amino acid was glycine as a spacer, and the last amino acid was L-lysine for the improvement of water solubility (Figure 2). After synthesis, they were purified with RP-HPLC. The purity of them was confirmed by gel permeation chromatography (GPC).

In this study we used antisense compounds for the region near the starting codon of NMDAR subunit NMDAR1 sequence (CAS), and fluoresceine-labeled poly A oligomers for resistance and metabolism (Figure 2).

We revealed that the cells dealing with these compounds targeting the NR1 protein-mRNA showed the loss of protein-production depending on the concentration of the cysteine derivatives.

In previous study, CAS can be transfected into cells without carrier, and we evaluated cell death by counting live cells and analyzed protein by Western blotting. Cell cultures were preincubated with oligomers, and incubated further with retinonic acids. After that, we carried out each measurements. As a result, it observed that expression of NMDAR detected by immunoperoxidase staining against NMDAR was decreased compared with control. However, we observed NMDAR1 mRNA by northern blotting. In addition, we could observe the resistance and metabolism. In conclusion, we revealed that this cysteine oligomer has the excellent ability as antisense reagent, and inhibited the production of protein by antisense mechanism.

CONCLUSION

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References