Nucleic Acid Analogues with Amino Acids

Shoko ABE* and Takuo CHIBA

(received November 21, 2001)

The recent antiviral agents were nucleic acid derivatives for the inhibitor of DNA replication and peptides for protease inhibitor in the virus cell. We synthesized the nucleic acid analogues (pyrimidine bases) with amino acids by the application of Biginelli reaction. Namely, the acetoacetyl amino acids, which were easily prepared by the reaction of diketene with amino acids, were used instead of acetoacetyl ethyl ester for the formation of pyrimidine ring. The synthesized pyrimidine bases were tested for the inhibition of several viruses.

Since a virus depends on its host cell for many functions of virus replication, it is difficult to inhibit virus multiplication without at the same time affecting the host cell itself. Because of this, the spectacular medical successes following the discovery of anti-bacterial agents have not been followed by similar success in the search for specific antiviral agents. A few antiviral compounds are successful in controlling virus infection in laboratory situations and certain of these have been used in restricted clinical cases; but no substance has yet been found with more than limited practical use. The chemical inhibition of virus action is known to occur at the stages of virus replication: for example, Kethoxal for free Influenza virus (FLUV); Amantazine for FLUV and Benzoyloxy carbonyl peptide for Measles at the stage of the entry of Nucleic acid; Benzimidazole and Guanidine for Poliovirus, Fluorodeoxyuridine, Iododeoxyuridine, and Ancylovis for Herpesvirus (HSV), and Azidothymidine for Human immunodeficiency virus (HIV) at the stage of the Nucleic acid replication; Isatin thiosemicarbazone for Smallpox virus at the stage of the late protein synthesis. At the stage of the adsorption and the release, however, none of the therapeutic antiviral agents are known.

The glycoprotein, designed F (fusion polypeptide), is involved in virus penetration, through fusion of viral and cell membranes, and in virus-induced cell fusion and hemolysis, activities which are activated by a host protease to yield two disulfide-linked polypeptides, F1 and F2.

The proteolytic cleavage that activates these biological properties of the virus generates a new N-terminus on the F1 polypeptide, which is the polypeptide whose C-terminus is associated with the lipid bilayer of the viral membrane. The following lines of evidence suggested that the structure of the new N-terminal is important for the expression of the biological activities of the F protein, each of which involves membrane fusion: (1) as indicated above, the expression of the activities is dependent on the cleavage; (2) the new N-terminal region is extremely hydrophobic, raising the possibility that it could be involved in interactions with the target cell membrane; (3) the amino acid sequence in this region is highly conserved among different parvoviruses; (4) in a mutant of Sendai virus which is cleaved and activated by a protease different from that which activates the wild type virus, the N-terminal amino acid sequence of F1 is the same as that of the wild type; i.e., Phe-Phe-Gly-providing further evidence for the requirement for a specific amino acid sequence in that region for biological activity; (5) finally, we noted a similarity between this N-terminal amino acid sequence of F1 and that of an oligopeptide, benzoyloxy carbonyl-D-Phe-L-Phe-L-(nitro)Arg was found in 1968 to inhibit plaque formation by

* Advanced course of ANCT
measles virus. Subsequently Norrby showed that this oligopeptide, and some others with similar observations, inhibited measles virus penetration and virus-induced cell fusion and hemolysis, and we made similar observations with another paramyxovirus, SV5. Because of the indication that the N-terminal region of F1 was involved in the biological activities of the protein, and with the hypothesis that it might be possible to inhibit these activities of the protein, we synthesized a pyrimidine base with peptides which resembled this region of F1 polypeptide, and investigated their ability to inhibit the replication of several different paramyxoviruses. The hemagglutinin protein of myxoviruses is also cleaved by a protease supplied by the host cell or present in the extracellular fluid, e.g., serum plasmin, and although this cleavage has no effect on hemagglutination, it activates the infectivity of the virus. As in the case of paramyxoviruses, the cleavage of the HA protein of influenza virus yields two disulfide-linked subunits, HA1 and HA2, and a new N-terminus is generated on the HA2 subunit, the C-terminus of which is embedded in the membrane. Thus, there is a structural and functional analogy between the paramyxovirus F protein and the myxovirus HA protein, an analogy that is strengthened by the fact that the sequences of the first nine amino acids of the N-terminus of the HA2 polypeptide of several influenza virus strains resemble that of the F1 polypeptide of paramyxoviruses, except that in influenza virus there is an N-terminal glycine that precedes phenylalanine, which is the N-terminus of the F1 polypeptide. Because of these structural and functional similarities between the HA and F proteins, pyrimidine base with peptides were synthesized which resembled the N-terminus of the influenza HA2 polypeptide and tested for their inhibitory activity.

Materials and Methods

Cells. The MDBK line of bovine kidney cells was grown in reinforced Eagle’s medium (REM) with 10% fetal calf serum as described. An epithelioid clone of CV-1 line of African green monkey cells obtained from Dr. E.L. Gershey was grown in Eagle’s minimal essential medium (MEM) as described by D’Alisa and Gershey. Viruses. The Edmonston strain of measles virus, originally obtained from Dr. E. Norby, was grown in Vero cells in the absence of serum as described. A mutant of this strain (R93) which was resistant to Z-Phe-Phe-(NO2) Arg was selected by Dr. M. C. Graves by three successive selection of plaques which formed in the presence of this oligopeptide at a concentration of 40 µM. Canin distemper virus (CDV), originally obtained from Dr. M. Appel, was supplied by Dr. W. W. Hall and propagated in CV-1 cells. Wild-type Sendai and Newcastle disease viruses were grown in the allantoic sac of 11-day-old embryonated chicken eggs inoculated with ~10⁶ egg-infective doses as described. The WSN strain of influenza A virus and W3 strain of SV5 were grown in MDBK cells.

Plaque assays. Plaque assays were done in CV-1 cells. Virus stocks were diluted to yield 100-200 plaques on monolayer in plastic petri dishes in the absence of oligopeptides, and aliquots of these dilutions were stored in 0.2% BSA at −70 degree until use. Cells were inoculated with 2 ml of virus in the presence or absence of designated pyrimidine bases, and after a 2 hr adsorption period at 37 degree, the medium was removed and replaced with an agar overlay containing REM and appropriate pyrimidine base. The overlay used in Sendai and influenza virus plaque assays also contained N-acetyltrypsin, 0.3 or 0.5 µg/ml, respectively. The monolayers were incubated for 2-3 days at 37 degree, and a second agar overlay containing REM, 2% fetal calf serum, and 0.004% neutral red was added. Plaques were counted 6-12 hr later.

Results. Biginelli reported that the reaction of ethyl acetoacetate with urea and benzaldehyde gave the 4-phenyldihydropyrimidone in a favorable yield. In this paper, the acetoacetyl amino acids easily prepared by the reaction of amino acids with diketene were used instead of
acetoacetyl ester. The reaction condition is almost the same as original Biginelli method, and we used the trace amount of concentrated hydrochloric acid as a catalyst. In the purpose of increasing of the solubility and the antiviral activity of the drugs, aromatic benzaldehyde was replaced by aliphatic isobutyl aldehyde, and thiourea was used instead of urea. In the case of using benzaldehyde and urea, the yields of phenyldihydropyrimidone were 12~71%, otherwise, the yields of isopropylidihydropyrimidone were low. In the case of using benzaldehyde and thiourea, phenyldihydropyrimidithiones were obtained in 7-69% yields.

It has become apparent that the Biginelli reaction could be applied for the synthesis of substituted pyrimidone derivatives. All drugs were tested for antiviral activity against HIV, HSV, FLUV-A, and Measles viruses. None of all compounds were cytotoxic for host cells as CC₅₀ 200 μg/ml. Unfortunately, active drugs were not found in this research.

References

8) P. Biginelli, Ber., 24, 1317 (1891).