Action of Proflavine on RNA Synthesis of Newcastle Disease Virus

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Proflavine inhibits DNA-dependent RNA synthesis in a way similar to actinomycin (1). It binds to DNA (2) and RNA (3, 4) and interferes with virus multiplication (4, 5). A dose-effect relationship has revealed that its binding capacity to DNA goes in parallel with the inhibition of cellular RNA synthesis and with the inhibition of the formation of fowl plague virus; while its binding capacity to RNA parallels the inhibition of protein synthesis and the inhibition of the formation of Newcastle disease virus (4). Therefore it has been suggested that proflavine inhibits fowl plague virus multiplication by the same mechanism as actinomycin does. The inhibition of Newcastle disease virus formation, however, should be due to an interference with an RNA template or t-RNA (6), inhibiting protein synthesis.

This investigation was concerned with the action of proflavine on the RNA-dependent RNA synthesis. The multiplication of Newcastle disease virus, strain ITALien, in primary chick fibroblast cells was chosen as a model. Cultures were infected, and different doses of proflavine were added at various times after infection. Viral RNA synthesis was measured by incorporation of $^3$H]uridine (2·18 c/m-mole) in the presence of actinomycin (2 µg./ml.) to suppress specifically cellular RNA synthesis (7). (For further details see references (4) and (8)). Proflavine (free base) was a gift of the Bayerwerke, Leverkusen, Germany; actinomycin was provided by Merck, Sharp and Dohme, New York.

When the inhibitor was added immediately after infection the synthesis of viral RNA was strongly inhibited with about 16 µg./ml. proflavine (Fig. 1). This agrees with former observations that between 16 and 32 µg./ml. proflavine inhibited the multiplication of Newcastle disease virus and strongly reduced the incorporation of $[^{14}$C]-leucine into cellular protein (4). After prolonged treatment higher doses of proflavine interfered markedly with the labelling of the pool of trichloracetic acid soluble nucleotides by $^3$H]uridine (Fig. 2 b) as has been found also with actinomycin (9). This reduced incorporation of $^3$H]uridine into soluble nucleotides in the presence of high doses of proflavine and of actinomycin accounts for the low radioactivity in the RNA (Fig. 1).

When proflavine was added to the cultures 5 hr after infection, considerably higher doses were necessary to show a significant effect on the RNA synthesis of Newcastle disease virus. But even 50 µg./ml. did not suppress viral RNA synthesis completely. If proflavine was added simultaneously with actinomycin to tissue cultures, the inhibitory action on cellular RNA synthesis of the latter was somewhat counteracted by the former (Fig. 1, control curve).

When 30 µg./ml. proflavine was added at various times after infection, viral RNA synthesis proceeded readily if the inhibitor was added later than 3 hr after infection but not if it was added 3 hr or earlier after infection. In another experiment there was some viral RNA synthesis already, when the inhibitor was added at 3 hr after infection.

About 2 to 3 hr elapses before the synthesis of a stable 'early protein' necessary for viral RNA synthesis starts in cells infected with Newcastle disease virus (10, 11).
Short communications

Thirty μg./ml. proflavine probably inhibited the synthesis of this ‘early protein’ by interfering with a template activity of a single stranded RNA or with t-RNA (Fig. 2). If this stable ‘early protein’ was synthesized, however, the same dose of proflavine had no or only little effect on the synthesis of viral RNA. Its action here resembled that of actinomycin which also does not interfere with the synthesis of RNA-dependent RNA (7). In contrast to actinomycin, however, proflavine binds to single-stranded RNA and in this way inhibits protein synthesis and the initial phase in the formation of Newcastle disease virus.

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Fig. 2. Influence of 30 μg/ml proflavine added at different times after infection on the RNA synthesis of Newcastle disease virus and on the RNA precursor pool. Experimental conditions as in Fig. 1. ×—×, infected cells; ●—●, uninfected cells.

REFERENCES


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