INHIBITION OF DIRECTED PROTEIN SYNTHESIS BY CHLORAMPHENICOL: EFFECT OF MAGNESIUM CONCENTRATION*

by

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The ability of chloramphenicol to inhibit induced protein synthesis in mammalian cell-free systems varies with the type of stimulatory RNA employed (Weisberger, Armentrout and Wolfe, 1963; Weisberger, Wolfe and Armentrout, 1964). Protein synthesis induced by polyuridylic acid (poly U) is more resistant to chloramphenicol inhibition than that induced by natural template RNA isolated by phenol extraction of reticulocyte ribosomes. The variations in sensitivity to chloramphenicol inhibition may be related to differences in base composition and to differences in ribosomal binding of the various RNA preparations employed. Accordingly the interaction of these RNA preparations with ribosomes may not necessarily reflect that which occurs with natural messenger RNA (mRNA) and the inhibitory effect of chloramphenicol may be peculiar to the RNA preparation used. It is therefore desirable to demonstrate that chloramphenicol can inhibit the function of natural mRNA capable of directing the synthesis of specific proteins in a mammalian system.

This report presents evidence for the inhibition of mRNA directed protein synthesis by chloramphenicol. Hybrid polysomes were prepared with

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ribosomes obtained from one mammalian species and mRNA obtained from a different mammalian species. These hybrid polysomes have been shown to synthesize polypeptides coded by the mRNA (Weisberger and Armentrout, 1966). Inhibition of this mRNA function by chloramphenicol is markedly sensitive to changes in magnesium ion concentration.

Material and Methods

Reticulocytosis was induced in New Zealand white rabbits and in North American White Tail deer (Odocoileus Virginianus) with phenylhydrazine and ribosomes were prepared. The ribosomes were stored in 0.25 M sucrose at -70° C. A messenger ribonucleoprotein fraction (mRNP), previously shown to contain mRNA capable of directing antigenically specific polypeptide synthesis was prepared from the deer ribosomes. The ratio of protein to RNA in the mRNP was 3:1 by weight.

The magnesium requirements for protein synthesis by rabbit reticulocyte ribosomes was determined, both with and without the addition of stimulatory mRNA. Three to five μgms of stimulatory RNA obtained from deer reticulocyte ribosomes was added to rabbit reticulocyte ribosomes and the ability of chloramphenicol to inhibit the induced protein synthesis in the presence of varying concentrations of magnesium was determined. Cell-free protein synthesis reaction mixtures, assay techniques and materials were previously described (Weisberger and Armentrout, 1966).

Results

The optimum magnesium concentration for cell-free protein synthesis in the system employed was 4.0 mM both with and without added mRNA. Complete inhibition of protein synthesis induced by the added mRNA was obtained with 0.1 mM chloramphenicol in the presence of 6 mM magnesium. Inhibition of induced protein synthesis was markedly affected by varying the magnesium concentration (Table 1).

Table 1

Inhibition of Directed Protein Synthesis by Chloramphenicol:

Effect of Magnesium Ion Concentration

	NET STIMULATION (C/M)*		
Magnesium acetate mM	Without Chloramphenicol	With Chloramphenicol (0.1 mM)	Inhibition By Chloramphenicol (%)
0	15	13	0
2	105	69	32
4	1011	130	87
6	630	2	100
8	765	439	28
10	766	720	15
12	767	717	8

*Incorporation of C^{14} leucine into hot trichloracetic acid precipitable material. This represents the increase in CPM above endogenous protein synthesis.

Almost complete inhibition of the induced protein synthesis was obtained with magnesium concentrations ranging from 4 to 6 mM. Considerably less inhibition of induced protein synthesis was obtained with higher magnesium concentrations, even though stimulation of protein synthesis by the added mRNA was constant. As previously observed, the inhibition by chloramphenicol of endogenous protein synthesis by rabbit reticulocyte ribosomes was negligible.

Comment

In bacterial systems, current evidence supports the hypothesis that chloramphenicol acts on a ribosomal site at a stage after binding of mRNA and during peptide synthesis to prevent the final condensation of amino acids and the growth of nascent polypeptide chains (Weisberger, 1967). None of the interpretations applied to the mode of action of chloramphenicol in bacterial

systems accounts for the resistance of endogenous protein synthesis in mammalian systems and the sensitivity to chloramphenicol which occurs only when stimulatory RNA is added. Accordingly the mechanism of action of chloramphenicol may not be precisely the same in microbial and in mammalian systems. It has been suggested that chloramphenicol may inhibit protein synthesis in mammalian systems by preventing the binding of mRNA to ribosomes. The magnesium requirements for optimal inhibition of mRNA function by chloramphenicol are compatible with such an hypothesis. Although magnesium concentration can affect a number of steps in protein synthesis, it is known that magnesium ions are essential for binding mRNA to ribosomes. Maximum inhibition of mRNA function occurred with 4 to 6 mM magnesium and it is suggested that the lack of inhibition by chloramphenicol of induced protein synthesis at higher magnesium concentrations might be due to competitive displacement of the antibiotic from ribosomes by magnesium during the binding process. At low magnesium concentrations, there is relatively little binding of mRNA to ribosomes and most of the protein synthesis is attributable to RNA already bound to ribosomes (endogenous). As has been previously noted, the function of mRNA bound to mammalian ribosomes is not susceptible to chloramphenicol inhibition and endogenous protein synthesis is not appreciably inhibited by the drug.

The mRNA preparations employed in these studies have been shown to be capable of directing the synthesis of specific proteins in a mammalian cell-free system (Weisberger and Armentrout, 1966). The interaction of this mRNA with reticulocyte ribosomes more closely represents binding of natural mRNA than that which occurs with synthetic template RNA preparations such as poly U. The ability of chloramphenicol to inhibit this induced protein synthesis is direct evidence that the drug interferes with the function of a natural mRNA preparation.

References

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