THE BINDING OF CHLORAMPHENICOL BY RIBOSOMES FROM BACILLUS MEGATERIUM

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It has been previously reported that ^{14}C-labelled chloramphenicol (^{14}C-CAP) is taken up by Staphylococcus aureus and Bacillus megaterium. After breakage and centrifugal fractionation all the radioactivity was found to be associated with the ribosomes and soluble fraction. Binding of ^{14}C-CAP by ribosomes also occurred in cell-free systems. This binding was immediate, not energy dependent, unaffected by the incubation temperature and very easily reversible (Vazquez, 1963).

Further studies have now been made on the binding of ^{14}C-CAP (1.3×10^6 counts/min/μmole) to B. megaterium ribosomes. The bacteria were broken either by ultrasonic disintegration or by osmotic breakage of protoplasts obtained by treatment with lysozyme. Essentially similar results were obtained with ribosomes prepared using either method of breakage. The ribosomes were found to require K^+ ions for the binding of ^{14}C-CAP. The concentration of K^+ ions required was higher when buffer containing 10^{-2} M Mg^{++} was used (Fig.1) than in the case of buffer containing 10^{-4} M Mg^{++} (Fig.2) suggesting that the ratio Mg^{++}/K^+ is important in the association of chloramphenicol to the ribosomes. The finding that 0.2M K^{++} was the optimal concentration for the binding to the 70s ribosomes is interesting as this corresponds to the internal

concentration of K^+ that has been previously reported in Escherichia coli (Schultz and Solomon, 1961). If there is a close relationship between the binding of ^{14}C-CAP to the ribosomes and the inhibition of protein synthesis the concentration of K^+ should be taken into account. K^+ had always been present in cell-free systems in which an inhibition of protein synthesis by chloramphenicol was observed. The concentrations of K^+ ranged from 3×10^{-2} M (Nathans and Lipmann, 1961) to 0.2 M (Rendi and Ochoa, 1962), but it is difficult to compare the inhibitions obtained because the systems were otherwise slightly different.

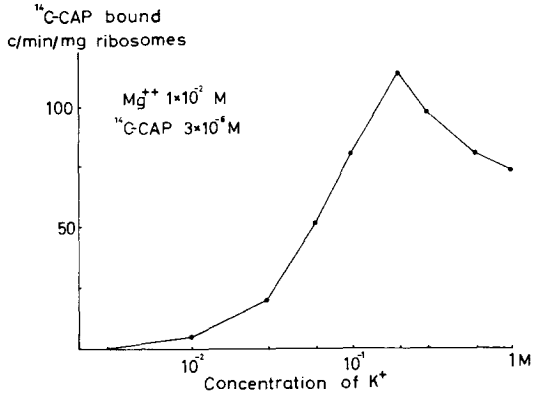

Fig.1 The effect of K^+ concentration on the binding of ^{14}C-CAP to ribosomes in buffer containing 10^{-2} M Mg^{++}.

^{14}C-CAP (3×10^{-6} M) was added to a preparation of ribosomes in 10^{-2} M Tris/HCl buffer pH 7.4 containing 10^{-2} M Mg^{++} and different concentrations of KCl. After 2 min at 4° the suspension was centrifuged at 150,000g for 60 min and the radioactivity in the pellet determined. In control experiments non-radioactive chloramphenicol (3×10^{-4} M) was present during the incubation period and ^{14}C-CAP was added at the end. Volume of incubation mixture was 2ml containing 4mg of ribosomes.

Studies of the effect of other monovalent cations on the binding of ^{14}C-CAP by the ribosomes showed that Li^+, Na^+ or Cs^+ ions cannot replace K^+ ions; no association of chloramphenicol

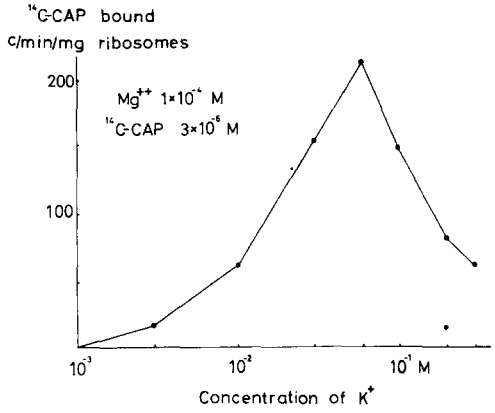

Fig.2 The effect of K^+ concentration on the binding of ^{14}C-CAP to ribosomes in buffer containing 10^{-4} M Mg^{++}.

The experimental conditions were the same as for Fig.1 with the exception that the concentration of Mg^{++} was reduced to 10^{-4} M. The ribosomes were centrifuged down at 150,000g for 130 min. Volume of the incubation mixture was 2ml containing 3mg of ribosomes.

with the ribosomes was found in the presence of these cations at concentrations ranging from 6 x 10^{-3} M to 6 x 10^{-1} M. However Rb^+ or NH_4^+ ions could replace K^+ ions and with NH_4^+ ions the binding was enhanced (Fig.3). This result is consistent with the finding that NH_4^+ can replace K^+ in some biological systems (Muntz, 1947). It has been reported that Rb^+ ions can replace K^+ ions for the growth of some bacteria (Lester, 1958). The effect of the various monovalent cations on the binding of ^{14}C-CAP to the ribosomes may be correlated with the radii of the hydrated ions; the radii of hydrated K^+, Rb^+ and NH_4^+ ions are very similar but differ from those of Li^+, Na^+ and Cs^+ ions.

In order to test whether chloramphenicol is bound to the 30s or 50s ribosomal component or to both, these components were partially separated by differential centrifugation and finally purified by centrifugation through a sucrose gradient. The fractions

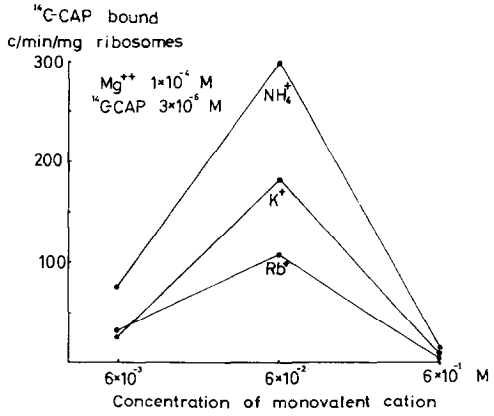

Fig.3 The effect of monovalent cations on the binding of ^{14}C-CAP to ribosomes in buffer containing 10^{-4} M Mg^{++}.

The experimental conditions were the same as for Fig.2. The effect of Li^+, Na^+, K^+, NH_4^+, Rb^+ and Cs^+ at three different concentrations (6×10^{-3} M, 6×10^{-2} M and 6×10^{-1} M) is shown. Binding of ^{14}C-CAP was not found in the presence of Li^+, Na^+ or Cs^+. Volume of the incubation mixture was 2ml containing 2.5mg of ribosomes.

corresponding to 50s and 30s peaks were taken independently and the ability of the ribosomes in these fractions to bind ^{14}C-CAP was studied in 10^{-2} M Tris/HCl buffer pH 7.4 containing either 10^{-2} M Mg^{++} and 0.2 M K^+ or 10^{-4} M Mg^{++} and 6×10^{-2} M K^+. It was found that 1 mμmole of ^{14}C-CAP was bound per 4 mg 50s ribosomes in 10^{-4}M Mg^{++} buffer and half this amount in 10^{-2}M Mg^{++} buffer. 30s ribosomes bound less than 1/20 of this amount under either condition.

No association of ^{14}C-CAP with any other structure or macromolecule in B. megaterium has been found. Binding of ^{14}C-CAP to polyuridylic acid could not be detected by equilibrium dialysis. Pretreating 70s ribosomes with polyridylic acid did not reduce the binding of ^{14}C-CAP to these ribosomes.

It has been shown that polyuridylic acid binds to the 30s ribosomes (Okamoto and Takanami, 1963) and that sRNA binds to 50s

ribosomes (Cannon, Krug and Gilbert, 1963). Jardetzky and Julian (1964) found 17% reduction in the binding of ^{14}C-labelled polyuridylic acid to 70s ribosomes in the presence of 20µg chloramphenicol/ml and 5 x 10^{-2} M K^{+}. The present work shows that chloramphenicol binds preferentially to the 50s ribosomes. Although polyuridylic acid and chloramphenicol bind to different sub-units, the presence of chloramphenicol on the 50s component may interfere with the functioning of the 70s aggregate.

References

Cannon, M., Krug, R. and Gilbert, W. (1963) J.mol.Biol., 7, 360.

Jardetzky, O. and Julian, G.R. (1964) Nature (Lond)., 201, 397.

Lester, G. (1958) J. Bacteriol., 75, 426.

Muntz, J.A. (1947) J. biol. Chem., 171, 653.

Nathans, D. and Lipmann, F. (1961) Proc. Nat. Acad. Sci. U.S., 47, 497.

Rendi, R. and Ochoa, S. (1962) J. biol. Chem., 237, 3711.

Okamoto, T. and Takanami, M. (1963) Biochim. biophys. Acta, 76, 266.

Robinson, R.A. and Stokes, R.H. (1955) Electrolyte solutions, Butterworths Scientific Publications, London.

Schultz, S.G. and Solomon, A.K. (1961) J.gen. Physiol., 45, 355.

Vazquez, D. (1963) Biochem. biophys. Res. Comm., 12, 409.