ACTINOMYCIN INHIBITION OF IN VITRO PROTEIN SYNTHESIS IN RAT LIVER

A. Korner and A.J. Munro

Department of Biochemistry
University of Cambridge, England

Received April 2, 1963

Actinomycin inhibits the synthesis of RNA in bacteria and in mammalian cells in culture (Kirk, 1960; Reich, Franklin, Shatkin and Tatum, 1962; Tamaoki and Mueller, 1962; Harbers and Muller, 1962; Hurwitz, Furth, Malamy and Alexander, 1962; Goldberg, Rabinowitz and Reich, 1962). At least three types of RNA are concerned in protein biosynthesis so that inhibition of RNA synthesis should be reflected in inhibition of protein synthesis (cf. Kirk, 1960). Meritz (1963) has recently shown that actinomycin inhibits all RNA synthesis in rat liver and the evidence presented below indicates that actinomycin inhibits protein synthesis in rat liver.

METHODS

Male albino rats were injected intraperitoneally with 0.3 mg. actinomycin D dissolved in 0.2 ml. propylene glycol. They were killed at various times afterwards and the livers removed rapidly into ice-cold 0.25 M sucrose. All operations were at 2° or less. The liver, weighed by displacement in sucrose, was homogenized and the homogenate centrifuged for 10 minutes at 15,000g. A portion of the supernatant was used to prepare cell sap by centrifuging it at 105,000g. for 4 hours and passing it through a sephadex (G 25) column in 0.05 M-tris buffer pH 7.6 containing 10 mM - Mg acetate, 100 mM - KCl, 40 mM - NaCl and 6 mM - mercaptoethanol (medium M). The remainder was treated with $\frac{1}{9}$th volume of 10% sodium deoxycholate in 0.05 M-tris buffer pH 8.2 and half was used to prepare ribosomes by methods previously described (Korner, 1961). The ribosomes were washed by respinning in medium M. The rest of the deoxycholate supernatant was used to prepare 'heavy' ribosomes (polysomes, ergosomes) (see Wettstein, Staehelin and Noll, 1963) by adding $\frac{1}{9}$th volume of 10 x M and layering 12 ml. on to a sucrose two-step gradient of 6 ml. 2.0 M and 6 ml. 0.5 M sucrose containing medium M and centrifuging at 100,000g for 4 hours. Ribosomes and polysomes were suspended

in M and were diluted with M to contain the same amount of 260 absorbing material. 0.3 ml. of ribosomes or polysomes were incubated at 37° with 0.5 ml. sap and 0.2 ml. of a 'cocktail' in M containing constituents which gave final concentrations in the 1 ml. of incubation fluid of 0.1 mM - GTP, 5.0 mM - ATP and 0.5 μC DL - [1-^{14}C] leucine. Reaction was stopped after 45 minutes by addition of 0.5 N - $HClO_4$ containing leucine and the protein worked up and its radioactivity assessed as described previously (Korner, 1961). Protein was determined by the Lowry method (Lowry, Rosebrough, Farr and Randall, 1951) and RNA as described before (Korner, 1961).

RESULTS and DISCUSSION

Actinomycin inhibits amino acid incorporation into protein in a ribosome or polysome cell-free system 6 and 12 hours after injection of 0.3 mg. of it into the rat (Table I; lines 1, 2, 3 and 6, 7, 8).

TABLE I

Cell constituents from rats given 0.3 mg. actinomycin at the times stated (in hours) before death.

	polysomes	ribosomes	sap	cpm/mg protein/mg particle RNA incubated	% inhibition
1.	0	-	0	4515	0
2.	6	-	6	2400	46.8
3.	12	-	12	1760	61
4.	6	-	0	3520	22
5.	12	-	0	3710	17.5
6.	-	0	0	3235	0
7.	-	6	6	880	72.8
8.	-	12	12	325	89.9
9.	-	6	0	2170	38.5
10.	-	12	0	1235	64.8
11.	-	0	6	1660	48.8
12.	-	0	12	980	69.7

Part of this inhibition is explained by the decreased ability of liver sap from actinomycin-treated rats to prepare amino acids for incorporation

into protein, for ribosomes from untreated rats (lines 11 and 12) showed 49% and 70% inhibition when incubated with sap from 6 hour and 12 hour actinomycin-treated rats, compared with the incorporation obtained when these ribosomes were incubated with control sap. Synthesis of s-RNA (apart from addition of terminal nucleotides) is inhibited in livers of rats given 1 mg. actinomycin seven hours before death (Meritz, 1963). The dose used in our experiments is 30% of this but it seems reasonable to ascribe the lessened activity of the liver sap of actinomycin-treated rats to lack of s-RNA. An alternative possibility is that synthesis of an essential and labile protein (one or more of the amino acid activating enzymes or one of the transfer enzyme(s), for example) may be inhibited and that lack of this factor accounts for the low activity of the liver cell sap of actinomycin-treated rats.

Not all of the inhibition can be explained by changes in the sap; part at least must be ascribed to changes in the ribosomes and polysomes, for particles from actinomycin-treated rats incorporate less amino acid into protein than those from control rats when incubated with sap from untreated rats (lines 1, 4, 5; 6, 9, 10). This inhibition cannot be explained in terms of actinomycin inhibition of synthesis of ribosomal structural RNA (though this is occurring; Meritz, 1963) since the ribosomes are less active per mg. RNA than those from untreated rats. Something must have occurred, other than an affect on the bulk of the RNA of the particles, and which inhibits incorporation. One possibility is that less messenger RNA is available in the liver of rats treated with actinomycin.

Ribosomes are inhibited by actinomycin to a greater extent than are polysomes (Table I) when the results are calculated per mg. particle RNA incubated. We have found that most of the 83 S particles are held up by the gradient used in preparing polysomes so that active particles are concentrated into the polysome pellet. In preparing polysomes from the livers of actinomycin-treated rats the more active particles are selected so that the activity of the material, per mg. RNA, is not greatly different from that from untreated rats although, of course, less of the active material is present in each gm. of liver. That the polysomes show inhibition may imply that more inactive particles are present in the liver from the actinomycin-treated rat and that more of these particles pass through the gradient. Present ideas about polysomes (Wettstein et al. 1963; Gierer, 1963; Warner, Knopf and Rich, 1963) suggest that a messenger RNA molecule has several 83 S particles attached to it. Our results suggest that one effect of actinomycin treatment of the rat is to inhibit the synthesis of messenger RNA in rat liver and that this causes the fall

in incorporation ability of the ribosome preparation and a fall in the number of polysomes.

ACKNOWLEDGMENTS

We thank Mrs B. Brown for highly skilled assistance, the Medical Research Council for financial help and the Royal Society for a grant to purchase a counter.

REFERENCES

Geirer, A., J. Mol. Biol., 6, 148 (1963).

Goldberg, I.H., Rabinowitz, M.L. and Reich, E., Proc. Nat. Acad. Sci., 48, 2094 (1962).

Harbers, E. and Muller, W., Biochem. Biophys. Res.Comm., 7, 107 (1962).

Hurwitz, J., Furth, J.J., Malamy, M. and Alexander, M., Proc. Nat. Acad. Sci., 48, 1222 (1962).

Kirk, K.M., Biochim. Biophys. Acta., 42, 167 (1960).

Korner, A., Biochem. J., 81, 168 (1961).

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., J.Biol. Chem., 193, 265 (1951).

Meritz, I., Biochem. Biophys. Res. Comm., 10, 254 (1963).

Reich, E., Franklin, R.M. Shatkin, A.J. and Tatum, E.L., Proc. Nat. Acad. Sci., 48, 1 (1962).

Tamaoki, T. and Mueller, G.L., Biochem.Biophys.Res.Comm., 9, 451 (1962).

Warner, J.R., Knopf, P.M. and Rich, A., Proc. Nat. Acad. Sci., 49, 122 (1963).

Wettstein, F.O., Staehelin, T. and Noll, H., Nature. 197, 430 (1963).