COLD-INDUCED "RUN-OFF" RIBOSOMES OF ACHETA DOMESTICUS

M.S.KAULENAS

Department of Zoology, University of Massachusetts, Amherst, Mass. 01002

Received April 20, 1971

Summary Cricket "run-off" ribosomes have a lower Mg^{2+} optimum and are approximately six times more active than other monomers in poly U-directed protein synthesis. Both differences can be eliminated by washing "run-off" ribosomes with 0.5 M NH_4Cl. A component in the salt wash is capable of partially restoring the enhanced activity.

Studies on the effect of heat on mammalian cells[1] and of cold on bacterial cells[2] have demonstrated that extremes of temperature inhibit initiation to a greater extent than other phases of protein synthesis. In both types of cell the inhibition of initiation is characterized by extensive "run-off" of polysomes and the accumulation of ribosomes in a state favourable for the rapid resumption of initiation on return to optimal temperature. A similar "run-off" of polysomes may be noted in cold-treated cricket nymphs; an effect which is rapidly reversed on return to higher temperatures. The possibility of stopping and restarting protein synthesis at the point of initiation makes this type of system attractive for the study of the mechanisms of initiation, especially since such mechanisms may well differ in important details from bacterial ones[3]. Further, investigation of "run-off" ribosomal intermediates may provide an explanation for the reported ready re-use of these ribosomes in *in vivo* protein synthesis, in contrast to the relative inactivity of the bulk of the cellular 80S monomers[4,5]. Some features of "run-off" ribosomes of cold-treated *Acheta* nymphs are described.

MATERIALS AND METHODS *Acheta domesticus* fifth and sixth instar nymphs were used. For cold-treatment the animals were kept at 4°C for 21 or

24 h. Control crickets were collected at room temperature. Standard and 0.5 M NH_4Cl washed ribosomes were prepared[6,7]. The procedures for the assay of endogenous and poly U-directed protein synthesis and gradient centrifugation have been reported[6,8].

RESULTS AND DISCUSSION The stringent conditions necessary for the isolation of ribosomes from crickets result in the disruption of polysomes[6]. As illustrated in Fig. 1, ribosomal preparations consist mainly of monosomes, although a substantial capacity for protein synthesis is retained (Table 1), indicating that messenger RNA is associated with some of the monosomes. The sedimentation profiles of control and cold-treated ribosomes are virtually identical under the ionic conditions used, showing that the previous temperature history of the animals does not result in a preferential maintenance of polysomes during isolation in either type of preparation.

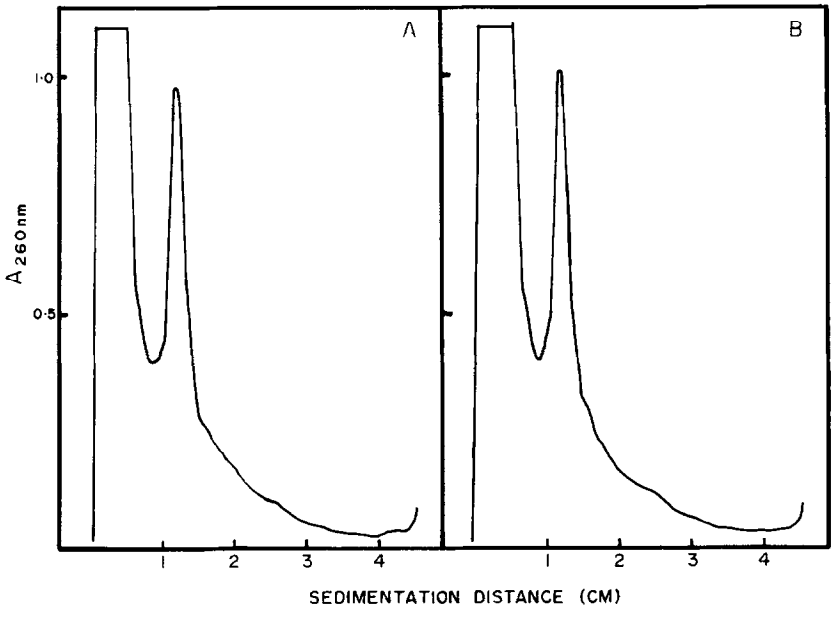

Fig. 1. Sedimentation patterns of post-mitochondrial fractions isolated from control (A) and cold-treated (24 h, 4°C) nymphs (B). Gradients of 15-45% sucrose, containing 50 mM Tris (pH 7.8), 10 mM $MgSO_4$ and 150 mM KCl. Centrifugation was for 45 min at 50,000 rev/min at 4°C in the Spinco SW-50 rotor. The direction of sedimentation is from left to right.

TABLE 1. Endogenous incorporation of (^{3}H)phenylalanine by ribosomes isolated from control and cold-treated nymphs.

Ribosomes	Incorporation pmoles Phe/mg RNA
W-K	50.8
W-NH_4	56.8
C24-K	20.6
C24-NH_4	24.8

Phenylalanine incorporation was assayed as previously described[6]. The figures are mean values from five experiments. W-K; control standard ribosomes. W-NH_4; control, 0.5 M NH_4Cl washed ribosomes. C24-K; standard ribosomes, isolated from nymphs exposed to 4°C for 24 h. C24-NH_4; 0.5 M NH_4Cl washed ribosomes isolated from nymphs exposed to 4°C for 24 h.

In this[6] and other types of tissue[9], sedimentation of ribosomes through gradients containing low levels of Mg^{2+} and moderately high KCl concentrations (1 mM Mg^{2+}, 150 mM K^+) results in the dissociation of peptide-free monomers, while ribosomes resulting from polysome rupture, bearing messenger RNA fragments and nascent peptides, remain undissociated. Inspection of the sedimentation pattern of ribosomes in such gradients allows an estimate of the fraction of the ribosomal population originally derived from polysomes. Fig. 2A shows that about two-thirds of control ribosomes are resistant to dissociation in 1 mM Mg^{2+}-150 mM K^+ gradients, and that therefore this proportion of ribosomes was engaged in protein synthesis on polysomes. Exposure of the nymphs to cold, however, results in a drastic decrease in polysomal ribosomes, and a corresponding increase in ribosomes which are dissociated into subunits in 1 mM Mg^{2+} (Fig. 2B), but exist as monomers at 10 mM Mg^{2+} (Fig. 1B).

The decrease in the amount of polysomes as the result of cold-treatment is confirmed by assaying the endogenous protein synthetic activity of the ribosomes (Table 1). Controls exhibit an approximately 60% greater capacity for protein synthesis than do ribosomes isolated from

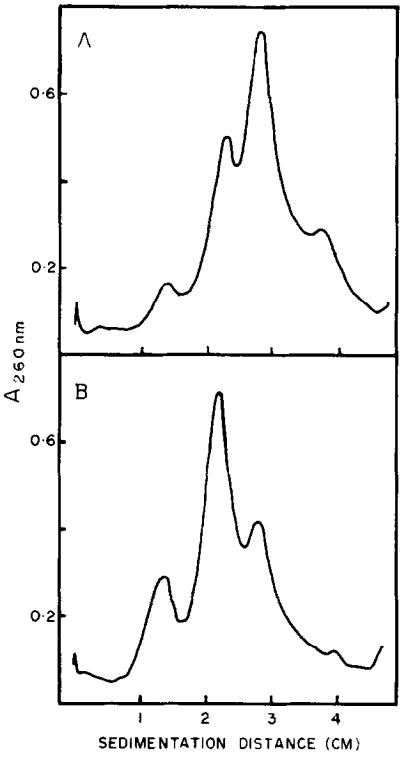

Fig. 2. Sedimentation patterns of standard ribosomes isolated from control (A) and cold-treated (24 h, 4°C) nymphs (B). Gradients of 8-30% sucrose, containing 50 mM Tris (pH 7.8), 1 mM $MgSO_4$ and 150 mM KCl. Centrifugation was for 75 min at 50,000 rev/min at 4°C in the Spinco SW-50 rotor. Approximately 2.0 A_{260nm} units in a volume of 0.05 ml were loaded on each gradients. Other conditions as in Fig. 1.

TABLE 2. Poly U-directed phenylalanine incorporation shown by ribosomes isolated from control and cold-treated nymphs.

Ribosomes	% dissociable in 1 mM Mg^{2+}-150 mM K^+ gradients	Incorporation of Phe	
		pmoles/mg RNA	pmoles/mg RNA in dissociable ribosomes
W-K	35	212	606
W-NH_4	40	201	502
C24-K	67	1366	2038
C24-NH_4	69	326	553

Conditions as in Table 1, except that (^{3}H)phenylalanine incorporation was assayed at 9.5 mM Mg^{2+} in the presence of saturating amounts of poly U.

cold-treated animals. Washing the ribosomes in 0.5 M NH_4Cl has no effect on endogenous incorporation.

A striking difference between ribosomes isolated from control and cold-treated nymphs is detected when their ability to use poly U is assayed (Table 2). Controls incorporate 212 pmoles Phe/mg RNA. If it assumed that ribosomes with endogenous messenger RNA cannot use poly U[10], a closer approximation to true specific activity may be obtained by expressing incorporation as a function of the amount of ribosomes without endogenous messenger (i.e., dissociable in 1 mM Mg^{2+}). On this basis, the specific incorporating activity of control ribosomes is 606 pmoles Phe/mg RNA. Washing control ribosomes with 0.5 M NH_4Cl does not produce a statistically significant difference in the ability of such ribosomes to use poly U as template. While cold-treatment doubles the amount of ribosomes dissociable in 1 mM Mg^{2+}, phenylalanine incorporation increases to 1366 pmoles/mg RNA, or 2038 pmoles/mg RNA in ribosomes lacking endogenous messenger. Clearly, this represents only the average specific incorporating ability of the ribosomal population. If it is assumed that the additional 32% (67 minus 35%, Table 2) of ribosomes lacking endogenous messenger RNA are responsible for all of the increase in poly U response (from 212 to 1366 pmoles/mg RNA), then their specific incorporating activity can be calculated as 3600 pmoles Phe/mg RNA. It is evident that "run-off" ribosomes produced by cold treatment can use poly U much more efficiently than controls. However, this increased capacity is totally lost if the ribosomes are washed with 0.5 M NH_4Cl. The average incorporating ability of washed ribosomes becomes comparable to that of controls.

The above differences in the response to poly U are accentuated when the Mg^{2+} optima for the reactions are compared (Fig. 3). Control ribosomes (Fig. 3B) show maximum incorporation at approximately 12 mM Mg^{2+}, a value similar to that found for native subunits (Fig. 3A). Ribosomes

isolated from cold-treated nymphs, however, have a Mg^{2+} optimum of 9 mM (Fig. 3C). Strikingly, washing such ribosomes with 0.5 M NH_4Cl not only reduces their capacity to use poly U as template but also shifts the Mg^{2+} optimum to 12 mM (Fig. 3D).

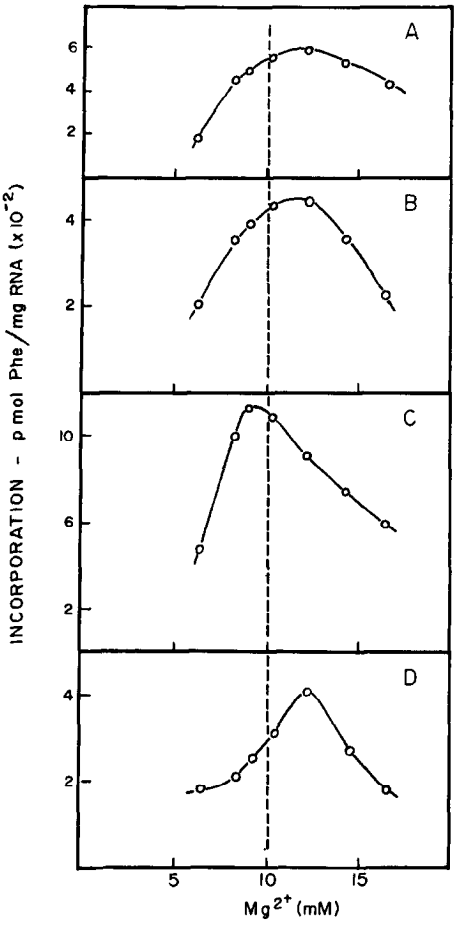

Fig. 3. Magnesium ion concentration optima for poly U-directed phenylalanine incorporation by cricket ribosomes. Assay conditions were as described[6], except that the Mg^{2+} concentration was varied between 6.4 and 17 mM. Control values for endogenous incorporation at each Mg^{2+} concentration in the absence of poly U have been subtrated. (A) Native subunits. (B) Control, standard ribosomes. (C) Standard ribosomes isolated from nymphs exposed to 4°C for 24 h. (D) as in (C), except that the ribosomes were washed in 0.5 M NH_4Cl.

Since washing in high salt concentrations may remove some protein from ribosomes[11], it was possible that material facilitating the response of ribosomes to poly U was removed also by this treatment. To

TABLE 3. Partial restoration of enhanced response to poly U by the 0.5 M NH_4Cl ribosomal wash.

Ribosomes	Additions	Incorporation pmoles Phe/mg RNA
C21-K	-	959
C21-NH_4	-	248
C21-NH_4	18 µg wash protein	343
C21-NH_4	45 µg wash protein	357
C21-NH_4	90 µg wash protein	230
-	45 µg wash protein	0

Conditions as in Table 2, except that cold exposure of nymphs was for 21 h at 4°C. Untreated 105,000 g supernatant fraction from the 0.5 M NH_4Cl wash was used as the source of wash protein.

test this possibility the untreated supernatant fraction from the ribosome wash was included during incubation of washed ribosomes (Table 3). It is clear that some component in the wash is capable of partially restoring the enhanced poly U response shown by "run-off" ribosomes. Large amounts of wash protein are inhibitory, possibly due to nuclease contamination[12]. Attempts to purify this factor(s) have so far led to inconclusive results; ammonium sulfate precipitable material from the ribosome wash has shown high activity in some experiments, although the active component appears to be highly unstable.

The results allow the following conclusions. Cold-treatment of nymphs results in the accumulation of endogenous messenger-free "run-off" ribosomes, which are capable of using poly U at a much greater efficiency and at a lower Mg^{2+} level than controls. Since washing "run-off" ribosomes in 0.5 M NH_4Cl eliminates both characteristics, and a component in the wash is capable of at least partially restoring the enhanced activity, it may be reasonable to assume that "run-off" ribosomes have associated with them a factor(s) which allows more efficient initiation with poly U. The enhanced activity seems to be a general feature of

"run-off" ribosomes[13], although whether this can be eliminated by a high salt wash is unknown. It may be noted that some of the initiation factors isolated from eukaryote ribosomes also promote a "Mg^{2+} shift" in the poly U system[14], and factors with an affinity for poly U are also found on bacterial ribosomes[15]. It is possible, therefore, that "run-off" ribosomes may be characterized by an association with one or more initiation factors; a possibility which would explain their preferential re-use in *in vivo* protein synthesis[4].

Supported in part by NSF grant GB-8450.

REFERENCES

1. McCormick, W. and Penman, S., J.Mol.Biol., 39, 315 (1969).
2. Friedman, H., Lu, P. and Rich, A., Nature, 223, 909 (1969).
3. Shafritz, D.A., Laycock, D.G. and Anderson, W.F., Proc.Natl.Acad. Sci., 68, 496 (1971).
4. Adamson, S.D., Howard, G.A. and Herbert, E., Cold Spring Harbor Symp.Quant.Biol., 34, 547 (1969).
5. Okubo, C.K. and Raskas, H.J., Biochemistry, 9, 3458 (1970).
6. Kaulenas, M.S., J.Insect Physiol., 16, 2103 (1970).
7. Kaulenas, M.S., Anal.Biochem., in the press.
8. Kaulenas, M.S., Biochim.Biophys.Acta, 224, 276 (1970).
9. Lawford, G.R., Biochem.Biophys.Res.Commun., 37, 143 (1969).
10. Loeb, J.N., Arch.Biochem.Biophys., 139, 306 (1970).
11. Moldave, K. and Skogerson, L., Methods in Enzymol., 12, 478 (1967).
12. Mangan, K.F. and Kaulenas, M.S., J.Insect Physiol., 16, 1155 (1970).
13. Falvey, A.K. and Staehelin, T., J.Mol.Biol., 53, 21 (1970).
14. Shafritz, D.A., Pritchard, P.M., Gilbert, J.M. and Anderson, W.F., Biochem.Biophys.Res.Commun., 38, 721 (1970).
15. Smolansky, M. and Tal, M., Biochim.Biophys.Acta, 213, 401 (1970).