PLANT RIBOSOME-BOUND PROTEINS: A FRACTION FROM LEAVES THAT DISSOCIATES BEAN RIBOSOMES

Gene R. Williams and J. A. Maser

Department of Plant Sciences
Indiana University
Bloomington, Indiana 47401

Received May 11, 1973

SUMMARY

Partial purification is described for a fraction from the salt wash of leaf ribosomes that dissociates bean monoribosomes to subunits. The active fraction is heat-labile and of high molecular weight. Under the assay conditions used, a linear relationship exists between the amount of factor and the level of dissociation observed. Apparent heterogeneity within the dissociation fraction was found with column chromatography on DEAE-cellulose. Similarities between the properties of this fraction and those expected for ribosome-bound peptide initiation factors are discussed.

The specific involvement of ribosomal subunits and bound proteinaceous factors in the initiation of peptide biosynthesis has been demonstrated with ribosomes from both prokaryotes (1,2) and mammals (3,4). One of these factors (IF3) interacts with the smaller subunit and, under the appropriate assay conditions, leads to accumulation of subunits from competent monosomes (5,6). Since stoichiometric amounts of the factor are involved, IF3 has been postulated to exert some quantitative control on protein synthesis by regulating the level of ribosomal subunits in the cell. In addition, IF3 mediates the selection and attachment of natural mRNA (7,8) and, therefore, also has been ascribed a qualitative regulatory role with respect to the kinds of messenger translated.

Because of its potential significance, we seek to establish the existence of this factor and examine its role in developing higher plant tissues. This report describes the partial purification of a putative factor associated

with leaf ribosomes and presents evidence that this protein fraction exhibits a high level of dissociation (DF) activity with leaf ribosome monomers.

MATERIALS AND METHODS

Actively-growing trifoliate leaves from greenhouse grown beans (Phaseolus vulgaris L. var. Kentucky Wonder) were used as the source of the protein fractions. All operations were performed at 4° unless noted otherwise.

Leaf tissue was homogenized in 10-g batches in 2.7 volumes of Grinding Medium (200 mM Tris-HCl, pH 8.4 at 4°, containing 450 mM sucrose, 6 mM $MgCl_2$, 15 mM KCl, 5 mM phosphate (K+), and 10 mM 2-mercaptoethanol). After centrifuging the brei at 48,000 x g for 10 min, ribosomes were pelleted from the supernatant by a 90 min centrifugation at 150,000 x g. The pellets, including the loosely-packed sediments, were suspended in Sucrose Solution (5 mM phosphate (K+), pH 7.5, containing 250 mM sucrose, 0.10 mM EDTA (K+), and 1.0 mM dithiothreitol). After slowly adjusting the suspension to 500 mM with respect to KCl and stirring 60 min, it was centrifuged 150 min at 150,000 x g and the upper 75% of the supernatant was recovered. The protein precipitated by $(NH_4)_2SO_4$ at 70% saturation was suspended in and dialyzed 2 hr against 50 mM KCl in Buffer B (10 mM Tris-HCl, pH 7.6 at 4°, containing 5 mM phosphate, 0.1 mM EDTA, and 1.0 mM dithiothreitol).

The dialyzed protein fraction was loaded on a DEAE-cellulose column (1.0 x 10.0 cm, previously washed 24 hr with the loading buffer). After washing through unadsorbed protein, batch fractions were eluted with 150 mM KCl followed by 400 mM KCl (both in Buffer B). The latter fraction, which contained nearly all the activity, was precipitated with $(NH_4)_2SO_4$. The pellet was dissolved in a small volume (ca. 2 ml/100 g leaves used) of Medium C (10 mM Tris-HCl, pH 7.6 at 4°, containing 100 mM KCl, 1.0 mM dithiothreitol, and 0.1 mM EDTA) and dialyzed against two changes of the same medium for a total of 4 hr. The crude factor was used within 12 hr or was stored under liquid N_2.

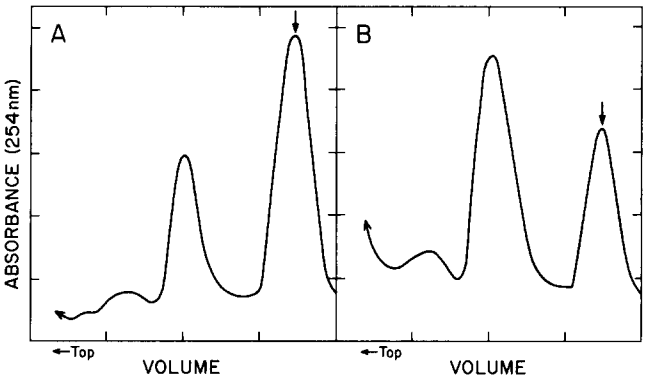

Fig. 1. Effects of leaf heat-labile DF on the dissociation of bean monoribosomes. Shown are sucrose gradient profiles of ribosome material (subunit + monomer) after treatment with heated DF (panel A) and after treatment with fresh DF (panel B). The assay procedure was the same as that described in Table I. The position of the monomer peak is indicated by the inverted arrow (↓) in each case.

Ribosomes for the dissociation assay were prepared from young etiolated bean leaves as described previously (9) except that the final pellets were suspended in 10 mM Tris-HCl (pH 7.6 at 4°, containing 6 mM $MgCl_2$, 100 mM KCl and 1.0 mM dithiothreitol). Sucrose gradients contained 10 mM Tris-HCl (pH 7.6 at 4°), 3.0 mM $MgCl_2$, and 15 mM KCl. After centrifugation, gradients were pumped through a flow cell and the area under each peak on the resultant tracings was measured with a planimeter. In some instances, the smaller subunit was not resolved completely from UV-absorbing material near the top of the gradient and so, for uniformity, the total subunit level was assumed to be 1.4 times that of the larger subunit.

RESULTS AND DISCUSSION

Fig. 1 illustrates the subunit level found after treatment with heat-inactivated DF (panel A, 40% subunit) and that produced by treatment with the same amount of DF (panel B, 66% subunit). The DF activity is clearly heat-labile. The smaller increment of dissociation produced by heated DF as compared with ribosomes incubated without DF (see Table I) is the result of incomplete dialysis and is abolished completely by passage over

TABLE I

SOME STABILITY PROPERTIES OF THE BEAN LEAF DISSOCIATION FACTOR ACTIVITY

Data are expressed as the percentage of total (subunits + monomer) ribosome material represented by the subunit. Each assay tube contained in a final volume of 0.2 ml: the protein fraction and ribosomes (0.8 absorbancy units) suspended in reaction buffer which included in μmoles: Tris-HCl, pH 7.6 at 4°, 2.0; $MgCl_2$, 0.6; KCl, 20; dithiothreitol, 0.2; EDTA, 0.01. The dissociation reaction was initiated by adding the protein fraction. After 10 min at 28°, 1.5 volumes of cold reaction buffer were added, and mixture was loaded immediately on a 10-30% linear sucrose gradient and centrifuged 2.0 hr at 41,000 rpm in a Spinco SW 41 rotor.

TREATMENT	SUBUNIT LEVEL (%)
- DF	35
Heated DF	49
DF (Fresh)	75
- DF	36
Heated DF	46
DF (liquid N_2, 12 hr)	79

a molecular sieve column. The heat-labile DF recovered off BioGel P-4 is in the void volume. Most of the activity of purified DF preparations remains after freezing in liquid N_2 (Table I) or after standing on ice for up to 12 hr (see Table II and compare with the activity of fresh DF in Table I). Preparations frozen for several weeks still show partial activity.

Under the conditions generally used for the assay, the extent of dissociation observed is directly proportional to the DF level (Table II).

TABLE II

STOICHIOMETRY OF THE DISSOCIATION FACTOR ACTIVITY FROM BEAN LEAVES

Subunit level data are expressed as the percentage of total (subunits + monomer) ribosome material represented by the subunits. Observed DF activity represents changes in subunit level attributable to heat-labile DF activity. Relative DF activity is based on the overall observed increment of 21.0% (assumed to be 1.00). Assay conditions are the same as described in Table I, except that this preparation of DF was stored 12 hr at 4° prior to this assay.

TREATMENT	SUBUNIT LEVEL (%)	DF ACTIVITY	
		OBSERVED	RELATIVE
- DF	43	-	-
Heated 1.0 DF	45	0.0	0.00
0.1 DF	45	1.8	0.08
0.3 DF	50	6.6	0.31
0.6 DF	58	13.8	0.66
1.0 DF	66	21.0	1.00

None of our DF preparations, however, have yielded more than 80% subunit content.

We have been unable to demonstrate activity in purification stages prior to the DEAE-cellulose batch elution procedure. Normally, very crude protein fractions seem to possess degradative activity that converts the small fraction of polysomes present in the assay ribosomes to monomers. In addition, degradation of assay ribosomes is observed when the crude fractions employed are highly concentrated; in these instances, the existing subunits seem more susceptible to such attack. Thus, any change in total level of monomer + subunit material that accompanies the assay of very

crude fractions has been associated invariably with a decrease in the relative proportion of subunits. On the other hand, the purified fraction retained by DEAE-cellulose in 50 mM KCl and used in these studies does not alter the total level of monosome + subunit material during the assay period.

Attempts to purify the DF activity further on DEAE-cellulose columns by KCl gradient elution (in Buffer B) yielded a broad region of activity that eluted between 0.23 M and 0.47 M KCl and appeared to contain at least two overlapping peaks of DF activity (maxima were at 0.29 M and 0.40 M KCl). In addition, a sharp band with low activity was observed consistently at 0.12 M KCl. The possibility that this heterogeneity is an artifact of chromatography rather than a partial resolution of multiple DF activities has not yet been fully excluded.

Clearly, while we have not demonstrated that the purified fraction described in this report participates directly in the initiation reactions of peptide biosynthesis, it does, however, possess a number of properties in common with known initiation factors from microbial and mammalian sources. Indeed, insofar as we know, all endogenous ribosome-bound heat-labile activities that have been shown to promote ribosome dissociation are also initiation factors. Work is now underway to establish the role of our purified fraction in ribosome functioning.

ACKNOWLEDGEMENT

We gratefully acknowledge the expert and faithful assistance of Alice Williams. This work was supported by grant GB 25366 from the National Science Foundation.

REFERENCES

1. Sabol, S., Sillero, M.A.G., Iwasaki, K. and Ochoa, S., Nature, 228, 1269 (1970).
2. Dubnoff, J.S. and Maitra, U., Proc. Natl. Acad. Sci. U.S., 68, 318 (1971).
3. Prichard, P.M., Gilbert, J.M., Safritz, D.A. and Anderson, W.F., Nature, 226, 511 (1970).

4. Schreier, M.H. and Staehelin, T., Nature New Biol., 242, 35 (1973).
5. Subramanian, A.R. and Davis, B.D., Nature, 228, 1273 (1970).
6. Noll, M. and Noll, H., Nature New Biol., 238, 225 (1972).
7. Lee-Huang, S. and Ochoa, S., Nature New Biol., 234, 236 (1971).
8. Revel, M., Aviv (Greenshpan), H., Groner, Y., and Pollack, Y., FEBS Lett., 9, 213 (1970).
9. Williams, G.R. and Novelli, G.D., Biochim. Biophys. Acta, 155, 183 (1968).