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EFFECT OF DIETHYLPYROCARBONATE AND SODIUM DEOXYCHOLATE ON POLYRIBOSOMES FROM COTYLEDONS OF *VICIA FABA*

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Key Word Index—*Vicia faba*; Leguminosae; broad bean; ribosomal RNA; breakdown products.

Abstract—Degradation of *r*RNA during the isolation of polyribosomes in the presence of either sodium deoxycholate or diethylpyrocarbonate gave breakdown products with MW corresponding to 1·13, 1·02, 0·93, 0·79, 0·61, 0·52, 0·44, 0·42, 0·30, 0·28, 0·24, 0·20 and 0·12 × 10^6 daltons.

INTRODUCTION

THE ANALYSIS of *r*RNA, extracted from isolated ribosomes by centrifugal techniques, reveals apparently undegraded molecules.[1–4] Recently, using polyacrylamide gel electrophoresis, Payne and Loening[5] described seven *r*RNA degradation products from pea root *r*RNA which were undetected by sucrose gradient sedimentation analysis. In previous work many *r*RNA preparations were shown to contain specific degradation products, formed during ribosome isolation.[6–10] In the present study *r*RNA degradation products from bean cotyledons have been characterized and the effect of detergents and diethylpyrocarbonate (DEP) on polyribosome and *r*RNA profiles has been investigated.

RESULTS

Normal breakdown of rRNA during polyribosome isolation

Isolation of polyribosomes, either directly from the Triton X100 treated post-mitochondrial supernatant or from Triton X100 microsomes yielded essentially similar profiles on sucrose gradient analysis (Fig. 1). Analysis of the nucleic acids of such preparations on 2·6% acrylamide gels showed that in contrast to the nucleic acids extracted directly from developing cotyledons (Fig. 2a), the *r*RNA was degraded. This was indicated by the

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appearance of the 1·02 × 10^6 and 0·61 × 10^6 daltons degradation components of the 25*S* and 18*S* *r*RNAs respectively (Fig. 2b). Incubation of microsome preparations at 0° for 24 hr revealed a loss of polyribosomes as compared to non-incubated samples. The nucleic acids extracted from 24 hr-incubated microsome preparations, when analysed on 2·6% acrylamide gels, showed a variety of breakdown products not previously detected. The degradation products observed were determined to have MW of 1·02, 0·93, 0·61, 0·52, 0·44 and 0·20 × 10^6 daltons respectively.

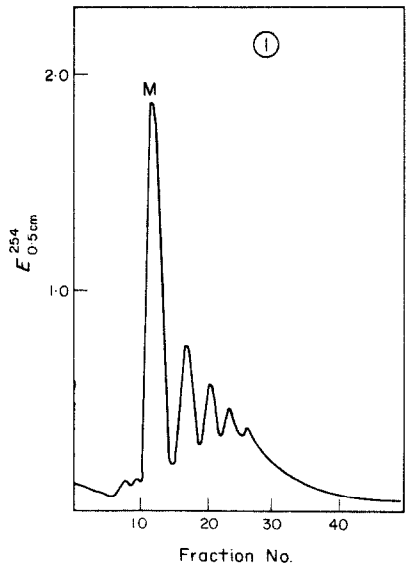

FIG. 1. SUCROSE-GRADIENT SEDIMENTATION PROFILE OF TRITON X100 TREATED POLYSOMES FROM 60-DAY-OLD COTYLEDONS OF *Vicia faba*.
Suspended polysomes (1 mg) were layered onto the gradient and centrifuged at 65000 *g* for 2 hr. M = monosome fraction.

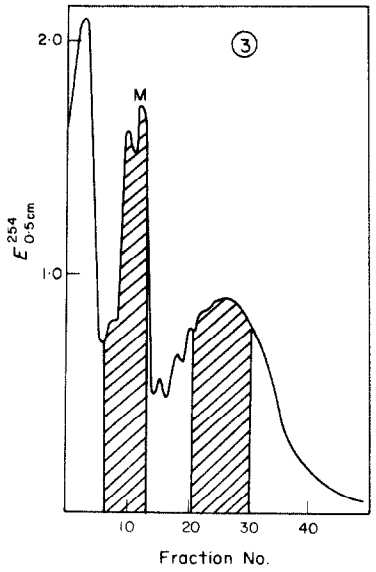

FIG. 3. SUCROSE-GRADIENT SEDIMENTATION PROFILE OF TRITON X100 DISRUPTED MICROSOMES FROM 60-DAY-OLD COTYLEDONS.
Microsomes were extracted in the presence of DEP. Triton X100 disrupted microsomes (2 mg) were layered onto the gradient and centrifuged at 65000 *g* for 2 hr. The shaded areas were collected and the RNA extracted.

Effect of sodium deoxycholate

The isolation and analysis of polyribosomes, following treatment of the post-mitochondrial supernatant with the ionic detergent deoxycholate (DOC), showed a conversion of polyribosomes to monosomes and subunits, when compared to the effect of the non-ionic detergent, Triton X100. Following the isolation and extraction of the monosome fraction, analysis of the nucleic acids on 2·6% acrylamide gels demonstrated that substantial degradation of the *r*RNA had occurred. The degradation products had MW of 1·02, 0·61, 0·44 and 0·30 × 10^6 daltons. The latter two components appeared to be heterogeneous and were further resolved following heat treatment of the RNA. The 0·44 × 10^6 daltons fraction was resolved into two components with MW of 0·44 and 0·42 × 10^6 daltons. The 0·30 × 10^6 daltons component was not completely resolved but contained a shoulder with a MW of 0·28 × 10^6 daltons. Other fragments only detected after heat treatment had MW of 1·13, 0·79 and 0·12 × 10^6 daltons.

Effect of diethylpyrocarbonate

The isolation of microsomes in the presence of DEP exerts a protective effect on polyribosomal structure (Fig. 3), though some subunit formation occurs. Analysis of the subunit-monosome or polyribosome fraction nucleic acids on 2·6% acrylamide gels resulted in essentially identical traces. Both the 25*S* and 18*S* *r*RNA had degraded in a manner similar to that observed in the DOC-treated preparation except that the $0{\cdot}30 \times 10^6$ daltons component was not detected in DEP-treated samples. Melting of the RNA resulted in the release of the hydrogen-bonded fragments, and as with the DOC-treated sample, an apparent molar excess of the 18*S* *r*RNA over the 25*S* *r*RNA. The observed pattern of breakdown following heat treatment was similar to that of the DOC-treated sample. However, in the DEP-treated samples the $0{\cdot}28 \times 10^6$ daltons component was resolved from the $0{\cdot}30 \times 10^6$ daltons component and a previously undetected component was separated with an estimated MW of $0{\cdot}24 \times 10^6$ daltons.

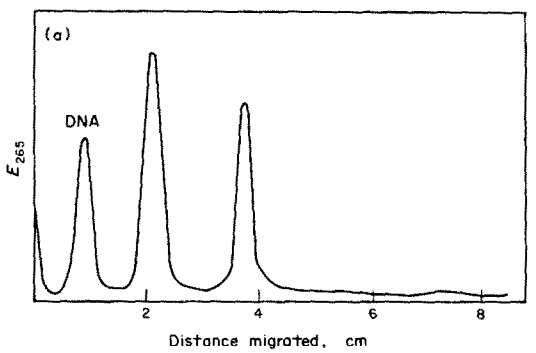

FIG. 2(a). NUCLEIC ACIDS FROM 60-DAY-OLD COTYLEDONS.
The sample was prepared directly from whole cotyledons. 20 μg nucleic acids were electrophoresed on a 2·6% gel for 3 hr.

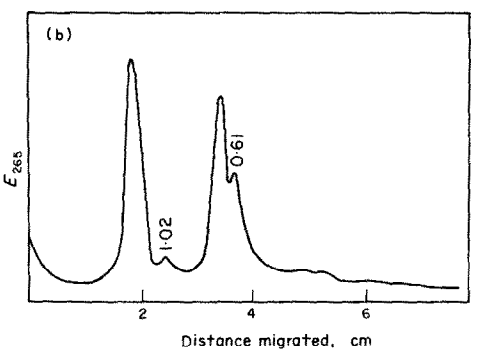

FIG. 2(b). NUCLEIC ACIDS OF POLYRIBOSOMES ISOLATED FROM 60-DAY-OLD COTYLEDONS IN THE PRESENCE OF TRITON X100.
20 μg sample electrophoresed on a 2·6% gel for 3 hr. The numbers above the peaks refer to their MW × 10^6 daltons.

DISCUSSION

Bentonite, which has often been used in the preparation of plant polyribosomes,[11,12] was used initially in the current study in an attempt to inhibit RNase by selective adsorption. The ability of bentonite to remove RNase was assessed by analysing the nucleic acids of the ribosomes by polyacrylamide gel electrophoresis. It was evident by comparison with nucleic acids extracted directly from the cotyledons that degradation had occurred during polyribosome isolation. Further, the incubation of preparations at low temperatures resulted in an even greater amount of *r*RNA degradation, indicating an association of RNase activity with the particles. Hsiao[13] has previously demonstrated a similar association of RNase activity with *Zea mays* ribosomes.

The omission of bentonite and the use of the ionic detergent DOC induces a partial destruction of ribosome units into monosomes and subunits when compared to the non-ionic

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detergent Triton X100. This destruction of polyribosomes has previously been observed by Burka,[14] Golub and Clegg,[15] and Olsnes *et al.*[16] and the treated particles have been found to have a reduced sedimentation rate through sucrose gradients.[6,16] The observable increase in *r*RNA degradation following isolation of polyribosomes in the presence of DOC as compared to Triton X100 and DEP can be explained by a DOC-mediated loss of ribosomal protein.[14] This is in keeping with the reduced sedimentation rate of DOC-treated particles, and is supported by the work of Dingman *et al.*,[6] who showed that DOC mediated the release of RNA species from rat liver microsomes. These RNAs are *r*RNA breakdown products according to the more recent work of Takagi *et al.*[10]

The nuclease inhibitor, DEP, which was first reported to have a protective effect on polyribosome structure during the isolation of wheat embryo polyribosomes,[17] has recently been shown to protect *V. faba* polyribosomes.[18] In preparations which included DEP there was a marked increase in ribosomal subunit content, supporting earlier suggestions[17] that DEP causes partial disaggregation of the ribosome units. In this respect it is similar to the detergent DOC. Analysis of the nucleic acids either from the monosome or polysome fraction gave profiles virtually identical to the profile obtained when *r*RNA degradation had been induced by the omission of bentonite and the addition of DOC. Some variation in the amount of *r*RNA degradation was observed between preparations, although in all instances degradation of the 18*S r*RNA occurred. This conflicts with the observation of Anderson and Key,[19] who showed that DEP inhibited the breakdown of the 18*S r*RNA. This difference could reflect a difference in the RNase content of the tissue. The incubation of DEP-extracted polyribosomes does not alter their profile and indicates that RNase activity is absent from the preparations. The observed *r*RNA degradation must therefore have arisen during the earlier part of the extraction procedure, indicating that RNase is not completely inhibited in the initial homogenate. It is therefore apparent that polyribosomes isolated from *V. faba* cotyledons in the presence of DEP are not representative of the *in vivo* distribution.[18] The inability of DEP to inactivate RNase in tissue homogenates is due to its limited solubility and the high protein concentration.[20,21]

Melting of the *r*RNA isolated from either the DOC-extracted polyribosomes or the DEP-extracted polyribosomes produced a dramatic change in the optical density profile due to the release of hydrogen-bonded RNA fragments; the 18*S r*RNA becoming the prominent species, being in molar excess over the 25*S r*RNA. This type of profile could arise in two ways: firstly, a large number of available cleavage points on the 25*S r*RNA molecule resulting in many low MW fragments and relatively few cleavage points on the 18*S r*RNA, or, secondly, a highly specific cleavage point in the 25*S r*RNA which results in a fragment equivalent in size to the 18*S r*RNA. The latter explanation is favoured by the fact that there was an apparent increase in the 18*S* RNA species following heat treatment. The cleavage of the heavy *r*RNA into a fragment which is equivalent in size to the light *r*RNA, has been demonstrated in *E. coli*[22] and *Tetrahymena pyriformis*.[23] This leads

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to a molar excess of the small *r*RNA over the heavy *r*RNA, a distribution which also occurs following the isolation of *r*RNA from chloroplast ribosomes.[7,24]

EXPERIMENTAL

Biological materials. Seeds of *Vicia faba* L. var. Triple White were grown in the open. Beans of an average cotyledon weight for a particular age were used.

Chemicals. Chemicals were obtained from British Drug Houses Ltd., Poole, Dorset, U.K., except for DEP, which was a gift from Bayer Chemicals Ltd., Richmond, Surrey.

Isolation of polyribosomes. 10 g tissue was homogenized in a mortar for 5 min in 3 vol. of 50 mM Tris-HCl, 0·5 M sucrose, 16 mM KCl, 5 mM $MgCl_2$, pH 7·5, containing sodium bentonite to a final concn of 1 mg/ml. Sodium bentonite was omitted if DEP was added. DEP was added to a final concn of 1% (v/v) and the Tris concn was adjusted to 0·1 M by the addition of 2 M untitrated Tris. The brei was filtered through silk and the filtrate was centrifuged at 38000 *g* max. for 15 min. For the isolation of microsomes the 38000 *g* supernatant was recentrifuged at 105000 *g* av. for 90 min. For the isolation of polyribosomes the 38000 *g* supernatant was made 4% (v/v) with respect to Triton X100 or 0·2% (w/v) with respect to DOC and then centrifuged at 105000 *g* av. for 90 min. The resulting supernatant was decanted and the centrifuge tube walls dried with absorbent paper. The pellets were washed once and then carefully resuspended in a small volume of 10 mM Tris-HCl, 25 mM KCl, 1 mM $MgCl_2$, pH 7·6 using a loose-fitting Teflon homogenizer. Particle concentrations were estimated by the method of Ts'o and Vinograd.[25] Prior to the analysis of microsomes on sucrose gradients the suspension was made 4% (v/v) with respect to Triton X100 by the addition of 0·4 vol. 10% buffered Triton X100. All operations were carried out between 0° and 4°.

Sucrose gradient analysis. Convex exponential sucrose gradients were prepared using 10 ml 8% (w/v) sucrose and 19 ml 40% (w/v) sucrose (both containing 10 mM Tris-HCl, 25 mM KCl, 1 mM $MgCl_2$, pH 7·6) by the method of Henderson.[26] Gradients were centrifuged in the 3 × 23 ml rotor of an MSE Superspeed 65, and were subsequently fractionated into 0·4 ml fractions at 0·5 ml/min with an Isco density gradient fractionator model 180. $E^{254}_{0\cdot5\,cm}$ was measured with an Isco UV analyser model 222 attached to a Servoscribe chart recorder.

RNA extraction. Total nucleic acids were extracted from cotyledons by the method of Parish and Kirby[27] as modified by Loening.[28] Polysomal RNA was extracted by the method of Oda and Joklick.[29]

Polyacrylamide gel electrophoresis. Nucleic acids were fractionated in 2·6% acrylamide gels at room temp. as described by Loening.[28,30] Gels were scanned at 265 nm in a Joyce Loebl Chromoscan. The MWs of RNA components were determined by assuming that electrophoretic mobility is inversely related to the logarithm of the MW.[28] The MWs of *V. faba* *r*RNA were $1{\cdot}29 \times 10^6$ and $0{\cdot}70 \times 10^6$ relative to *Escherichia coli* *r*RNA, $1{\cdot}08 \times 10^6$ and $0{\cdot}56 \times 10^6$. The RNA of brome grass mosaic virus (kindly given by Dr. J. Bancroft) was used as a MW marker. It contains three components of MW $1{\cdot}07 \times 10^6$, $0{\cdot}76 \times 10^6$ and $0{\cdot}33 \times 10^6$ daltons.[31]

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