Phytochemistry, 1979, Vol. 18, pp. 1617–1619. © Pergamon Press Ltd. Printed in England. 0031-9422/79/1001-1617 $02.00/0

ENDOSPERMS AND EMBRYOS OF MATURE DRY CASTOR BEAN SEEDS CONTAIN ACTIVE RIBOSOMES

J. DEREK BEWLEY and KAREN M. LARSEN
Dept. of Biology, University of Calgary, Calgary, T2N 1N4, Alta., Canada

(*Received* 26 *January* 1979)

Key Word Index—*Ricinus communis*; Euphorbiaceae; castor bean; ribosomes; endosperm; dry seed; desiccation.

Abstract—Mature dry endosperms and embryos of castor bean contain ribosomes which are capable of catalysing protein synthesis *in vitro*. This observation contradicts previous reports that ribosomes are absent from mature dry endosperms. On hydration of the mature seeds the polyribosome content of both embryos and endosperms increases. These polyribosomes are lost on subsequent dehydration but, again, ribosomes are conserved.

INTRODUCTION

It has been observed that dry seeds, spores and moss gametophytes generally conserve the components of their protein synthesizing complex [1]. In seeds, for example, ribosomes have been shown to be present in dry lettuce achenes [2], dry embryos of wheat [3], red pine [4] and rye [5], and in dry cotyledons of peanut [6]. On the other hand, polyribosomes are absent from dry seed tissues. An apparent exception to this observation has been reported for the endosperm of castor bean. It has been claimed that mature dry castor beans contain no polyribosomes and few, if any, ribosomes [7–10]. If, however, castor bean seeds are allowed to hydrate for 40 hr, and then dehydrated, ribosomes can be extracted from the dried endosperms [11]. We decided to investigate further the status of ribosomes in mature dry endosperms and in endosperms of hydrated–desiccated castor bean seeds, and also to extend these studies to the status of ribosomes in the dry embryo.

RESULTS

Ribosome content of mature dry endosperms and embryos

Sucrose gradient analysis of the ribosomal pellet extracted from mature dry endosperms and embryos of castor beans revealed the presence of a single ribosomal peak (R), a subunit peak (S), but no polyribosomes (P) (Fig. 1, A and B).

Ribosomal pellet activity was assayed by determining its ability to stimulate the synthesis of polyphenylalanine *in vitro* utilizing poly(U) as message. Ribosomes from dry endosperms and embryos have considerable activity, those from the dry embryos being the more active (Table 1). Similar amounts of RNA from the embryos and endosperms were added to the *in vitro* system. In the absence of wheat germ supernatant, ribosomes, or of added messenger RNA, no appreciable polypeptide synthesis occurred. The amount of RNA in the ribosomal pellet extracted from the dry embryo was 2.24 μg RNA/mg dry wt and from the endosperm 0.97 μg RNA/mg dry wt. The difference is, in part, a reflection of the greater dry wt of the storage material (lipid and protein) in the endosperms.

The above observations obviously contradict those reported previously [7–10]. We considered that this might be because our mature seeds were not as dry as those used in the previous studies. Hence we dissected endosperms from 3 mature dry seeds and embryos from 8 mature dry seeds, and kept them over activated Si gel for 48 hr. The loss in weight from the endosperms was 15.9 mg (original weight, 836 mg), and from the embryos,

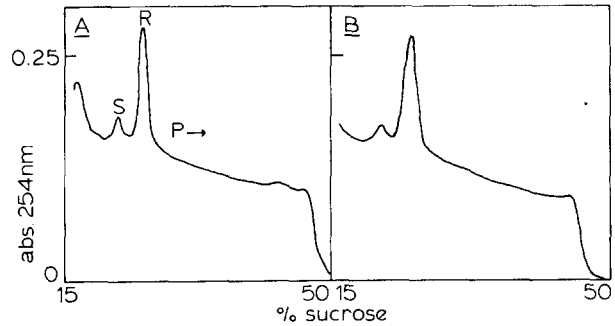

Fig. 1. Sucrose gradient analysis of the ribosomal pellet from (A) endosperms and (B) embryos of mature dry castor bean seeds.

Table 1. *In vitro* activity of ribosomes from dry mature embryos and endosperms of castor bean

Components	Incorporation of phenylalanine (pmol/mg RNA)
Dry embryos	
Complete system	101.5
Minus supernatant	0.2
Minus poly(U)	1.2
Dry endosperms	
Complete system	70.1
Minus supernatant	0.3
Minus poly(U)	0.2

For components of the complete system see Experimental. Amount of RNA from dry embryos and dry endosperms added to the incubation mixture was 13.0 and 13.4 μg, respectively. In the absence of ribosomes there was $<2.5\%$ incorporation of the complete system.

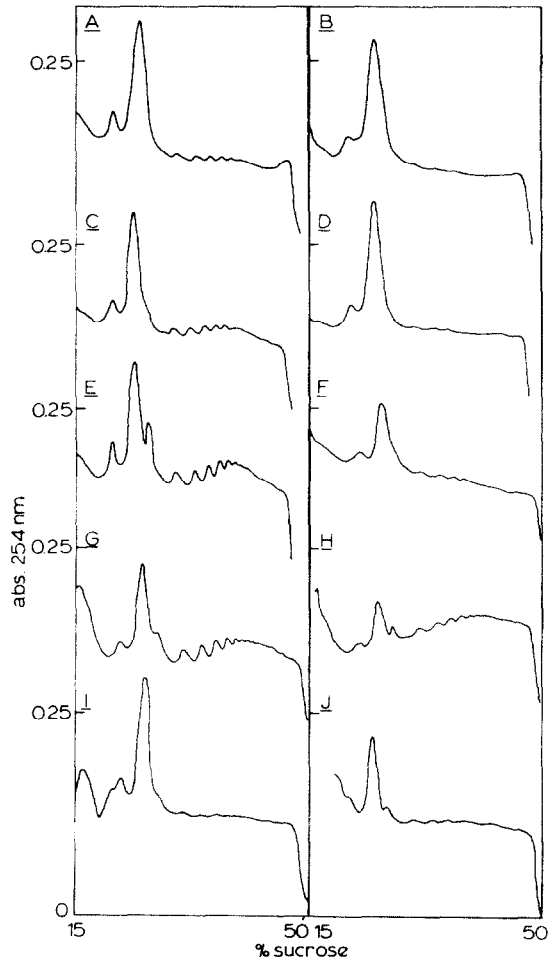

Fig. 2. Sucrose gradient analysis of the ribosomal pellet from (A, C, E, G, I) endosperms and (B, D, F, H, J) embryos of castor bean hydrated for (A, B) 2 hr, (C, D) 4 hr, (E, F) 12 hr and (G, H) 19 hr, or (I, J) hydrated for 19 hr before dehydration for 48 hr over Si gel. Because of the presence of residual Triton X-100, and of heparin in the solution in which the ribosomal pellet was suspended, absorbance readings to determine RNA concentration could not be taken. Hence, loading on the sucrose gradient was by volume, and some variability in total RNA occurred as can be observed by differences in the total area under the peaks.

5.9 mg (original weight, 134 mg). Extraction and analysis of the ribosomal pellet, however, resulted in an identical pattern to that obtained in Fig. 1, A and B (data not presented).

Ribosomes and polyribosomes in hydrated and subsequently dehydrated endosperms and embryos

With increasing time after hydration of the mature dry seeds there is increased formation of polyribosomes within the endosperms and the embryos (Fig. 2, A–H). Polyribosome formation appears to be more pronounced in the endosperms at earlier times (2–12 hr) after rehydration. After 19 hr from initial hydration, polyribosome content of both parts of the seed is considerable (Fig. 2, G and H). Endosperms and embryos dissected from 19 hr hydrated seeds and placed over Si gel for 48 hr decline in polyribosome content, while single ribosomes increase (Fig. 2, I and J). The ribosomes from these hydrated–dehydrated endosperms and embryos catalyse polyphenylalanine synthesis to a similar extent as shown in Table 1 (data not presented).

DISCUSSION

Our results show that mature dry endosperms and embryos of castor bean contain ribosomes which are capable of catalysing protein synthesis. We cannot readily explain why previous workers [7–10] were unable to detect the presence of ribosomes within the mature dry endosperm. Our studies suggest that the degree of 'dryness' of the mature seed was not a factor. Nor does it seem that seed age in storage could have been a factor for we, and others [7], used seeds from the previous year's harvest. We suggest our use of improved techniques for polyribosome extraction and *in vitro* analysis accounts for our ability to detect ribosomes in the dry seed tissues.

We confirmed that ribosomes are conserved in the endosperms after hydration followed by dehydration, and extended this observation to the embryos. A few polyribosomes appear to be present in the 19-hr hydrated–dehydrated seed parts. This may be related to the speed of dehydration to which these tissues were subjected, for in other plant tissues it has been shown that some polyribosomes remain after rapid desiccation over Si gel [12].

In conclusion, it is apparent from these studies that ribosomes are conserved in an active state in mature dry and in dried endosperms and embryos of castor bean. This is the usual situation in dry, desiccation-tolerant tissues. Hence castor bean no longer can be claimed as an exception.

EXPERIMENTAL

Seeds of castor bean (*Ricinus communis*) cv Hale, 1977 harvest, were purchased from McNair Seed Co., Plainview, Texas and stored at 5° until used.

Polyribosome and ribosome extraction. This was based upon two previously used techniques for dry tissues [13, 14]. Endosperms from 3 seeds, or embryos from 8 seeds, were placed in an ice-cold mortar and pestle and ground in 2 ml grinding soln (250 mM sucrose, 40 mM KCl, 5 mM Mg acetate, 50 mM Tris–Cl pH 8.1, 5 mM mercaptoethanol, 1% Triton X-100, and 1000 units/ml heparin (Na salt, Sigma). Two more lots (3 ml each) of grinding soln were added. The homogenate was transferred to a Duall ground-glass homogenizer where the grinding

was completed. The 8 ml of homogenate was centrifuged at 21000 *g* for 10 min after which the supernatant was layered onto a 2.5 ml 36% sucrose pad made up in the same salts as the grinding soln, plus 5 mM mercaptoethanol. After centrifugation for 90 min at 165000 *g* in a Beckman Ti50 rotor, the supernatant and sucrose pad were removed by vacuum. The remaining pellet was resuspended in 0.6 ml 40 mM KCl, 5 mM Mg acetate, 50 mM Tris–Cl pH 8.1, 5 mM mercaptoethanol and 100 units/ml heparin. An aliquot was loaded onto a 15–50% sucrose gradient containing the same salts as the grinding soln, plus 5 mM mercaptoethanol. The gradients were spun for 75 min at 200000 *g* using a Beckman SW50.1 rotor. Polysome and ribosome content of the gradients was determined by monitoring the gradients at A_{254} in a modified continuous flow ISCO UA2 analyser.

In vitro *activity of ribosomes.* Ribosome activity was followed using an *in vitro* polyphenylalanine synthesis assay. Details of the ribosome extraction technique, prepn of wheat germ (Maple Leaf Mills Ltd., Calgary) supernatant, and the *in vitro* system are to be found in ref. [15].

Acknowledgements—This work is supported by NSERC grant A6352 and E2550 to J. D. B.

REFERENCES

1. Bewley, J. D. (1979) *Annu. Rev. Plant Physiol.* **30**, 195.
2. Fountain, D. W. and Bewley, J. D. (1973) *Plant Physiol.* **52**, 604.
3. Marcus, A., Luginbill, B. and Feeley, J. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **59**, 1243.
4. Sasaki, S. and Brown, G. N. (1971) *Plant Cell Physiol.* **12**, 749.
5. Roberts, B. E., Payne, P. I. and Osborne, D. J. (1973) *Biochem. J.* **131**, 275.
6. Jachymczyk, W. J. and Cherry, J. H. (1968) *Biochim. Biophys. Acta* **157**, 368.
7. Marré, E., Cocucci, S. and Sturani, E. (1965) *Plant Physiol.* **40**, 1162.
8. Sturani, E. and Cocucci, S. (1965) *Life Sci.* **4**, 1937.
9. Sturani, E. (1966) *G. Bot. Ital.* **73**, 343.
10. Sturani, E. (1968) *Life Sci.* **7**, 527.
11. Sturani, E., Cocucci, S. and Marré, E. (1968) *Plant Cell Physiol.* **9**, 783.
12. Bewley, J. D. (1973) *Plant Physiol.* **51**, 285.
13. Akalehiywot, T., Gedamu, L. and Bewley, J. D. (1977) *Can. J. Biochem.* **55**, 901.
14. Bewley, J. D. (1973) *Plant Sci. Letters* **1**, 303.
15. Gwóźdź, E. A. and Bewley, J. D. (1975) *Plant Physiol.* **55**, 340.