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BASE COMPOSITION AND COLUMN CHROMATOGRAPHY STUDIES OF RIBONUCLEIC ACID DIFFERENTIALLY EXTRACTED FROM PEA ROOTS WITH SODIUM LAURYL SULFATE OR *p*-AMINO SALICYLATE*

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Abstract—Three-day-old pea roots, aseptically grown, were incubated for 2 hr in ^{32}P phosphate and extracted by the phenol method in the absence of sodium lauryl sulfate or *p*-amino salicylate. One or the other of these compounds was then added and the residue was re-extracted. RNA obtained by the re-extraction with either compound was of higher specific activity and contained a higher mole percent AMP than the RNA from the first extraction. A small part of the AMP-rich RNA eluted with DNA during chromatography on methylated albumin kieselguhr columns, but most of it adhered to the columns. Presence of unlabelled carrier nucleic acids had no measurable effect on the elution pattern.

INTRODUCTION

ONE of the difficult problems in studying plant mRNA (messenger) has been to separate it from the other nucleic acids, especially the rRNA (ribosomal). Several investigators have approached the problem by using differential extraction, without and then with SLS (sodium lauryl sulfate) or PAS (*p*-amino salicylate), to partially effect the separation. Kirby[1] found that PAS, SLS, and various other organic anions increased the yield of DNA obtained by phenolic extraction of animal tissues. It has since become common in many laboratories to add PAS or SLS when extracting nucleic acids by the phenol method. Cherry and Chroboczek[2] studied the effects of SLS concentration during the extraction of nucleic acids from peanut cotyledons. They showed increasing specific activity of RNA and increasing yield of DNA as SLS concentration was increased from 0 to 4 per cent in the extracting medium.

Kidson *et al.*[3] extracted rat livers by the phenol method without PAS and then re-extracted the insoluble residue in the presence of PAS, thus obtaining an RNA fraction characterized by high specific activity after short periods of labelling. This method was adopted by Kidson and Kirby[4,5] to provide a preliminary separation of mRNA and DNA from other nucleic acids prior to countercurrent distribution studies of mRNA. Ingle *et al.*[6] used the same kind of differential extraction technique to remove from soybean hypocotyls an RNA fraction rich in AMP. However, MAK (methylated albumin on kieselguhr) chromatography indicated

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[1] K. S. KIRBY, *Biochem. J.* **66**, 495 (1957).
[2] J. H. CHERRY and H. CHROBOCZEK, *Phytochem.* **5**, 411 (1966).
[3] C. KIDSON, K. S. KIRBY and R. K. RALPH, *J. Mol. Biol.* **7**, 312 (1963).
[4] C. KIDSON and K. S. KIRBY, *J. Mol. Biol.* **10**, 187 (1964).
[5] C. KIDSON and K. S. KIRBY, *Cancer Res.* **24**, 1604 (1964).
[6] J. INGLE, J. L. KEY and R. E. HOLM, *J. Mol. Biol.* **11**, 730 (1965).

that a mixture of nucleic acids was obtained when the insoluble residue was re-extracted in the presence of SLS, especially when incubation with radioisotope was for 7 hr rather than 1 hr. A similar mixture was found by Cherry and Chroboczek[2] in nucleic acids differentially extracted with SLS from peanut cotyledons after 2-hr incubation periods. Both groups of workers reported that differential extractions depending upon re-extraction at higher temperature or higher pH produced about the same effects as did re-extraction with SLS.

The purpose of the present study was to determine the base composition of RNA fractions obtained by extracting plant tissue in the absence of SLS and PAS with those obtained by a re-extraction in the presence of one of these compounds. The crude extracts as well as fractions separated by MAK chromatography were subjected to base analysis. Different RNAase inhibitors were utilized for the SLS and PAS experiments in accordance with procedures previously developed for each.[3,7] Pea (*Pisum sativum* L.) roots were selected as the experimental tissue because of their rapid uptake of ^{32}P and because they could readily be grown free from bacterial contamination.

RESULTS

Effect of SLS on Nucleic Acid Extractions

Before beginning the differential extraction experiments, nucleic acids were extracted from pea roots by the procedure of Cherry *et al.*[7] and Chroboczek and Cherry,[8] with SLS added during the initial extraction. The MAK chromatography profile of the nucleic acids (Fig. 1) followed the usual pattern for plant tissues. Designation of sRNA (soluble), DNA-RNA, lrRNA (light ribosomal), hrRNA (heavy ribosomal), and mRNA fractions is based upon characterization of corresponding fractions obtained from peanut cotyledons[7–9] and soybean hypocotyls.[6]

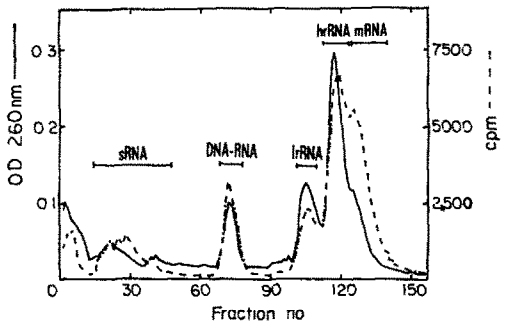

FIG. 1. SEPARATION ON MAK COLUMNS OF NUCLEIC ACIDS EXTRACTED IN PRESENCE OF SLS.

After 2 hr incubation with ^{32}P, thirty-six aseptically grown pea roots were homogenized on ice in a mixture containing 9 ml 0·01 M tris-HCl, pH 7·6, 0·06 M KCl, 0·01 M $MgCl_2$; 1 ml bentonite suspension (20 mg); 2·4 ml 11% SLS ("dupanol"); and 9 ml phenol (washed with the tris-buffer). The aqueous layer was drawn off after centrifugation and re-treated with cold phenol. The nucleic acids were precipitated with ethanol and potassium acetate, dissolved in 0·05 M sodium phosphate, pH 6·7, and dialyzed 2 days against the same buffer. Seven-tenths of the sample, representing 24 O.D. units, was placed on a MAK column and eluted with a linear gradient of NaCl from 0·35 M to 1·05 M in 0·05 M sodium phosphate, pH 6·7. Fractions containing 5 ml were collected and assayed for absorbancy at 260 nm and for radioactivity.

[7] J. H. CHERRY, H. CHROBOCZEK, W. J. G. CARPENTER and A. RICHMOND, *Plant Physiol.* **40**, 582 (1965).
[8] H. CHROBOCZEK and J. H. CHERRY, *J. Mol. Biol.* **19**, 28 (1966).
[9] J. H. CHERRY, *Science* **146**, 1066 (1964).

Omission of SLS from the extracting medium greatly reduced the DNA–RNA peak in the MAK profile (Fig. 2(a)). A small additional quantity of nucleic acids was removed from the residue in the phenol layer by adding SLS and re-extracting (Fig. 2(b)). The ratio of nucleic acids extracted without SLS to those obtained in the re-extraction was 22:1 as estimated from u.v. absorbancy. The ratio between the radioactivities was only 13:1, reflecting the higher specific activity of nucleic acids extracted with SLS. Note that because of the small amount of nucleic acids obtained by the re-extraction, data in Fig. 2(b) are plotted to a different scale than that used in Fig. 2(a). The DNA–RNA peak in Fig. 2(b) comprises a much greater proportion of the total nucleic acids than was the case in Fig. 2(a), but a nucleic acid fraction of high specific activity is also apparent in the rRNA and mRNA zones (tubes 114–142).

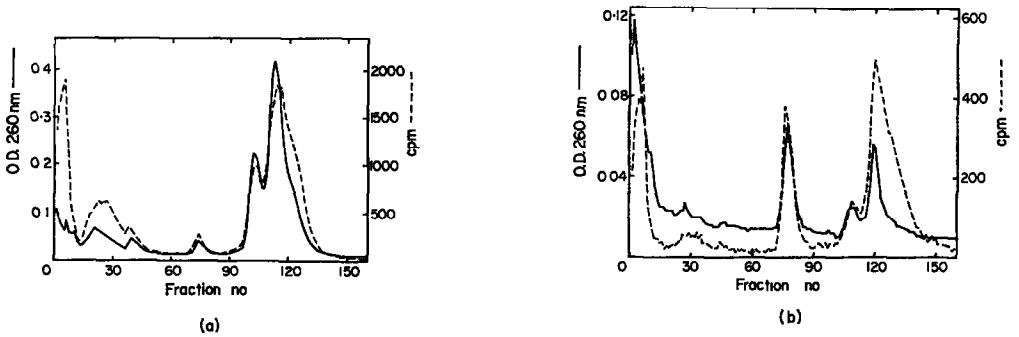

FIG. 2. MAK CHROMATOGRAPHY OF NUCLEIC ACIDS DIFFERENTIALLY EXTRACTED WITH SLS.

(a) Nucleic acids were extracted as in Fig. 1, except that SLS was omitted and the phenol layer was re-extracted with the tris-buffer solution, which was then combined with the first aqueous layer. After further phenol treatment, ethanol precipitation, and dialysis, 26 per cent of the nucleic acids were placed on a MAK column. The remainder of the extract was taken for KOH hydrolysis (Fig. 3(a)).

(b) The insoluble residue in the phenol layer from (a) was treated with SLS, tris-buffer solution, and bentonite and re-extracted. The aqueous phase was twice treated with more phenol, the nucleic acids were precipitated and dialyzed, and 80 per cent of the solution was placed on a MAK column. The remainder was taken for KOH hydrolysis (Fig. 3(b)).

Dowex-1 chromatography of the hydrolyzed RNA provided good separation of the four mononucleotides. Sample profiles are shown in Fig. 3. Base composition of the total extracts and of the various fractions obtained from MAK chromatography are presented in Table 1. For comparison the last line of the table gives the base composition of the mRNA fraction from a preparation like that shown in Fig. 1, i.e. from a single extraction with SLS present. The percentage recoveries of radioactivity were calculated by dividing the sum of the cpm in the four peaks by the total cpm (adjusted for decay) in the fractions taken for KOH hydrolysis. Thus, in addition to any losses during manipulation, the ^{32}P not recovered could have been in the form of DNA, inorganic phosphate, "background" between the peaks, or material adhering to the Dowex—1.

The crude extracts obtained by differential extraction with SLS were distinctly different in base composition (see the first two data lines in Table 1). It was difficult to explain the conspicuously high AMP content of the SLS–RNA from an inspection of the base compositions of the MAK fractions shown in the remainder of Table 1. The lrRNA, hrRNA, and mRNA differentially extracted with SLS had somewhat more AMP than the equivalent fractions

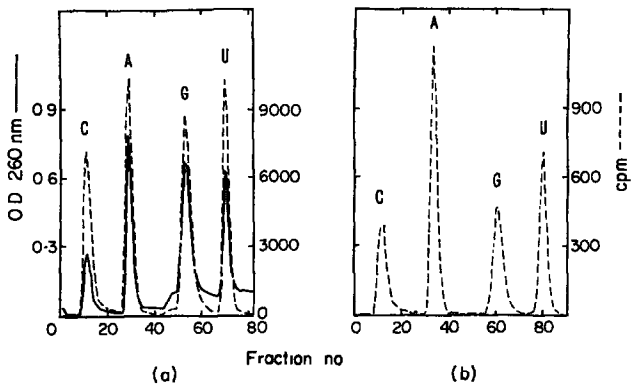

FIG. 3. SEPARATION OF MONONUCLEOTIDES ON DOWEX-1 COLUMNS.

Mononucleotides obtained from KOH hydrolysis were placed on columns of Dowex 1 × 8 charged with formate. CMP, AMP, and GMP were removed by stepwise elution with 0·1 N, 1 N, and 3 N formic acid, respectively. UMP was eluted with 4 N formic acid containing 0·1 N ammonium formate. Four or 5-ml fractions were collected. Failure of the O.D. profile to return to the base line after the second peak is due to absorbance by formate.

(a) Nucleotides from hydrolysis of RNA extracted without SLS.

(b) Nucleotides from hydrolysis of RNA obtained by re-extraction in presence of SLS.

TABLE 1. BASE COMPOSITION BEFORE AND AFTER MAK CHROMATOGRAPHY OF RNA EXTRACTED IN ABSENCE OF SLS, COMPARED WITH BASE COMPOSITION OF EQUIVALENT FRACTIONS RE-EXTRACTED IN THE PRESENCE OF SLS*

Nature of extraction	Fraction from MAK	Mole per cent† C	A	G	U	A+U/C+G	Recovery (%)
Without SLS	Crude	22	25	30	24	0·94	77
Re-extracted with SLS	Crude	16	37	22	25	1·64	77
Without SLS	DNA-RNA	17	25	29	28	1·14	57
Re-extracted with SLS	DNA-RNA	(11)	(41)	(21)	(28)	(2·2)	5
Without SLS	lrRNA	17	25	31	26	1·04	86
Re-extracted with SLS	lrRNA	15	28	29	28	1·28	79
Without SLS	hrRNA	20	26	33	21	0·90	76
Re-extracted with SLS	hrRNA	18	29	31	23	1·06	68
Without SLS	mRNA	18	28	29	25	1·11	79
Re-extracted with SLS	mRNA	15	34	25	27	1·51	76
With SLS (first time)	mRNA	20	27	30	23	1·00	82

* Aliquots of extracts described in Fig. 2 were analyzed as in Fig. 3 to obtain base composition of total (crude) RNA before MAK chromatography. Also analyzed were fractions from the MAK columns. For convenience, the MAK fractions are arbitrarily assigned the names shown in Fig. 1.

† The sum of the cpm in the four peaks was set at 100 per cent. Any odd nucleotide which might have been present in addition to the four common ones would have been erroneously counted with one of these four or ignored, depending upon where it eluted. Inorganic phosphate elutes immediately after the 3 N formic acid is added, at least ten tubes ahead of the AMP, and is not included in the cpm sum. Values in parentheses are approximated because of low radioactivity.

extracted without SLS, but the difference was less than in the crude extracts. From the percentage recoveries (cpm released by KOH hydrolysis) it seems that most of the radioactivity in the small DNA–RNA peak obtained without SLS was RNA rather than DNA. By contrast, very little of the radioactivity in the same peak differentially extracted with SLS was recovered as ribonucleotides. In spite of the low radioactivity it was obvious that the AMP content was distinctly higher in the latter fraction.

Effect of PAS on Nucleic Acid Extractions

Kirby[10] used a modified phenol method to extract nucleic acids from rat liver at 20°. Since it was not clear whether Kidson *et al.*[3] also worked at room temperature or on ice with their differential extraction of rat liver by PAS, and since extraction temperature affects the nucleic acids obtained without detergent,[2] we first tried the extractions in the PAS experiments

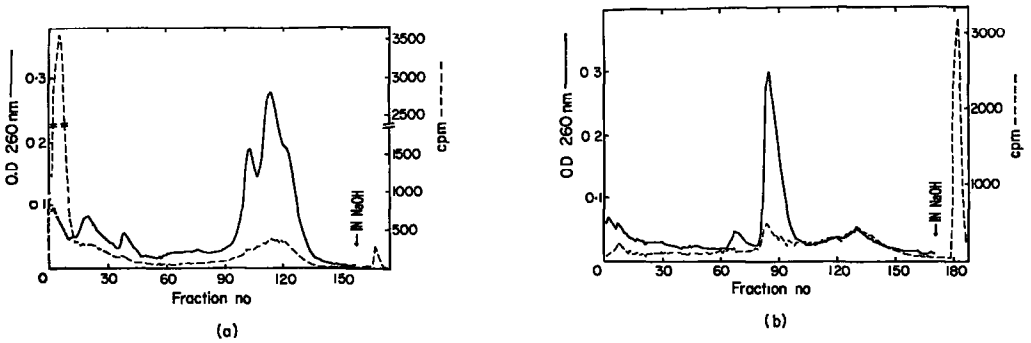

FIG. 4. MAK CHROMATOGRAPHY OF NUCLEIC ACIDS DIFFERENTIALLY EXTRACTED AT ROOM TEMPERATURE WITH PAS.

(a) Nucleic acids were extracted as in Fig. 2(a), but with the following changes: the aqueous phase consisted of 0·5% NDS; the phenol phase contained 0·1% 8-hydroxyquinoline; and the extraction was at room temperature rather than on ice. Following further phenol treatment, precipitation, and dialysis, 14 per cent of the nucleic acid solution was put on the MAK column.

(b) The residue at the interface from (a) was re-extracted as in Fig. 2(b), except that PAS was substituted for SLS and the re-extraction was at room temperature. All of the nucleic acids were put on the MAK column.

at room temperature. All solutions were kept at 0–5° except during the actual extractions. At the end of the regular NaCl elution gradient 90 ml of 1 N NaOH was passed through each MAK column and the resulting nucleic acid fractions were collected for radioactivity measurements.

As expected, the MAK elution profile for nucleic acids extracted without PAS (Fig. 4(a)) had no DNA–RNA peak and the sRNA, lrRNA, and hrRNA peaks were well defined. However, the elution of radioactive materials did not coincide well with the u.v. absorbancies. The bulk of the radioactivity was eluted at the beginning of the gradient where oligonucleotides would be expected. There was radioactivity generally coinciding with the rRNA peaks, but no sharp separation was apparent between lrRNA and hrRNA. Some further radioactivity was eluted from MAK by NaOH.

Nucleic acids differentially extracted with PAS had a large u.v. absorbancy peak in the DNA–RNA area (Fig. 4(b)). There was also a suggestion of rRNA peaks. While the rRNA

[10] K. S. KIRBY, *Biochim. Biophys. Acta* **55**, 545 (1962).

fraction of the PAS nucleic acids (Fig. 4(b)) had a much higher specific activity than the rRNA extracted without PAS (Fig. 4(a)), it is extremely interesting that nearly one-third of the radioactivity of the PAS nucleic acids was eluted from the MAK column with NaOH.

We suspected that the high radioactivity in the oligonucleotides and the indistinct cpm profile in the rRNA area of Fig. 4(a) indicated serious degradation by RNAase. Therefore the experiment was repeated with the exception that the extraction procedure was carried out at 1–5° and *m*-cresol was added to the phenol to further inhibit RNAase.[11] As can be seen

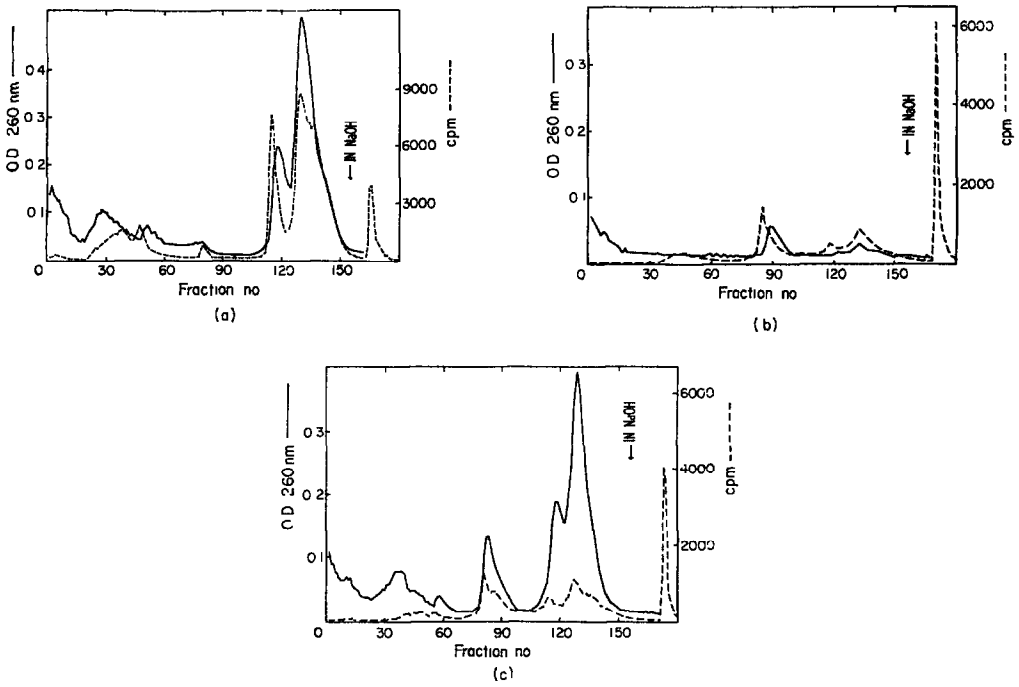

FIG. 5. MAK CHROMATOGRAPHY OF NUCLEIC ACIDS DIFFERENTIALLY EXTRACTED ON ICE WITH PAS AND PHENOL-*m*-CRESOL.

(a) Same as Fig. 4(a), except all steps performed on ice and *m*-cresol added to the phenol (12:88, vol./vol.). One-sixth of the nucleic acid solution was put on the MAK column.

(b) Same as Fig. 4(b), except for the changes noted in (a). Half of the nucleic acid solution was put on the MAK column. No carrier nucleic acids added. Note that scales are different from Fig. 4(a).

(c) Same as (b), but with unlabelled nucleic acids from pea cotyledons added prior to MAK chromatography.

from Fig. 5(a), the radioactivity in the presumed oligonucleotides was virtually eliminated by this treatment, and lrRNA and hrRNA were sharply defined. Figure 5(b) depicts the results of the differential extraction of nucleic acids with PAS. Note that due to the small quantity of nucleic acids present in this fraction it was again necessary to change the plotting scale in order to show detail. The rRNA from the differential extraction with PAS was about one-fourth higher in specific activity than the rRNA from the extraction without PAS. However, the most conspicuous feature of Fig. 5(b) is the large amount of radioactivity which was not eluted by the saline solution but which was removed by NaOH. Calculation of percentage recovery

[11] C. KIDSON and K. S. KIRBY, *Cancer Res.* **25**, 472 (1965).

of radioactivity after KOH hydrolysis and base analysis of mononucleotides indicated that nearly all of the radioactivity in the NaOH effluent was present as RNA. This was confirmed in later experiments.

Asano[12] showed a shift in the position of mRNA elution from MAK columns depending upon whether or not rRNA was present. To determine whether a similar effect could be detected with the nucleic acids differentially extracted by PAS, unlabelled nucleic acids from pea cotyledons were added to one aliquot (Fig. 5(c)), but not to another (Fig. 5(b)), before MAK chromatography. A comparison of Fig. 5(b) with Fig. 5(c) indicates that in this case the elution of radioactivity was not significantly affected by the addition of carrier nucleic acids.

Base compositions of the RNA fractions differentially extracted by PAS are presented in Table 2. As in the SLS experiments, the crude extracts are markedly different in AMP content. However, the RNA extracted by PAS was rich not only in AMP but also in UMP, with the consequence that the ratio of A+U to C+G was greater than 2. There was too little radioactivity in the DNA–RNA peak shown in Fig. 5(a) for base analysis. The DNA–RNA radioactivity peak from Fig. 5(b), and especially from Fig. 5(c), gave an indication that two overlapping peaks might be present. Therefore fractions 78–83 from the DNA–RNA peak shown in Fig. 5(c) were analyzed separately from fractions 84 to 93. The recovery of cpm as ribonucleotides from the first and second portions of the peak was 12 per cent and 15 per cent, respectively. As shown in Table 2, base compositions of the two portions were also similar. Both resembled the crude extract in AMP and UMP content.

TABLE 2. BASE COMPOSITION BEFORE AND AFTER MAK CHROMATOGRAPHY OF RNA EXTRACTED IN ABSENCE OF PAS, COMPARED WITH BASE COMPOSITION OF EQUIVALENT FRACTIONS RE-EXTRACTED IN THE PRESENCE OF PAS*

Nature of extraction	Fraction from MAK	Mole per cent†				A+U/ C+G
		C	A	G	U	
Without PAS	Crude	23	22	29	26	0·94
Re-extracted with PAS	Crude	13	33	19	35	2·12
Without PAS	sRNA	21	18	34	27	0·82
Re-extracted with PAS	DNA-RNA, 1st 6 tubes	11	33	22	35	2·06
Re-extracted with PAS	DNA-RNA, next 10 tubes	10	31	25	34	1·85
Without PAS	lrRNA	12	23	37	28	1·01
Re-extracted with PAS	lrRNA	13	28	34	25	1·14
Without PAS	hrRNA	18	26	34	23	0·96
Re-extracted with PAS	hrRNA	16	28	34	22	0·99
Without PAS	mRNA	18	26	34	22	0·92
Re-extracted with PAS	mRNA	13	28	33	25	1·15
Without PAS	NaOH eluate	20	25	31	23	0·93
Re-extracted with PAS	NaOH eluate	15	44	19	22	1·96

* Aliquots of extracts described in Fig. 5 were analyzed as in Fig. 3 to obtain base composition of total (crude) RNA before MAK chromatography. Also analyzed were fractions from the MAK columns.

† See second footnote, Table 1.

[12] K. ASANO, *J. Mol. Biol.* **14**, 71 (1965).

Differences in AMP content between RNA extracted with or without PAS were slight or non-existent for lrRNA, hrRNA, and mRNA fractions. In contrast, there was a striking difference in the AMP content of RNA eluted by NaOH. Where nucleic acids were differentially extracted with PAS, the AMP content of the NaOH eluate was 19 mole per cent higher than in the equivalent fraction obtained by extracting in the absence of PAS.

DISCUSSION

Excised pea roots afforded a convenient test system for studying differential extraction of nucleic acids from plant tissues. The MAK profiles obtained from pea roots were similar to those from other plants; their high incorporation of ^{32}P phosphate into nucleic acids facilitated the base analyses of small MAK fractions; and the problem of bacterial contamination, as indicated by placing randomly selected roots on potato dextrose agar, was essentially eliminated without resort to antibiotics.

Differential extractions by the SLS and PAS procedures gave similar results. In agreement with previous reports,[2, 6] re-extractions with SLS yielded MAK profiles which contained lrRNA and hrRNA as well as a DNA–RNA fraction composed mainly of DNA. The same was true for re-extractions with PAS. Also agreeing with earlier work, the differentially extracted RNA was of higher specific activity and contained a much greater proportion of AMP than when SLS and PAS were absent. However, examination of the base compositions of fractions ordinarily separated by MAK chromatography did not account for the high AMP content of the RNA extracted with SLS or PAS. Only the RNA associated with the DNA peak was as rich as the crude extracts in AMP, and it represented a small part of the total RNA.

Thus it appeared that an RNA fraction high in AMP was lost during MAK chromatography. Koch and Kubinski[13] reported that a rapidly labelled RNA from polio virus adhered to MAK, and Asano[12] found that a large part of phage mRNA could not be eluted from MAK with saline solution. From these papers it seemed likely that the AMP-rich fraction was remaining on the MAK after NaCl elution. Therefore in the PAS experiments NaOH was added at the end of the saline gradient to determine if a tightly bound nucleic acid could be eluted. Such proved to be the case, since significant amounts of radioactivity were removed by NaOH. Judging from percentage recoveries after KOH hydrolysis, most or all of this material was RNA rather than DNA. For RNA extracted without PAS, the NaOH eluate was not greatly different from other MAK fractions in base composition. In contrast, RNA obtained by differential extraction with PAS had a much greater tendency to remain on the MAK, and it had a surprisingly high AMP content. This fraction had nearly twice as much A + U as C + G, whereas the ratio of A + T to C + G in pea DNA is only about 1·5,[14] and the highest A + U/C + G previously reported[15] in RNA from pea roots was approximately 1·4. It is clear that much of the AMP-rich RNA obtained by differential extraction with PAS is not removed from MAK by 1·2 M NaCl. No doubt the loss of AMP-rich RNA from the SLS experiments can be explained in the same way.

A second fraction high in AMP was RNA associated with the DNA peak of nucleic acids differentially extracted with SLS or PAS. In the PAS experiments this fraction was high in UMP as well as in AMP. Cherry[9] showed that the DNA–RNA peak from peanut cotyledons was composed of at least three components: rapidly labelled DNA, bulk DNA, and rapidly

[13] VON G. KOCH and H. KUBINSKI, *Z. Naturforsch.* **19**, 683 (1964).
[14] J. K. HEYES, *Proc. Roy. Soc.* **B152**, 218 (1960).
[15] U. E. LOENING, *Proc. Roy. Soc.* **B162**, 121 (1965).

labelled RNA. The present study indicates that the RNA can be further divided into one portion which is extracted in the absence of SLS or PAS and a different portion which contains much more AMP and which is extracted when SLS or PAS is present.

It is interesting to speculate whether the AMP-rich RNA in the DNA–RNA peak and that eluted by NaOH might be forms of mRNA. Two papers which appeared after this work was completed are relevant to the question. The first[16] is a report of the isolation of poly A (polyadenylic acid) from rat liver. The poly A was not extracted with phenol at neutral pH but was soluble at pH 9·0. Since differential extraction by pH manipulation gives effects similar to differential extraction with SLS,[2, 6] it is possible that part or all of the high AMP content of the RNA which was extracted with SLS and PAS in these experiments was due to the presence of poly A rather than to a DNA-like base sequence. The second paper of particular interest is by Ellem,[17] who obtained from mammalian tissues an RNA fraction which was not removed from MAK by saline elution. This fraction had a high ratio of A+U to C+G.

In plant tissues it has been shown that the fraction eluting after hrRNA, when compared to the other MAK fractions, is highest in AMP and has a base composition most closely resembling that of DNA;[8, 18] hybridizes to the greatest extent with homologous DNA;[19] is most sensitive to actinomycin D;[8] is most rapidly synthesized and has the shortest half-life;[6, 8] is associated to the greatest degree with polyribosomes;[20] is best correlated with growth of excised plant tissues;[18] and is most capable of supporting protein synthesis *in vitro* (Jackymczyk and Cherry, unpublished data). Thus it possesses the characteristics expected of mRNA to a greater extent than do any of the other MAK fractions eluted by 1·2 M NaCl. At least some of these characteristics could apply to a precursor of mRNA as well as to mRNA, so that the findings are not necessarily in conflict with Ellem's[17] suggestion.

Another possibility is that part of the mRNA elutes after the rRNA while additional mRNA adheres to the MAK.[12] If this is the case these two fractions might represent two classes of RNA messengers. At any rate, it would seem desirable to subject the PAS nucleic acid fraction eluted by NaOH to the tests which have been used to characterize fractions eluted from MAK by the saline gradient. So far, its base composition is the only indication that this fraction might be a type of mRNA.

The virtual disappearance of radioactivity in the presumed oligonucleotide peak when *m*-cresol was added to phenol and extractions were made on ice suggests that the labelled oligonucleotides are mainly degradation products resulting from RNAase activity. If so, the combination of NDS, 8-hydroxyquinoline, and *m*-cresol used in the PAS experiments appears to inhibit RNAase even more satisfactorily than does bentonite.

EXPERIMENTAL

Alaska pea seeds were agitated for 2 min in 1% sodium hypochlorite and rinsed repeatedly with sterile, distilled water. The seeds were placed in sterile petri dishes lined with moist tissue paper and allowed to germinate in the dark at 24°. After 3 days, 100–150 radicles, each 3–5 cm long, were excised and placed in a heat-sterilized medium containing 0·03 M sucrose, 0·01 M tris-HCl at pH 6·5, 0·01 M $MgCl_2$, 0·06 M KCl, and 3–5 mc carrier-free $NaH_2\,^{32}PO_4$. Roots were incubated 2 hr in a shaker-bath at 27°. Aseptic technique was used for all operations from hypochlorite treatment through incubation. Immediately after incubation, tissue was rinsed with distilled water and homogenized in one of the media described below for 2 min with a VirTis blender. Except as noted, homogenization and all successive operations preparatory to MAK chromatography were carried out at 0–5°.

[16] A. Hadjivassiliou and G. Brawerman, *J. Mol. Biol.* **20**, 1 (1966).
[17] K. A. O. Ellem, *J. Mol. Biol.* **20**, 283 (1966).
[18] J. L. Key and J. Ingle, *Proc. Natl. Acad. Sci. Wash.* **52**, 1382 (1964).
[19] R. B. van Huystee and J. H. Cherry, *Biochim. Biophys. Res. Commun.* **23**, 835 (1966).
[20] C. Y. Lin, J. L. Key and C. E. Bracker, *Plant Physiol.* **41**, 976 (1966).

Two methods were used for differential extraction of nucleic acids. Where SLS was employed, tissue was homogenized in a medium prepared by mixing 20 ml 0·01 M tris-HCl at pH 7·6, 0·06 M KCl, 0·01 M $MgCl_2$ with 1 ml bentonite (40 mg) and 18 ml cold phenol previously washed with the tris buffer. The aqueous phase was removed and treated with more phenol and bentonite, and the phenol phase was mixed with more of the tris solution and bentonite. The latter mixture was stirred vigorously and centrifuged, and the aqueous phase was combined with the aqueous phase from the first centrifugation. After two further phenol treatments and centrifugations, nucleic acids were precipitated from aqueous solution with 2 vol. of cold ethanol in the presence of 0·2 M potassium acetate. The precipitated nucleic acids were dissolved in 0·05 M sodium phosphate buffer, pH 6·7, and dialyzed against the same buffer. The resulting nucleic acids are referred to as "extracted without SLS".

The phenol phase from the second centrifugation was treated with more tris solution and bentonite, and with 4 ml of an 11 % SLS solution (prepared from "dupanol"). After vigorous stirring the mixture was centrifuged and the phenol phase discarded. The aqueous phase was treated twice more with phenol, and nucleic acids were precipitated from the aqueous with ethanol and dialyzed as outlined above. These nucleic acids are referred to as "extracted with SLS".

Where PAS was substituted for SLS, the procedure was modified to conform more closely to that described by Kidson and Kirby.[11] Instead of tris buffer and bentonite, the aqueous phase of the extracting medium was 0·5 % NDS (naphthalene-1,5-disulphonate) adjusted to pH 7·6. The phenol reagent was prepared by washing phenol with the NDS, making it 0·1 per cent for 8-hydroxyquinoline, and—after the initial experiment—adding to 88 parts of the resulting mixture 12 parts *m*-cresol (vol./vol.). (The *m*-cresol was not added in the first experiment with PAS, when the extractions were made at room temperature.)

Roots were homogenized in 24 ml of each of the aqueous and phenolic phases and the homogenate was centrifuged as above. The aqueous phase was removed and treated with 8 ml 11 % SLS and a further 16 ml of phenol reagent, while the phenol phase was mixed with more 0·5 % NDS. The latter mixture was stirred and centrifuged, and the aqueous phase was combined with the aqueous phase from the first centrifugation. After further phenol treatment and centrifugation the nucleic acids were precipitated and dialyzed as before. The resulting nucleic acids are referred to as "extracted without PAS".

The phenol phase from the second centrifugation was washed with more 0·5 % NDS. The interfacial residue was removed and stirred with equal volumes of 6 % PAS, pH 7·6, and the phenol reagent. The nucleic acids "extracted with PAS" were then obtained by following a procedure parallel to that outlined in the case of SLS.

MAK column chromatography was done according to the method of Mandell and Hershey.[21] Elution was at room temperature using a linear gradient of NaCl from 0·35 M to 1·10 M NaCl in 0·05 M sodium phosphate, pH 6·7.

Nucleotide composition was determined following hydrolysis in 0·5 N KOH for 18 hr at 37°. Hydrolysates were neutralized in the cold with perchloric acid and centrifuged. The mononucleotides in the supernatent were separated by step-wise elution with formic acid from Dowex-1 columns in the formate form.[6,9] Base compositions were estimated from the relative amount of ^{32}P in each of the four nucleotide peaks. Nucleic acids from MAK column chromatography fractions were precipitated with ethanol and dialyzed prior to KOH hydrolysis.

[21] J. D. MANDELL and A. D. HERSHEY, *Anal. Biochem.* 1, 66 (1960).