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TRANSFER RIBONUCLEIC ACID FROM *SCENEDESMUS OBLIQUUS* D3: PURIFICATION OF THE MAJOR FORMYLATABLE METHIONINE-ACCEPTING SPECIES

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Key Word Index—*Scenedesmus obliquus* D3; Chlorophyceae; purification $tRNA^{Met}$; arginine–agarose; BD-cellulose.

Abstract—The major formylatable methionine *t*RNA of *Scenedesmus obliquus* has been purified using two column chromatographic steps: (1) chromatography on arginine–agarose, which gave a 15-fold purification of the methionine accepting activity at 76% recovery of the major activity peak and (2) chromatography on BD-cellulose which separated the major formylatable methionine *t*RNA from at least one other methionine *t*RNA species. This major species is estimated to be at least 85% pure. Nucleoside analysis shows that it lacks the nucleoside ribothymidine. It is proposed that this methionine *t*RNA species is the cytoplasmic initiator *t*RNA ($tRNA_i^{Met}$) of *S. obliquus*.

INTRODUCTION

Since the first *t*RNA species was purified and its primary structure determined [1, 2], the structural elucidation of some 95 *t*RNA species from different sources has been achieved [3]. It has been found that all the sequences fit the clover leaf secondary structure proposed by Holley and the *t*RNAs have certain common features, one being the sequence of nucleosides —GTψC— in loop IV [3]. It has been found, however, that the cytoplasmic initiator *t*RNAs from eukaryotes are distinguished from other *t*RNAs by the replacement of the above sequence by —GAUC— [5–10].

Of the sources from which *t*RNAs have been isolated and studied the green algae have been largely neglected. Recently we have isolated the *t*RNA from the green alga, *Scenedesmus obliquus* and reported on its general properties [11]. Here we report on the fractionation of this *t*RNA and the purification and nucleoside analyses of the major formylatable methionine *t*RNA species.

RESULTS AND DISCUSSION

Initial attempts to purify the methionine-acceptor *t*RNAs of *S. obliquus* by column chromatography on DEAE-cellulose, DEAE-Sephadex A25 or DEAE-Sephadex A50 were unsuccessful. In each case the methionine-accepting material was found in fractions where the bulk of the *t*RNA was eluted from the column and only slight overall purification was achieved.

Fractionation of S. obliquus *tRNA on arginine–agarose*

Arginine–agarose has been used successfully for the purification of methionine accepting *t*RNA from *E. coli* [12, 13], and here also it proved useful. *S. obliquus* *t*RNA was separated into 3 peaks as shown by the absorbance profile (260 nm) in Fig. 1. Two peaks of methionine accepting activity, labelled I and II, in the ratio of 95:5 respectively were found using an homologous enzyme preparation. Peak I was eluted at the rear part of the first absorbance peak with a substantial purification of the methionine acceptor *t*RNA. Fractions 93–100 inclusive representing 76% of peak I, have an average specific activity of 304 pmol/A_{260} unit which represents a 15-fold purification.

The percentage of the methionine acceptor *t*RNA in the peaks which could be formylated was assayed by formylation of [^{14}C]methionyl-*t*RNA in the fractions using [^{3}H]formyltetrahydrofolate as formyl donor and a crude transformylase preparation from *E. coli*. Fig. 2 shows the distribution of formylatable material in the fractions and the percentage formylation related to methionine acceptor activity. Here the methionine accepting activity was assayed using an *E. coli* synthetase preparation and it will be seen that the pattern of methionine acceptor *t*RNA is very similar to that obtained with the homologous synthetase preparation (Fig. 1). Methionyl-*t*RNA in fractions of the early part of peak I are formylated very little, but in fractions of the latter part of this peak the methionyl-*t*RNA is formylated to *ca* 25% and the percentage formylation is about the same in each of the latter fractions. This would indicate that in these latter fractions there is a single species of methionine *t*RNA. Overall there would appear to be at least two species of methionine *t*RNA in peak I. One is an early running non-formylatable species and another is a later running species which is formylated to a lowish level. Although peak II is small, its presence was confirmed by repeated assays and accounted for *ca* 5% of the total methionine accepting activity. The methionyl-*t*RNA in peak II was formylated to *ca* 70% with the *E. coli* transformylase preparation. It would appear therefore that the formylatable methionyl-*t*RNA species in peak II is a different species of *t*RNA to the one found in peak I since it is formylated much more efficiently.

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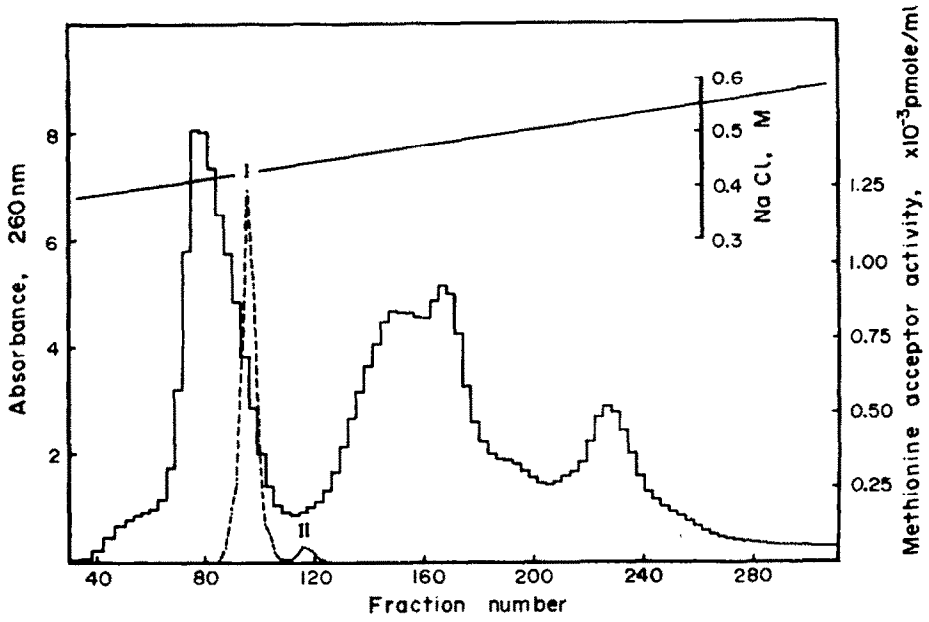

Fig. 1. Purification of *S. obliquus* methionine-accepting *t*RNA on arginine–agarose. 3020 A_{260} units of crude *t*RNA with a methionine-acceptingactivity of 22.2 pmol methionine/A_{260} unit of total *t*RNA were chromatographed on an arginine–agarose column (1.5 × 80 cm) at room temp. using 1.6 l. linear gradient of 0.35–0.75 M NaCl in 20 mM Tris–HCl pH 7.5, 10 mM $MgCl_2$. Fractions of 3 ml were collected at a flow rate of 26 ml/hr. -·---- Methionine acceptor activity, assayed as described in Experimental.

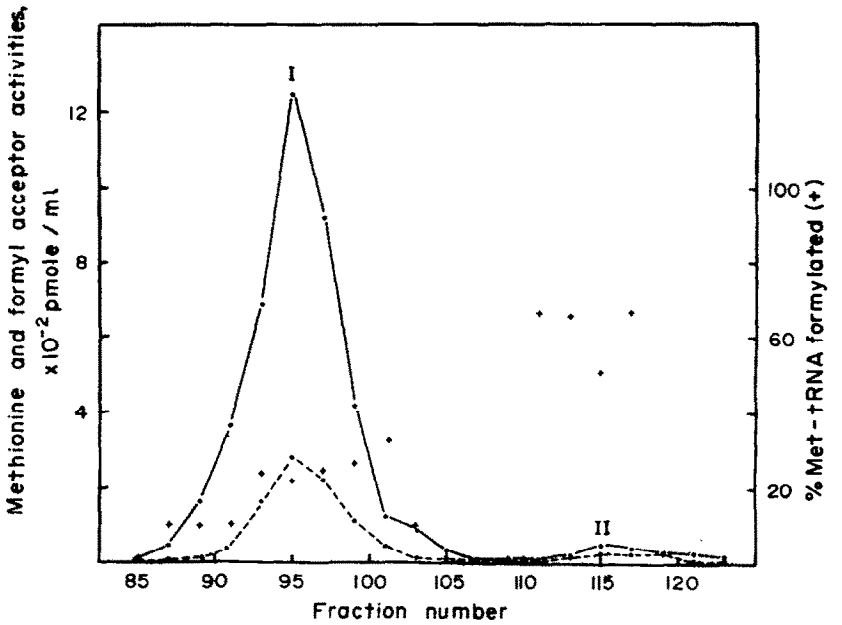

Fig. 2. Formylation of methionyl-*t*RNA in peaks I and II of Fig. 1. Formyl (-·---·) and methionine (·——·) accepting activities were assayed as described in Experimental. + Represents the percentage formylation as related to the methionine accepting activity.

Purification of methionine tRNA on BD-cellulose

Partially purified *S. obliquus* methionine *t*RNA obtained by arginine–agarose chromatography (peak I, Fig. 1) was fractionated by chromatography on BD-cellulose [14]. Two methionine-accepting activity peaks labelled A and B (Fig. 3) were obtained and peak A corresponds to a peak in the absorbance profile. Peaks A and B were present in a ratio of 73:27 and accounted for 95% of the total methionine accepting activity applied to the column. In peak A fractions 56–60 representing *ca* 45% of the total activity of peak A each had a similar methionine accepting activity of 1360 pmol/A_{260}. From this methionine acceptor activity, the $tRNA^{Met}$ in fractions 56–60 was judged to be about 85% pure. This has recently been

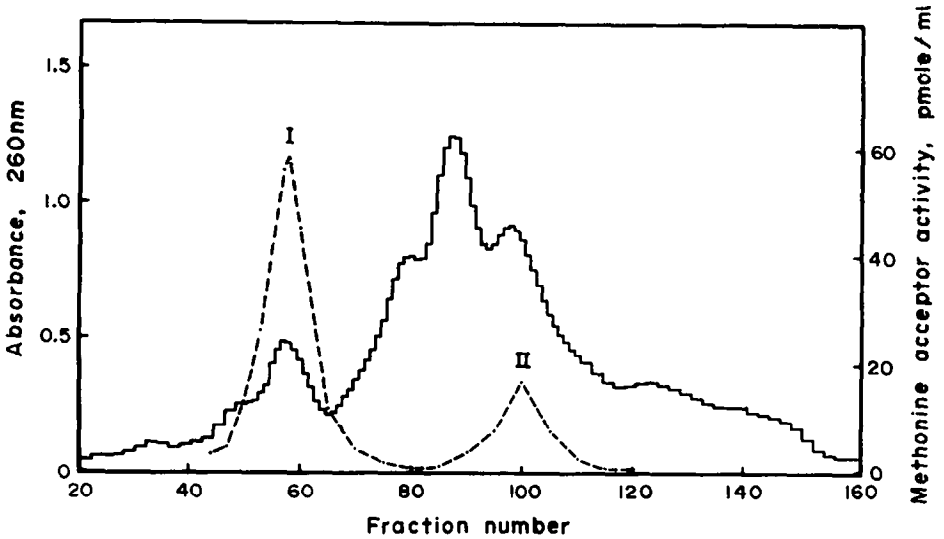

Fig. 3. Purification of methionine *t*RNA on BD-cellulose. Material (136 A_{260} units) in peak I (fractions 91–102, 205 pmol/A_{260}) of Fig. 1 was chromatographed on a BD-cellulose column (1.5 × 80 cm). The sample was eluted with a 1.5.1. linear gradient of 0.45–1.00 M NaCl in 20 mM Tris–HCl pH 7.5, 10 mM $MgCl_2$ at room temp. Fractions of 3.5 ml were collected at a flow rate of 26 ml/hr. – – – –. Methionine acceptor activity, assayed as described in Experimental.

confirmed by gel electrophoresis and by examination of a RNase T_1 digest of the material in these fractions (Olins and Jones, unpublished results). The percentage formylation of methionine *t*RNA in each of the fractions 45–65 using an *E. coli* transformylase preparation was found to be the same at 15%. Since this species of *t*RNA is a major species it is most probably of cytoplasmic origin. The cytoplasmic initiator *t*RNAs from yeast and animal sources are formylated by an *E. coli* transformylase preparation [15–18]. However, those isolated from higher plants are not recognised by the bacterial transformylase [19–22]. Although wheat germ cytoplasmic initiator *t*RNA is not formylated using *E. coli* transformylase it can be formylated to a low level using a transformylase from *Anacystis nidulans* (a blue-green alga) [23]. It is possible therefore that the $tRNA^{Met}$ in peak A (Fig. 3) is the *S. obliquus* cytoplasmic initiator *t*RNA. If this is the case either it differs from the cytoplasmic initiator species of higher plants since it is formylated to a low level by the *E. coli* transformylase or it is contaminated by the chloroplastic or mitochondrial initiator *t*RNA. The latter is unlikely since each of the fractions in the peak is formylated by the *E. coli* transformylase to the same degree.

Nucleoside composition of the major formylatable $tRNA^{Met}$

From the results of the formylation experiments it was suspected that the major formylatable $tRNA^{Met}$ species is the cytoplasmic initiator *t*RNA of *S. obliquus* ($tRNA_i^{Met}$). If it conforms to the pattern found for other eukaryotic cytoplasmic initiator *t*RNAs, it should lack the modified nucleoside ribothymidine. It will be seen (Table 1) from the nucleoside composition, determined according to the method of Randerath *et al.* [24] of the material from fractions 56–60 (Fig. 3) that *S. obliquus* $tRNA_i^{Met}$ species has a low proportion of ribothymidine, which is only 7.5% of that required for one mole to be present per mole of *t*RNA. By comparison, material taken from the forerunning fractions (87–91) of peak I (Fig. 2), which contains partially purified non-formylatable methionine *t*RNA ($tRNA_m^{Met}$), has a ribothymidine composition close to that calculated for one mole of nucleoside per mole *t*RNA. The small amount of ribothymidine found in the $tRNA_i^{Met}$ species probably represents slight contamination from another species of *t*RNA.

The absence of ribothymidine in the major formylatable methionine species supports the proposal that it is the cytoplasmic initiator *t*RNA ($tRNA_i^{Met}$). This conclusion is confirmed by preliminary results from sequence studies, which indicate the absence of the oligonucleotide —TψCG— in the RNase T_1 digest (Olins and Jones, unpublished results).

Table 1. Proportion of nucleosides in *t*RNA isolated from *S. obliquus*

Nucleoside	*S. obliquus* $tRNA_i^{Met}$	*S. obliquus* $tRNA_m^{Met}$
A	26.6	21.5
C	26.3	21.7
G	27.5	30.3
U	9.3	13.0
m^1A	0.5	0.5
m^6A	0.7	0.4
m^5C	1.5	1.6
m^1G	1.1	0.9
m^2G	1.9	0.6
m^7G	0.4	0.4
m_2^2G	0.2	0.6
D	0.9	0.9
m^5U(T)	0.1	1.0
ψ	2.1	2.8

The proportions of nucleosides in the *t*RNA were determined according to the method described by Randerath *et al.* [24] and are expressed as a percentage of the total radioactivity recovered in the nucleoside triols. $tRNA^{Met}$ represents *t*RNA in fractions 56–60 (Fig. 3), $tRNA_m^{Met}$ represents *t*RNA in fractions 87–91 (Fig. 2).

The results shown in Fig. 2 suggest that beside the $tRNA_i^{Met}$ species there are at least two other methionine acceptor tRNAs. One is the non-formylatable methionine tRNA in the forerunning part of peak I and the other is in peak II. Since the methionyl-tRNA in peak II is formylatable it would appear to be another initiator tRNA species. It is formylated much more efficiently with the *E. coli* transformylase than the Met-$tRNA_i^{Met}$ species, which is characteristic of a prokaryotic initiator tRNA [25]. Since also it is present in only a small amount, tentatively we would identify the formylatable methionine tRNA in peak II as either the chloroplastic or mitochondrial initiator tRNA of *S. obliquus*. We feel that it is more likely to be the former since the mitochondria make a much smaller contribution to the mass of the cell than either the cytoplasm or the chloroplast and as is the case in wheat germ [26] the mitochondrial tRNA will be difficult to detect in extracts from whole cells.

EXPERIMENTAL

Materials. Analytical grade chemicals were used where available. L-methionine [Me-^{14}C] (54 mCi/mmol), Na boro [^{3}H] hydride (11Ci/mmol) and Na formate [^{3}H] (250 mCi/mmol) were from The Radiochemical Centre, Amersham, U.K., Micrococcal nuclease was from Sigma (London) and snake venom phosphodiesterase and *E. coli* alkaline phosphatase were from Worthington Biochemical Corporation. Cellulose thin layers on a plastic backing and Blue Brand X-Ray Film were purchased from Eastman Kodak Co. Benzoylated DEAE cellulose (BD-cellulose) was prepared according to the method of ref. [14] and arginine–Sepharose according to the method of ref. [27]. [^{3}H]N^{10}-formyltetrahydrofolate was prepared by Dr. J. M. Old of this laboratory by the method of ref. [28] using a preparation of formyltetrahydrofolate synthetase which was a gift from Professor J. C. Rabinowitz, University of California, U.S.A. Crude *S. obliquus* tRNA [11], and the preparation of aminoacyl tRNA synthetase from *S. obliquus* [11] and *E. coli* [13] were obtained as described previously. Scintillation fluid contained 5g 2,5-diphenyloxazole and 0.3 g 1,4-bis(5-phenyloxazol-2-yl) C_6H_6/l. toluene.

Methionine and formyl-accepting activity assays. Methionine accepting activity of tRNA in the fractions from the column chromatographic steps was determined as described previously [11, 29] using a synthetase preparation from either *E. coli* or *S. obliquus* as indicated in the text. Formylation assays were performed in the same manner as the methionine accepting activity assay using the *E. coli* enzyme preparation except that unlabelled methionine replaced methionine-[^{14}C] and [^{3}H]N^{10}-formyltetrahydrofolate (249 μCi/μmol, 41 μCi/μl) was added to a final concn of 3.3 μM.

Column chromatography. Chromatography on arginine–Sepharose and BD-cellulose was performed using glass columns at atmos. pres. and room temp. The eluant was pumped from the bottom of the column. The *A* of the eluant in the individual fractions was measured at 260 nm.

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