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VALYL *t*RNA's OF *ANACYSTIS NIDULANS*

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Key Word Index—*Anacystis nidulans*; Cyanophyceae; blue–green algae; prokaryote; *t*RNA isoacceptors; valine.

Abstract—*Anacystis nidulans* was found to contain three $tRNA^{Val}$ isoacceptors which could be charged also in heterologous systems with aminoacyl synthetase preparations obtained from dark- and light-grown barley seedlings as well as from yeast.

INTRODUCTION

Interest in the biochemistry of blue–green algae is rapidly growing due to the unique phylogenetic position attributed to them in the symbiotic origin of chloroplasts of higher (eukaryote) plants [1,2]. A number of biochemical properties of the prokaryote blue–green algae are similar to those of the chloroplasts of higher plants, including the sedimentation constants of their ribosomes [3], the molecular size [4], maturation and instability of their ribosomal rRNAs [5–7], the mode of initiation of protein synthesis [8], and the presence of two photosystems [9]. Some unexpected features of metabolic regulation in these organisms [10,11] also warrants further studies on the degree of relationship of this group to bacteria and/or chloroplasts as well as eukaryotic cells. *t*RNAs and their cognate aminoacyl synthetases seem to present a system well suited for a study of this kind, because from a comparison of homologous and heterologous *t*RNA–synthetase systems one can draw conclusions as to the relatedness of the organisms and/or organelles from which the components are derived. Recognition in a heterologous system is regarded as a measure of relatedness. The validity of this idea was tested on the valyl *t*RNA isoacceptors of *Anacystis nidulans* in homologous and heterologous systems.

RESULTS AND DISCUSSION

^{14}C-aminoacylated-$tRNA^{Val}$ of *A. nidulans* charged with homologous synthetase was separated into 3 distinct isoacceptors eluting at 0·55, 0·58 and 0·63 M NaCl, respectively (Fig. 1). The elution patterns of ^{14}C-valyl-*t*RNA's of the same *t*RNA preparation charged with aminoacyl synthetases from yeast, dark-grown and light-grown

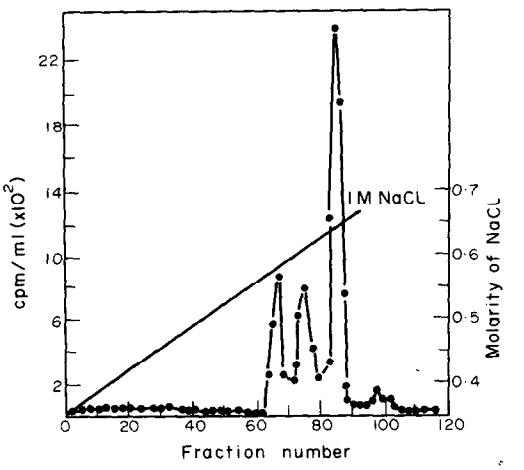

Fig. 1. RCP-5 Chromatography of $tRNA^{Val}$ isoacceptors of *Anacystis nidulans* charged with a homologous aminoacyl synthetase preparation.

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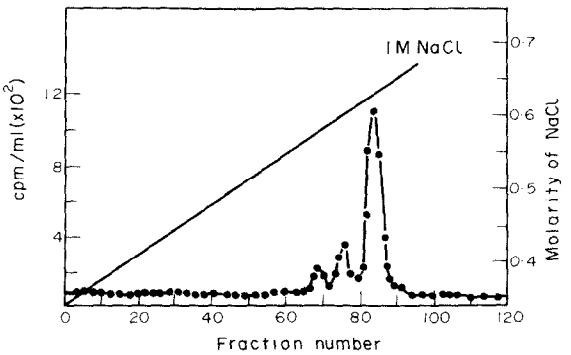

Fig. 2. RCP-5 Chromatography of $tRNA^{Val}$ isoacceptors of *Anacystis midulans* charged with an aminoacyl synthetase preparation isolated from yeast.

barley seedlings (see e.g. Fig. 2) showed that all three enzyme preparations aminoacylated all 3 $tRNA^{Val}$ isoacceptors. The extent of aminoacylation of the individual isoacceptors was, however, different in the homologous (Fig. 1) and heterologous systems (Fig. 2). The 2 $tRNA^{Val}$ isoacceptors eluting at lower NaCl molarities were charged in all heterologous systems to a relatively lower extent, as compared to the isoacceptor eluting at 0·63 M NaCl. The elution profiles were similar with all three heterologous systems.

The literature shows that prokaryotic *t*RNAs (from bacteria and chloroplasts) are preferentially, or often exclusively, charged by synthetases of prokaryotic origin [12–18]. For *A. nidulans*, a prokaryote, strict specificity was shown for the aminoacylation of $tRNA^{Leu}$ with synthetases of prokaryotic origin [19]. On the other hand, indication of some charging with rat liver enzyme of three *t*RNSs of *A. nidulans* (not resolved into isoacceptors) was also reported [20]. The present results show that at least one isoacceptor of $tRNA^{Val}$ of *A. nidulans* can be charged with enzymes of eukaryotic origin essentially to the same extent as with the homologous enzyme. The fact that the enzyme from green- and dark-grown barley seedlings as well as that from yeast equally aminoacylated the isoacceptor in question, suggests that an enzyme synthesized other than in the chloroplasts is responsible for charging. In addition, preliminary observations show that the aminoacyl synthetase of *A. nidulans* recognizes the $tRNA^{Val}$ isoacceptors isolated from barley root tissues in much the same way as the barley synthetase. Therefore, although the principle that prokaryotic *t*RNAs are preferentially recognized by prokaryotic enzymes seems to be generally valid, the results presented in this paper suggest that exceptions to this rule are more widespread than previously believed, and over-generalizations are not justified.

EXPERIMENTAL

Preparation of tRNA. *A. nidulans* cells grown as described by Szalay *et al.* [21] were collected by centrifugation and washed 2 × in 0·05 M Tris buffer, pH 7·8, containing 0·01 M $MgCl_2$, 0·14 M NaCl, 0·0001 M EDTA and 0·002 M $Na_2S_2O_3$. The washed cells were suspended in the same buffer (1 g fr. wt/4 ml) and an equal amount of buffer-saturated phenol was added. The suspension was shaken in the cold for 1 hr. After centrifugation the aq phase was collected. Phenol was removed from the aq phase with Et_2O extraction and the rest of the Et_2O by N_2 stream. The aq phase was applied on a DEAE-cellulose column equilibrated with 0·1 M acetate buffer, pH 4·8, containing 0·01 M $MgCl_2$, 0·01 M EDTA, 0·002 M $Na_2S_2O_3$ and 0·3 M NaCl. The column was washed with the above buffer containing 0·7 M NaCl and the UV absorbing material was collected. *t*RNA was precipitated with 2·5 vol. of cold EtOH.

Isolation of aminoacyl tRNA synthetase. The method of Parthier [22] and that of Barnett *et al.* [23] was used to isolate the enzyme from *A. nidulans* and barley seedlings, respectively.

Aminoacylation of tRNA. The reaction mixture contained 10 A_{260} units of *A. nidulans* *t*RNA (after deaminoacylation according to Guderian *et al.* [13]), 1·5 μmol of ^{14}C -valine (sp act 280 mCi/m mol), 30 μmol of $MgCl_2$, 0·3 μmol of ATP, 4·5 μmol of mercaptoethanol, 100 μmoles of Tris–HCl buffer, pH 7·5, and enzyme preparation to saturate the system. The mixture was incubated for 20 min at 37°. Then, the proteins were removed by passage of the solution through a DEAE-cellulose column as described by Yang and Novelli [24].

Separation of isoacceptors. RCP-5 chromatography was done at 30° as described by Pearson *et al.* [25]. The column size was 1 × 27 cm. 10 A_{260} units of aminoacylated *t*RNA dissolved in 0·01 M acetate buffer, pH 5·0, containing 0·01 $MgCl_2$, 0·002 M EDTA, 0·02 M mercaptoethanol, and 0·35 M NaCl were adsorbed on the RCP-5 column. *t*RNAs were eluted by a linear gradient of 0·35–0·65 M NaCl. The radioactivity of 1 ml fractions added to 10 ml Bray soln was counted in a Packard liquid scintillation spectrometer.

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