cysteine induces a conformational change in the enzyme, thus enhancing the formation of aminoacyl-tRNA".

J. M. O. gratefully acknowledges receipt of an M.R.C. studentship.


Transfer Ribonucleic Acid from Scenedesmus obliquus D3

DAVID S. JONES and FRANCIS T. JAY

Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

Scenedesmus obliquus is a green alga whose cells have a well-defined nucleus, and contain a chloroplast. Even when grown in the dark these cells retain their chloroplast.

Because of the toughness of the cell wall, extraction of tRNA requires either vigorous homogenization of the cells before phenol extraction or prolonged phenol extraction at 60°C. Determination of the amount of tRNA and its purity, estimated by measurement of the methionine-accepting tRNA per g of cells and per $E_{260}$ unit of isolated nucleic acid respectively, showed that the preferred method is by phenol extraction at 60°C. This method, however, is only acceptable when cells, harvested in the mid-exponential phase of growth, are used, since for older cells a toughening of the cell wall apparently prevents extraction of nucleic acid by phenol treatment.

Determination of the base composition by the method described by Katz & Comb (1963) shows that tRNA from S. obliquus contains a considerably higher proportion of UMP and a somewhat lower proportion of CMP than other tRNA species, e.g. that from Escherichia coli (Zubay, 1962), yeast or rabbit liver (Cantoni et al., 1962).

Optimal conditions for charging the tRNA with methionine by using a crude aminoacyl-tRNA synthetase isolated from S. obliquus requires 4mM-Mg$^{2+}$ and pH7.8. With a crude preparation of E. coli transformylase and $[^3H]N^{10}$-formyltetrahydrofolate approx. 20% of this methionyl-tRNA is formylated, whereas with a homologous enzyme preparation only 5% formylation is observed.

The species specificity of the tRNA and synthetase preparations from S. obliquus was studied by comparing the extent of $^{14}$C]methionyl-tRNA formation in homologous and heterologous systems. Whereas the synthetase from rat liver is able to charge the S. obliquus tRNA to an even greater extent than the homologous enzyme, synthetases from E. coli and Anacystis nidulans give a considerably lower charging level. The S. obliquus synthetase is able to charge methionine into tRNA from rat liver, wheat germ and E. coli to a degree only slightly lower than that obtained by using their homologous synthetases.

Conditions have been described (Marcu et al., 1973) whereby an unmodified uridine residue at position 23 from the 3'-end of the tRNA can be methylated specifically. This residue is normally the ribothymidine of the common sequence -G-T-Ψ-C-. By this procedure it is found that S. obliquus tRNA is methylated to about one-half of the extent to which rat liver and wheat-germ tRNA are methylated. Only a very low amount of E. coli tRNA is methylated, which agrees with the findings of Marcu et al. (1973). When tRNA, fractionated by BD-cellulose chromatography (Gillam et al., 1967), was examined by this method at least four separated species of S. obliquus tRNA were detected in which the uridine at this position is unmodified.
The Identification of Sulphated Glycoproteins in Rat Whole Saliva

GRAHAM EMBERY and ELAINE WHITEHEAD

Department of Dental Sciences, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

Although sulphated glycoproteins have been isolated from a wide variety of sources (Yosizawa, 1972), little information is available about those of salivary origin. Their presence in human saliva has been suggested (Martin et al., 1969; Schrager & Oates, 1971), and more recently Sonju & Rolla (1974) have isolated sulphated macromolecules from the sublingual secretions of the monkey Macaca irus. Earlier radioautographic studies on the uptake of injected sodium [35S]sulphate into rodents showed that labelled material localized in certain salivary glands of these animals (Davies & Young, 1954; Belanger, 1963). The nature of the subsequently secreted radioactivity was not established. The primary difficulty of obtaining sufficient quantities of the major salivas is further complicated by the possibility that the minor salivary glands may secrete radiactive sulphate (Hensten-Pettersen & Jacobsen, 1975).

In the present investigation, observations were made on the whole saliva of albino Harvard rats. The fluid was obtained by washing the mouths of the animals with ice-cold 0.9% NaCl at regular intervals during a 6h period after the intraperitoneal injection of sodium [35S]sulphate. Time-course studies showed that radioactivity appeared in the saliva after 10min and was maximal after 90min. At all time-intervals up to 6h, the major proportion of the secreted radioactivity was present as free sulphate, an average of 10% being represented as bound [35S]sulphate.

To isolate the bound radioactivity, pooled saliva from a number of rats was dialysed and concentrated by Amicon filtration by using a UM-05 membrane. Examination of the concentrate by isoelectric focusing on polyacrylamide-gel slabs in the pH range 3–10 produced 37 protein bands, with radioactivity present in the pH range 4.0–5.5. The concentrate was then applied to a column of Bio-Gel P-150 and eluted to yield two radioactive zones, peak 1 (mol.wt. 150000) and peak 2 (mol.wt. 75000). Both peaks were further resolved on DEAE-cellulose to give two further radioactive fractions each. The four fractions obtained were examined by cellulose acetate electrophoresis and isoelectric focusing and were shown to be electrophoretically distinct.

Analysis for hexosamine (Gatt & Berman, 1966), neutral hexose (Dubois et al., 1956), fucose (Pryce-Evans, 1960), sulphate (Doddson, 1961), sialic acid (Warren, 1959), and protein by summation of total amino acid residues, showed the compounds to be sulphated glycoproteins. Hexuronic acid was not detected in any of the samples (Bitter & Muir, 1962).

With the exception of one of the higher-molecular-weight compounds, sulphate contents were in the range 3.5–8.4% and the compounds had carbohydrate:protein ratios in the order of 2:1. In contrast, one of the peak-1 samples had only 18.0% carbohydrate and a sulphate content of 24.7%. The molecular size and sulphate content were not altered on recycling through the columns of Bio-Gel P-150 and DEAE-cellulose and the sulphate content was in accord with the high radioactivity of this preparation. There was little evidence to suggest that the peak-2 components were breakdown products of peak-1 material.