YEAST TYROSINE tRNA LABELED WITH OSMIUM AT THE ISOPENTENYL ADENOSINE RESIDUE

W. R. MIDDEN and E. J. BEHRMAN
Department of Biochemistry, The Ohio State University, Columbus, OH 43210, USA

Received 11 April 1979

1. Introduction

X-ray diffraction analysis of tRNA conformation requires the preparation of 3 or 4 isomorphous, heavy-atom derivatives. Preparation of these derivatives can be the most time-consuming and laborious part of the analysis due to our limited knowledge of predictable, specific heavy-atom reactions. The first heavy-atom derivative is the most important, particularly if it is labeled at a single site since information obtained with it provides rough phase information which facilitates the analysis of subsequent derivatives [1]. We describe here a procedure for preparing a specific, single-site, osmium derivative of yeast $tRNA^{Tyr}$. The osmium is covalently attached to the isopentenyl adenosine residue at the 3′ side of the anticodon. This procedure should be applicable to other tRNA species which contain isopentenyl adenosine. In related work, the isopentenyl group in yeast $tRNA^{Ser}$ has been labeled with iodine [2] while this residue has been spin-labeled in yeast $tRNA^{Tyr}$ [3].

2. Materials

Osmium tetroxide was obtained from Varlacoid Chemical Co. and standardized by its ultraviolet spectrum, $\epsilon_{275} = 1733\ cm^{-1}\ M^{-1}$ in pH 7.0 sodium phosphate buffer [4]. 2,2′-Bipyridyl was obtained from G. Frederick Smith and purified by sublimation under vacuum. Pyridine was purified by fractional distillation and stored over molecular sieves. Nucleosides, nucleotides, ribonuclease T_1 and T_2, and bacterial alkaline phosphatase were obtained from Sigma Chemical Co. Ribonuclease T_2 was dialyzed against 10 mM ammonium acetate and stored at 100 units/ml, 4°C. The phosphatase was dialyzed against 10 mM ammonium bicarbonate (pH 8.0). It was stored at 0.5 mg/ml, 4°C. Fleischmann's cake yeast, used to prepare aminoacyl tRNA ligase, was purchased at local grocery stores. Crude brewer's yeast tRNA was a product of Boehringer. Cellulose thin-layer chromatographic (TLC) sheets (plastic-backed), obtained from Eastman, were washed with 2 M acetic acid by attaching a pad of filter paper to the top of 2 sheets back-to-back with glass rods and rubber bands. The sheets were dried in air. This was followed by a final wash in distilled water and air-drying. [U-^{14}C]Tyrosine was a product of Amersham.

3. Methods

3.1. *General methods*

Crude aminoacyl tRNA ligase was prepared using a modification [5] of the procedure in [6]. Yeast $tRNA^{Tyr}$ was isolated from crude yeast tRNA on columns of BD-cellulose using the procedure [7] as modified [5]. Amino acid acceptor activity (1760 pmol/A_{260}) of the tRNA was determined by charging samples with [^{14}C]tyrosine, precipitating the tRNA onto cellulose discs with ethanol, washing with trichloroacetic acid, ethanol and ether, drying, and counting radioactivity in a scintillation cocktail (detailed in [5]).

3.2. *Preparation of the osmium derivative*

One A_{260} unit (1760 pmol) of lyophilized yeast

Elsevier/North-Holland Biomedical Press

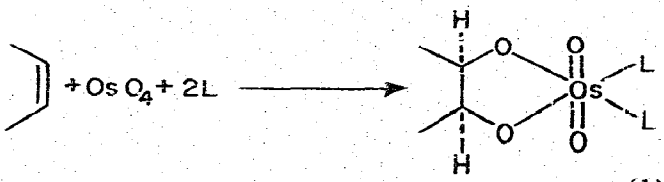
$tRNA^{Tyr}$ was dissolved in 10 μl water containing 1770 pmol osmium tetroxide and 1000 nmol pyridine. This mixture was incubated at 25°C for 1.5 h. The solution was evaporated to near dryness with a stream of air at room temperature (~1 h) to remove unreacted osmium tetroxide and most of the pyridine. The residue was redissolved in 25 μl saturated aqueous 2,2′-bipyridyl and incubated at room temperature for 2 h. The modified tRNA was either used at once or lyophilized and stored at −20°C. The modification was also carried out after preincubation in 5 mM $MgCl_2$, 100 mM ammonium bicarbonate (pH 7.5) for 1 h followed by addition of the osmium tetroxide–pyridine reagent and reaction as above.

3.3. *Analysis of the modified tRNA*

The position and extent of labeling was determined by hydrolyzing the tRNA to nucleosides with ribonuclease T_2 and phosphatase followed by separation of the nucleosides on cellulose TLC. Identical results were obtained whether or not the tRNA had been treated with $MgCl_2$ before modification with osmium. Conditions for hydrolysis: 1 A_{260} unit lyophilized tRNA dissolved in 10 μl solution containing 1 M ammonium acetate (pH 4.8), 100 μM EDTA, 25 μl ribonuclease T_2 (100 units/ml), 25 μl saturated aqueous 2,2-bipyridyl containing 1 mg crystalline bipyridyl. This mixture was incubated for 15 h at 38°C in a 2 ml polyethylene conical vial, followed by lyophilization and dissolution in a mixture of 25 μl phosphatase (0.5 mg/ml), 25 μl 100 mM NH_4HCO_3 (pH 8.0) and 25 μl saturated aqueous bipyridyl containing 1 mg crystalline bipyridyl for 15 h at 38°C. The mixture was lyophilized. The entire residue was redissolved in 2 μl deionized distilled water and spotted 2 cm from the bottom edge of a cellulose TLC strip. The TLC strip was developed with *n*-propanol conc. NH_4OH H_2O (80:10:10, by vol.) to 5 cm above the origin and air-dried. Nucleosides and osmium-modified nucleosides were visible under ultraviolet light. The detection limit was ~100 pmol. Nucleosides were identified by comparison with authentic samples. Spots containing osmium (VI) derivatives usually turned brown or gray in 1 or 2 days (apparently due to reaction with cellulose) and could be tentatively identified by this characteristic. Spots were quantitatively assayed for the presence of osmium by neutron activation analysis using the nuclear reactor at the University of Michigan. Samples were irradiated at ~1.5×10^{13} neutrons/cm^2. sflux for 2 hours. The activated samples were then analyzed between 7–21 days following irradiation using the neutron activation analysis facilities at The Ohio State University. Osmium was determined by integrating the peak at 129.4 keV characteristic of ^{191}Os and comparison with standards [8].

4. Results and discussion

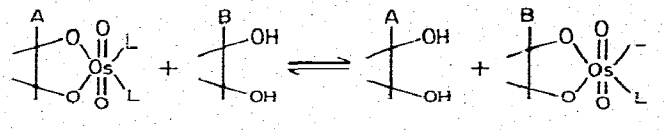
Yeast tyrosine tRNA contains 20 cytosine residues, 7 uracil residues, 3 pseudouridine residues, 1 thymine residue, and 1 5-methylcytosine residue in addition to the isopentenyl adenosine group. All of these groups are capable of reaction with an osmium (VIII)–ligand reagent to form an osmate (VI) ester according to eq. (1):



(1)

The data of table 1 show that we have been able to carry out a selective reaction with the isopentenyl adenosine residue using 1 equiv. OsO_4. This is the result of kinetic selectivity based on the finding [9] that the osmium (VIII)–pyridine reagent reacts with the isopentenyl sidechain ~4600-times more rapidly than with thymidine. Of the remaining groups mentioned above, all are less reactive than thymidine [10–13].

A further possible complication arises from the transesterification reactions as shown in eq. (2):



(2)

Thus, initial reaction of the osmium (VIII)–pyridine reagent with the isopentenyl residue could be followed by transesterification with the adenosine diol at the

Table 1
Chromatographic data for nucleosides, osmium derivatives of nucleosides and neutron activation analysis of a chromatogram of an enzymatic digest of osmium-modified yeast $tRNA^{Tyr}$

Compound	R_F	Osmium analysis	
		pmol Os	R_F range
(OsO_2)[a]	0.00	150 ± 100	0.00
Uridine osmate ester, H	0.08	Undetectable[d]	0.03–0.15
Guanosine	0.10		
Uridine	0.24	Undetectable	0.15–0.35
Ribothymidine osmate ester, H	0.24		
Cytidine	0.34		
Adenosine	0.42	Undetectable	0.35–0.60
$Os_2O_6(bipy)_2$	0.50		
Adenosine osmate ester, S	0.50		
Ribothymidine	0.54		
Isopentenyl adenosine osmate ester, O	0.66	750 ± 50	0.60–0.75
Isopentenyl adenosine[b,c]	0.92	Undetectable	0.75–1.00

[a] The osmium at the origin results from some decomposition during spotting. Esters of greater stability can be prepared using bathophenanthroline disulfonic acid instead of bipyridyl [16]

[b] Bipyridyl cochromatographs with isopentenyl adenosine but is easily removed because of its volatility

[c] The isopentenyl adenosine is absent in the derivatived sample

[d] The detection limit was 100 pmol

Abbreviations: H, an osmate ester formed by addition to the 5,6-double bond; S, the osmate ester formed at the ribose 2′,3′-diol; O, the osmate ester formed by addition to the double bond in the isopentenyl sidechain (see [9,14])

Judging from the quantity of osmium, the amount of tRNA hydrolysate used was equivalent to ~0.5 A_{260} units or 900 pmol

3′-terminus. The data of table 1 show that we have successfully prevented this reaction since no osmium (VI)–adenosine complex was found. This is in accord with [14,15] where bidentate ligands like 2,2′-bipyridyl were shown to form relatively inert osmium (VI) complexes.

Analysis of the modified and unmodified tRNA by digestion with ribonuclease T_1 and two-dimensional TLC showed the expected pattern of oligonucleotides. The chromatogram could not be analyzed for osmium because it was lost during chromatography. However, this experiment established that the tRNA was intact following modification.

Acknowledgements

This work was supported by the NIH, GM-20375.

We thank Jack Steehler for his valuable contributions to the isolation of the tRNA and Brian Hajek for assistance and use of the Nuclear Reactor Laboratory facilities.

References

[1] Sigler, P. B. (1975) Ann. Rev. Biophys. Bioeng. 4, 477–527, see especially 488–490.

[2] Horváth, P. and Dénes, G. (1977) Biochim. Biophys. Acta 474, 188–198.

[3] Weygand-Durašević, I., Nöthig-Laslo, V., Herak, J. N. and Kućan, Z. (1977) Biochim. Biophys. Acta 479, 332–344.

[4] Waldrop, A. A. (1977) PhD Dissertation, The Johns Hopkins University, p. 27. University Microfilms International no. 78–6279.

[5] Midden, W. R. (1978) PhD Dissertation, The Ohio State University, University Microfilms International no. 79–2186.
[6] Stephenson, M. L. and Zamecnik, P. C. (1961) Proc. Natl. Acad. Sci. USA 47, 1627–1635.
[7] Maxwell, I. H., Wimmer, E. and Tener, G. M. (1968) Biochemistry 7, 2629–2634.
[8] Raković, M. (1970) Activation Analysis (Cohen, D. transl.) Chemical Rubber Co., Cleveland.
[9] Ragazzo, J. A. and Behrman, E. J. (1976) Bioinorg. Chem. 5, 343–352.
[10] Beer, M., Stern, S., Carmalt, D. and Mohlhenrich, K. H. (1966) Biochemistry 5, 2283–2288.
[11] Burton, K. and Riley, W. T. (1966) Biochem. J. 98, 70–77.
[12] Burton, K. (1967) Biochem. J. 104, 686–694.
[13] Subbaraman, L. R., Subbaraman, J. and Behrman, E. J. (1971) Bioinorg. Chem. 1, 35–55.
[14] Daniel, F. B. and Behrman, E. J. (1975) J. Am. Chem. Soc. 97, 7352–7358.
[15] Daniel, F. B. and Behrman, E. J. (1976) Biochemistry 15, 565–568.
[16] Chang, C.-H., Beer, M. and Marzilli, L. G. (1977) Biochemistry 16, 33–38.