CHEMICAL MODIFICATIONS OF TRANSFER RNA SPECIES. METAL ION BINDING TO MONOPERPHTHALATE-TREATED tRNA.

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SUMMARY

Oxidation of the "exposed" adenosine moieties in intact tRNA's enhances the binding of heavy metal ions to the tRNA's. Experiments utilizing copper, cadmium and samarium ions indicated that binding enhancement was directly proportional to the degree of oxidation.

X-ray crystallographic studies of tRNA have progressed to a level of structural refinement (1,2) from which additional structural elaboration will be strongly dependent on successful isomorphous replacements with heavy atom substituents (3). Heavy atom derivatization of tRNA has been achieved by the addition of heavy metal ions to tRNA solutions, resulting in binding to a limited number of sites (4-6). The soaking of preformed tRNA crystals with lead ions has also resulted in tRNA's containing a single lead atom (6). In addition, one report has appeared concerning the covalent attachment of a heavy atom substituent to tRNA (7).

While the non-covalent binding of heavy metal ions to tRNA's may occur at several sites with only limited specificity, it should be possible to chemically alter the tRNA's to render such binding more specific. Thus Cramer and his coworkers (3,8) have shown that tRNA in which the terminal phosphodiester bond has been modified to a thiated phosphodiester bond exhibited enhanced binding of mercury ions at the site of modification. An additional example of a possible modification of this type seemed inherent in the work of Perrin (9), which indicated that adenine-N^1-oxide (1, purine portion) specifically bound manganese, iron,

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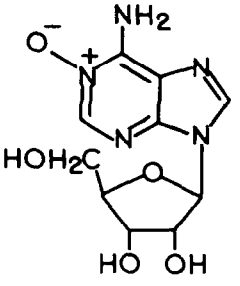

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cobalt, nickel, copper and zinc ions at neutral pH. The metals were bound between N^6 and either N-7 or the oxygen atom. This seemed especially promising for tRNA modification in light of the work of Cramer and his coworkers (10,11) which demonstrated that treatment of tRNA's with monoperphthalic acid resulted in the oxidation of "exposed" adenosine moieties to adenosine-N^1-oxides (1). The successful chelation of heavy metal ions to these modified tRNA's should afford heavy atom derivatives of tRNA's which would have a favorable chance of crystallizing in an isomorphous fashion, since the oxidized moieties are, by virtue of their mode of formation, on the outer surface of the molecule where they should have a minimal effect on tRNA folding interactions.

We wish to report that tRNA treated with monoperphthalic acid did exhibit enhanced binding for metal cations, that the degree of binding was proportional to the degree of oxidation of adenosine moieties, and that the newly formed metal ligands were apparently not disturbed by repeated ethanol precipitations of the tRNA.

MATERIALS AND METHODS

Monoperphthalic acid was prepared by the method of Stahmann and Bergmann (12), by treatment of phthalic anhydride with sodium perborate. The monoperphthalate oxidation of tRNA was carried out on tRNA derived from yeast (Saccharomyces cerevisiae, strain Y185), grown on minimal media in continuous culture with limiting glucose to force aerobic growth and shown to be free from bacterial contamination. The oxidation was carried out at 25° according to the method of Cramer et al. (10) in potassium phosphate buffer. The course of the reaction was conveniently

assayed by the removal of 3 mg aliquots of tRNA at regular time intervals. The aliquots were used to monitor changes in the A_{232}/A_{260} ratio (Figure 1). This ratio reflected the progress of the oxidation since, at neutral pH, adenosine-N^1-oxide (1) has an absorption maximum with a molar absorptivity of approximately 36,000 at a wavelength of 232 nm (13,14). Prior to ultraviolet measurement, each aliquot was isolated by repeated precipitations of the tRNA from aqueous solution with two volumes of cold ethanol. The reaction product consisted entirely of 4S RNA, as judged by polyacrylamide gel slab electrophoresis.

Measurement of the amount of metal specifically bound to the N-oxides was determined by measuring the total amount of metal associated with the tRNA, and adjusting this figure to reflect the amount of metal bound "non-specifically" to a zero-time (unoxidized) sample of yeast tRNA. Quantitative determination of the amount of metal present in the samples was carried out by neutron activation analysis. The samples were irradiated for 1-2 minutes in a pneumatic tube of the University of Wisconsin nuclear reactor. The thermal flux was 4.3×10^{12} n/cm^2/sec. The data, including that for standard samples containing known amounts of Cd, Cu and Sm, was counted on a multi-channel analyzer using a lithium-drifted germanium detector with a volume of approximately 30 cc; corrections were made where necessary.

RESULTS AND DISCUSSION

The oxidation of yeast tRNA and specific binding of cupric chloride to the adenosine-N^1-oxide moieties of aliquots of oxidized tRNA's is shown in Figure 1. The change in the A_{232}/A_{260} ratio shown in this figure corresponded to the introduction of about three adenosine-N^1-oxide moieties into an "average" tRNA. This result correlated fairly well with the work of Cramer (10,11). As may be seen from the figure, the net specific increase in copper binding due to the presence of these adenosine-N^1-oxide moieties was also about 3 moles/mole of tRNA. Similar results were obtained for cadmium and samarium, with binding increasing with time of oxidation, in parallel with the formation of adenosine-N^1-oxide moieties. Samarium exhibited a significantly higher level of oxidation-dependent binding then did cadmium or copper.

Each of the metals also exhibited varying levels of "non-specific" binding to the tRNA's in the absence of perphthalate

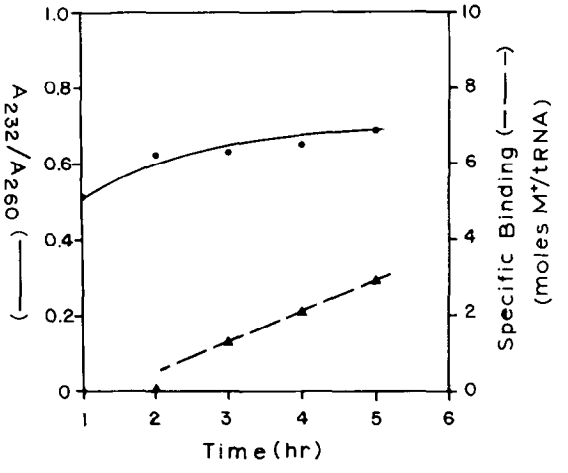

Figure 1. Formation of adenosine-N^1-oxide moieties in intact yeast tRNA species (———) and parallel increase in the specific binding of copper ions to the tRNA (— — —).

oxidation, presumably due to binding to certain heterocyclic bases (15-18) or to the phosphate ester anions (3,19) in the tRNA's. If the non-specific binding is the same in the oxidized and unoxidized tRNA's, and if isomorphous crystals can be obtained from the two species, difference Patterson and Fourier electron density maps should yield much useful information reflecting only the presence of the additional metals specifically chelated to the oxidized adenosine moieties in the tRNA's.

The approach has the feature that the metals associated with the adenosine-N^1-oxide moieties must be on the outer surface of the three-dimensional tRNA molecule, thereby maximizing the chances for normal tRNA folding and hence isomorphous replacement; also that a number of different metals may be conveniently introduced into specific sites on the tRNA. In addition, it is probable that the degree of oxidation of the tRNA's can be controlled by proper choice of reaction conditions so that, for example, a single site on the tRNA might be oxidized and utilized for isomorphous replacement.

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