THE ISOLATION AND CHARACTERIZATION OF N-[9-(β-D-RIBOFURANOSYL)-PURIN-6-YLCARBAMOYL]GLYCINE FROM YEAST TRANSFER RNA[1]

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Received July 17, 1970

SUMMARY

The modified nucleoside, N-[9-(β-D-ribofuranosyl)-purin-6-ylcarbamoyl] glycine, has been isolated from enzymic digests of unfractionated yeast tRNA. Structural elucidation was accomplished by detailed comparisons of natural and synthetic materials by paper chromatography, ultraviolet, nmr and mass spectra.

INTRODUCTION

Recently, several unusual nucleosides have been found in tRNA from various tissues. N^6-(Δ^2-isopentenyl)adenosine or its 2-methylthio derivative have been found in yeast, E. coli and rat liver tRNA (1-6) located next to the anti-codon in species responding to U-beginning codons, including tyrosine (2,5), serine (3,6) and phenylalanine (3) accepting tRNAs. A threonine-substituted adenine derivative, N-(purin-6-ylcarbamoyl)threonine (PCT)[a], as well as the corresponding 9-β-D-ribofuranoside (PCTR)[a], have been isolated and characterized from tRNA of several tissues (7,8).

Both the threonine-substituted adenine and the corresponding riboside have been found in human urine, and their chemical synthesis described (9).

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[a] Abbreviations used:
PCT, N-(purin-6-ylcarbamoyl)threonine
PCTR, N-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]threonine
PCGR, N-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]glycine
TEAB, Triethyl ammonium bicarbonate

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[1] Part of this work was completed by M.P.S. and K.McG. in the Department of Experimental Therapeutics, Roswell Park Memorial Institute. An account of this work was given at the Pacific Slope Biochemical Conference, University of California, San Diego, June, 1970.

Specific aminoacyl tRNAs responding to A-beginning codons have been found to contain this threonine derivative, including isoleucyl (10), lysyl, methionyl, and seryl (III) (11), again, at the 3' end of the anticodon.

We report here the isolation and characterization of a glycine-substituted adenosine, N-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]glycine (PCGR), from enzymatic digest of unfractionated yeast tRNA. The isolation procedures for obtaining this compound as well as PCTR have been improved in terms of time and yield by the use of anion exchange chromatography.

EXPERIMENTAL

Unfractionated yeast tRNA was purchased from Cal Biochem Company. Initially 10 g of this material was digested by Crotalus adamanteus venom (Ross Allen Snake Farm) and chicken intestinal alkaline phosphatase (Worthington) to the nucleosides with subsequent partition chromatography of the digest according to the method of Hall (12). PCGR was isolated from a five component mixture derived from the partition columns through the use of Dowex-2x8 (200-400 mesh, bicarbonate form, 2x40cm). After passing 2-3 liters of 10^{-3}M triethyl ammonium acetate to clear the column of uncharged material, consecutive application of 0.2, 0.8 and 1.0M triethyl ammonium acetate to the column resulted in elution of PCGR, in both 0.8 and 1.0M fractions. Subsequent column runs were performed using gradients of 0.2-1.0M triethyl ammonium bicarbonate (TEAB) because of its ease of removal by evaporation in vacuo; the enzymic digests, after concentration and centrifugation to remove guanosine, were immediately put on the columns to retain the carboxylate compounds. After elution, concentration and removal of the salt, the ureido nucleoside fractions (emerging at about 0.6M in the gradient) were purified by preparative paper chromatography. The yield of PCGR varied from 50-100 A_{268} units for the 10 g hydrolysates.

PCGR was synthesized in 50% yield by the reaction of ethylisocyanato acetate with tri-O-acetyladenosine. Further details of the synthesis and ion exchange method will be reported elsewhere (13). We are also indebted to Dr. G. B. Chheda, Roswell Park Memorial Institute, Buffalo, N.Y., for a synthetic sample of PCGR.

UV spectra were obtained on a Cary Model 14, nmr spectra on Varian A-60A and HA-100 spectrometers with a C-1024 computer for signal enhancement (chemical shifts were measured from external TMS). The mass spectra of the natural and synthetic nucleosides were recorded on a C.E.C. 21-110B instrument (Mattauch-Herzog geometry) using the direct introduction probe (source temperature: 290°C). The high resolution mass spectra were recorded on photographic plates and processed with an automatic comparator-densitometer via a DATEX tape unit.

RESULTS AND DISCUSSION

In all the characterization procedures below, the natural and synthetic PCGR preparations were essentially identical.

Paper chromatographic properties of PCGR are listed in Table I. Values for the threonine derivative, PCTR, are listed for comparison.

In paper electrophoresis experiments, PCGR migrated toward the positive electrode with one-half the mobility as 5'-AMP in 0.05 M TEAB, indicating a monoanion.

The uv spectrum of PCGR is shown in Fig. I. The spectral properties are

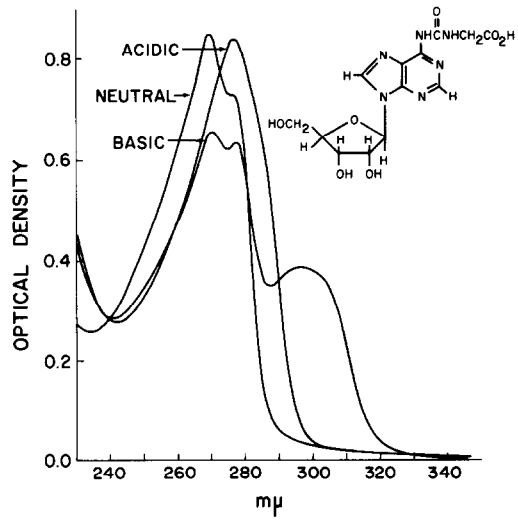

Figure I. UV spectrum of N-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]glycine (PCGR) from yeast tRNA.

Acidic, pH 1-2, λmax 276 nm, shoulder 268nm; neutral, pH 6-7, λmax 268 nm, shoulder 276 nm; basic, pH 10-11, λmax 269, 277 and 297 nm.

TABLE I. PAPER CHROMATOGRAPHY

	Solvent Systems (R_fx100)[a]					
	A	B	C	D	E	F
PCGR	41	37	42	27	0	42
PCTR	58	43	50	34	0	43

[a] Solvent Systems:

A) 55:10:35, 1-propanol:concentrated ammonium hydroxide:water
B) 2:1, 2-propanol:1% aqueous ammonium sulfate
C) 680:176:144, 2-propanol:concentrated hydrochloric acid:water
D) 7:2:1, 2-propanol:water:concentrated ammonium hydroxide
E) 4:1:2, ethyl acetate:1-propanol:water
F) 4:1:2, ethyl acetate:2-ethoxyethanol:16% formic acid

uniquely associated with the ureido adenosine chromophore, thus PCGR is quite similar to PCTR (9) and other ureido adenosines (G. B. Chheda, private communication).

Treatment of 129 μmoles PCGR with 6N HCl at 100°C for 15 hrs yielded 122 μmoles glycine as determined by quantitative ion exchange chromatography in the amino acid analyzer. Adenine was identified as the other product of this hydrolysis upon comparison of uv and chromatography with known material. When PCGR was subjected to basic hydrolysis, only 0.3-0.5 mole glycine per mole of nucleoside taken was liberated as determined in the amino acid analyzer, even using 1M solutions of KOH for 3 hrs at 100°C. Adenosine was the other product from this partial hydrolysis. Threonine is released from PCT or PCTR upon treatment with 0.1M NaOH for 3 hrs at 100°C. Apparently the participation of the β-hydroxyl in the case of the threonine compounds results in a more facile base catalyzed cleavage of the amino acid (7,8).

De-ribosylation of PCGR was effected by 0.5M HCl on the steam bath for 45 min. The uv spectra of the resulting purine base were identical to those of PCT (7-9). Inasmuch as ureido adenines have common uv characteristics, the purine base obtained here is undoubtedly N-(purin-6-ylcarbamoyl)glycine.

Time averaged 60 MHz nmr analysis of PCGR (3 mg/0.4ml DMSO-d_6) revealed

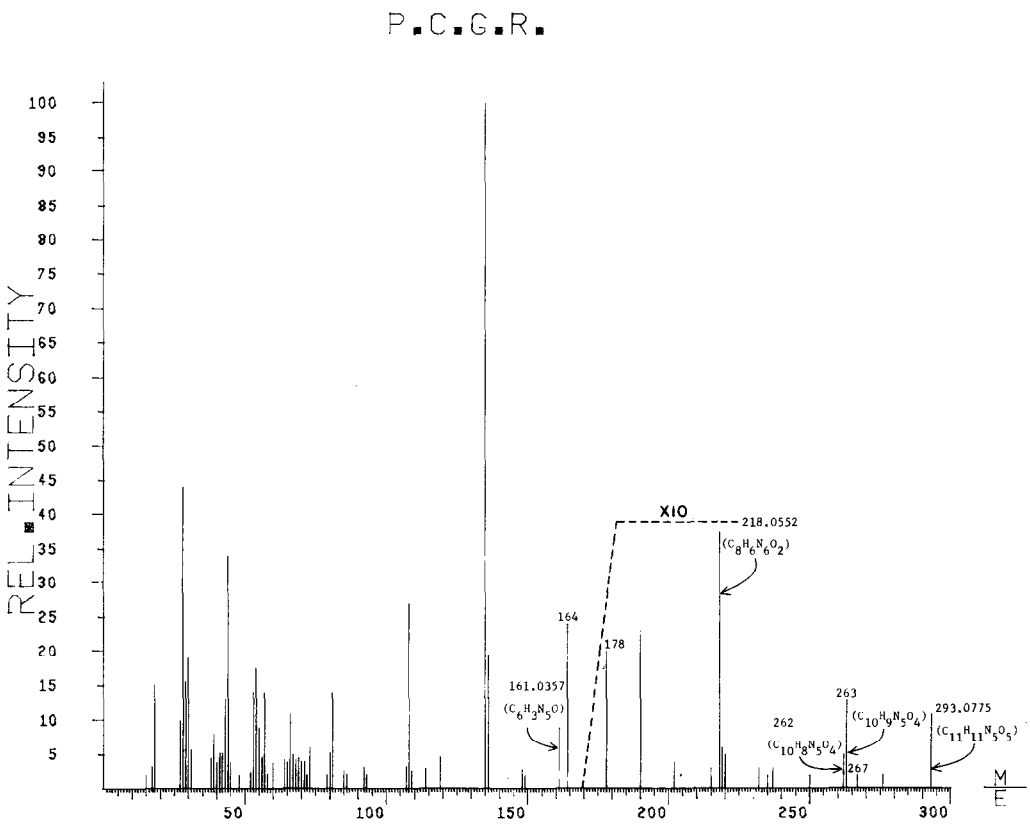

Figure II. Computer plotted mass spectrum of natural PCGR.

Ions characteristic of PCGR are listed with the observed m/e values and the corresponding elemental composition. Other ions at 267, 178 and 164 are typical of adenosine.

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the presence of the following pertinent protons in addition to those normally found in adenosine (14). A broad triplet was found at δ9.92 ppm, J = 5.0 Hz, which must be due to the α-NH, coupled to the glycyl methylene protons at δ4.20 ppm, a doublet with J = 5.0 Hz. Furthermore, the absence of a resonance at δ7.7 ppm, integrating for two protons, signifies that the glycine moeity is attached at the N-6 position of adenosine. It is possible that the N-6 proton is superimposed with the α-NH triplet, since this latter pattern is broad and unsymmetrical. N-6 protons of other ureido compounds have been found in the δ9.85-10.1 region (8).

The mass spectra of the natural and synthetic PCGR were nearly identical; however, there were some small differences in the intensities of certain ions. This is probably due to differences in rates of heating the samples and the amounts of sample used. The plotted spectrum of natural PCGR is shown in Fig. II

As in the case of PCT (8), the molecular ion was absent in the spectrum. The high resolution mass spectrum of the natural compound indicated that the ion with the largest m/e is 293.0775 (calc. 293.0760 for $C_{11}H_{11}N_5O_5$); it corresponds to the loss of glycine from the molecular ion. An important diagnostic ion appeared at m/e 218.0562 (calc. 218.0552 for $C_8H_6N_6O_2$) which corresponds to the dehydrated N-(purin-6-ylcarbamoyl)glycine. A similar behavior was observed for PCT (8). The 293 ion loses the elements of formaldehyde, probably from the sugar moiety, to yield the 263 and 262 ions ($C_{10}H_9N_5O_4$ and $C_{10}H_8N_5O_4$). All the ions of adenosine (15) are also present in the spectrum of PCGR. This indicates that at the temperatures required to vaporize the samples pyrolysis takes place to some extent and adenosine is formed in the mass spectrometer.

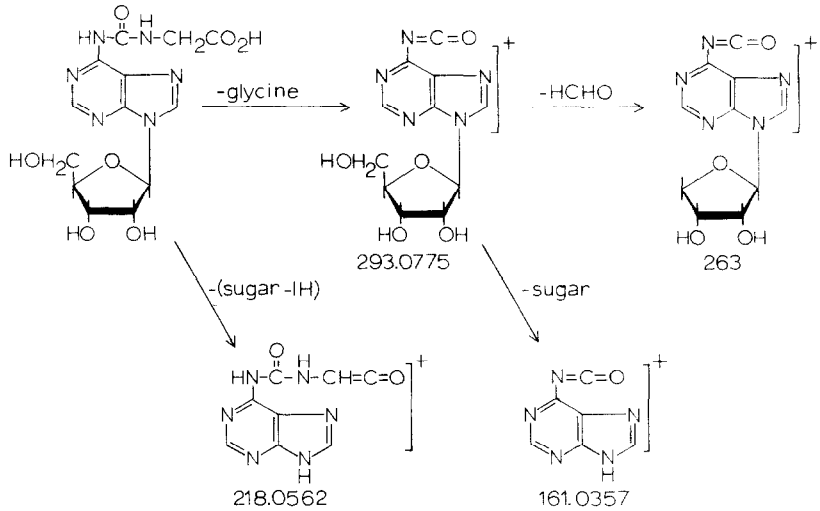

CHART I -- Fragmentation of PCGR

A schematic view of the fragmentation process is shown in Chart I.

N-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]threonine (PCTR) occurs in the tRNA of yeast, bacterial and mammalian tissue (7,8). We have only examined

Bakers yeast tRNA for the glycine analog, PCGR, but it is possible that this compound is present in the tRNA of other tissues. Ishikura et al (11) have found PCTR in E. coli tRNA specific for methionine, lysine and serine (III). Several other ureido adenosines, based upon the unique uv spectral properties of this class of compounds, have been found by these authors. Thus it is possible that "iso-accepting" tRNAs for methionine and lysine contain PCTR in one species and related ureido adenosines in the others.

We have found other fractions in hydrolysates of yeast tRNA in addition to PCTR and PCGR which display typical ureido adenosine uv spectra. Work on their identity continues.

ACKNOWLEDGEMENTS

We are grateful to Dr. William Chan, Department of Biochemistry, McMaster University, for the amino acid analysis, and to Prof. P. O. P. Ts'o, Johns Hopkins University, for use of the HA-100 spectrometer. We also appreciate the comments of Drs. Ross H. Hall, Department of Biochemistry, McMaster University, and G. B. Chheda, General Clinical Research Center, Roswell Park Memorial Institute. This work was supported in part by USPHS Grant No. CA-10835 and the Medical Research Council of Canada (Grant MT-2738).

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