POLY N^6-(Δ^2-ISOPENTENYL)-ADENYLIC ACID: SYNTHESIS AND CHARACTERIZATION

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SUMMARY: Blocked N^6-(Δ^2-isopentenyl)-adenosine was phosphorylated with 2-cyanoethylphosphate and N,N'-dicyclohexylcarbodiimide and the resulting 5'-nucleotide converted first to the 5'-phosphoromorpholidate and then to the 5'-diphosphate. The latter was polymerized using polynucleotide phosphorylase giving poly N^6-(Δ^2-isopentenyl)-adenylate. The polynucleotide had high molecular weight shown by $s^0_{20,w}$ = 12.0S and quantitative base hydrolysis to -3'(2')-nucleotide without observable end groups. The polynucleotide was highly hypochromic (relative to monomer) at pH 2, analogous to helical acid polyadenylate, but was less hypochromic than the latter at pH 7 with T_m = 13° and a second transition, 20°-85°, indicating less stable base-base interaction than polyadenylate.

Synthetic poly A[2] can exist as a random coil or a helix depending on the pH and ionic strength of the solution (1-3). Studies of related homopolyribonucleotides such as poly N^6-MeA (4) and poly N^6-HEA (5) show that these polymers have properties similar to poly A. Investigation of such model polynucleotides is not only valuable for understanding the effect of alkylation on base-base interactions in nucleic acids but might also provide information about possible functions of modified bases in tRNAs. The

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[2]Abbreviations used are poly A for polyadenylate, poly N^6-MeA for poly N^6-methyladenylate, poly N^6-HEA for poly N^6-hydroxyethyladenylate, poly IPA for poly N^6-(Δ^2-isopentenyl)-adenylate, IPA for the N^6-(Δ^2-isopentenyl)-adenylate residue in polynucleotide, DCC for N,N'-dicyclohexylcarbodiimide, IPAdo for N^6-(Δ^2-isopentenyl)-adenosine and derivatives, P for phosphoester, Tris for trishydroxymethylaminomethane, Na_2EDTA for disodium ethylenediaminetetraacetate.

function of IPA, located adjacent to the anticodon of several sequenced tRNAs, is poorly understood despite studies of the nucleoside (6,7), the nucleotide (8), two dinucleoside phosphates (9), and chemical (10) and enzymic (11,12) alteration of IPA in tRNA itself. In order to study the role of IPA in tRNA, we have synthesized and characterized the homopolyribonucleotide, poly IPA, which work is reported here.

MATERIALS AND METHODS

The 4-morpholine (distilled before use) and DCC were from Aldrich; tri-n-butylamine, triethylamine, and Spectroquality pyridine (stored with CaH_2) were from Matheson, Coleman, and Bell; DE-23 DEAE-cellulose was a Whatman product; primer-independent, M. luteus polynucleotide phosphorylase was from Miles Laboratories; snake venom and spleen phosphodiesterases and E. coli alkaline phosphatase were from Worthington and were used as outlined in the Worthington data sheets. All other reagents were of highest commercial grade.

Spectra were determined with a Hitachi-Coleman 124 recording spectrophotometer. Routine absorbance measurements and absorbance-temperature profiles were made with a Zeiss PMQII spectrophotometer with temperature controlled cell holder. Extinction coefficients were determined by removing aliquots from cuvettes on which spectra had been run and determining total phosphorus by a modification of the method of Lowry, et al. (13).

RESULTS

Starting IPAdo-5'-P was synthesized as previously described (9) and was converted to the 5'-phosphoromorpholidate by reaction with 4-morpholine and DCC (14). A quantitative yield of the 4-morpholine-N,N'-dicyclohexylcarboxamidinium salt was obtained

as demonstrated by single spots found in paper chromatography (3 solvents, Table I) and paper electrophoresis (product mobility 0.33 that of IPAdo-5'-P in 0.05 M triethylammonium bicarbonate, pH 7.5, 65 v/cm, 60 min.). Treatment of the IPAdo-5'-phosphoromorpholidate with 0.1 N H_2SO_4 (5 min., 25°) caused quantitative conversion to IPAdo-5'-P identified by paper chromatography.

TABLE I

PAPER CHROMATOGRAPHY OF IPAdo DERIVATIVES

Substance	R_f Solvent A[1]	Solvent B[1]	Solvent C[1]
IPAdo	0.87	0.89	0.80
IPAdo-5'-P	0.35	0.78	0.69
IPAdo-3'(2')-P	0.37		0.78
IPAdo-5'-phosphoromorpholidate	0.68	0.85	0.78
IPAdo-5'-diphosphate	0.28	0.62	0.63

[1] Solvent A is 7 : 1 : 2 isopropanol - 16 N NH_4OH - H_2O. Solvent B is 7 : 3 95% ethanol - 1 M NH_4OAc, pH 7.5. Solvent C is 6 : 3 : 1 95% ethanol - 2% H_3BO_3 - 16 N NH_4OH. Chromatograms were run descending on Whatman 1 paper at room temperature.

The IPAdo-5'-phosphoromorpholidate salt (290 μmoles) was reacted with 1.5 mmoles tri-n-butylammonium phosphate in anhydrous pyridine over 96 hrs. at 25° (14). The product mixture was chromatographed on a 2.5 x 30 cm column of DEAE-cellulose HCO_3^-, washing first with water and eluting with a one liter 0.0 → 0.5 M linear gradient of triethylammonium bicarbonate, pH 8.6.

The major product was eluted between 460 and 580 ml. The 485-550 ml fraction was dried on the rotary evaporator and buffer removed by dissolving in methanol and re-evaporating (repeated 3X). The recovered yield of IPAdo-5'-diphosphate was 53% (absorption spectroscopy). The product appeared homogeneous in paper chromatography and was quantitatively converted to IPAdo by E. coli alkaline phosphatase as determined by paper chromatography.

The IPAdo-5'-diphosphate (145 μmoles) was polymerized in a reaction mixture also containing 18 μmoles $MgCl_2$; 120 μmoles Tris·HCl, pH 9.0; 0.12 μmoles Na_2EDTA; 0.6 μmoles dithiothreitol; and 20 units of polynucleotide phosphorylase in a total volume of 1.2 ml. Incubation at 37° was for 9 hrs. at which time 3.2 ml H_2O was added and the mixture extracted with freshly distilled 90% phenol (3 ml, 3X). The aqueous layer was extracted with ether (5 ml, 4X) and then dialyzed against 3 1 liter changes 0.1 M NaCl - 0.001 M Na_2EDTA, pH 7.4, and finally against several changes of distilled H_2O over 48 hrs. at 4°. The yield of monomer incorporated into non-dialyzable poly IPA was 36 μmoles (25%) determined by total phosphorus analysis.

Hydrolysis of poly IPA with 0.3 N KOH (72 hrs., 25°) or 0.5 N NaOH (9 hrs., 37°) gave quantitative yields of IPAdo-3'(2')-P identified by paper chromatography in a borate solvent system which separates 3'(2')-nucleotides from 5'-nucleotides. No IPAdo or IPAdo-3'(2'),5'-diphosphate end groups were seen on these chromatograms indicating that the poly IPA molecules contained at least 30 nucleotides. The poly IPA was resistant to hydrolysis with snake venom or spleen (coupled with E. coli alkaline phosphatase) phosphodiesterases, only traces of monomer being seen on chromatograms of these enzymic digests. This low susceptibility to exonucleases might be due to precipitation of the Mg^{2+} salt of

poly IPA which was observed in these assays. The $s^0_{20,w}$ of the poly IPA in 0.15 M NaCl - 0.015 M Na citrate, pH 7.0, was measured by sedimentation - velocity as 12.0S. The high sedimentation coefficient coupled with the lack of observable end groups on alkaline hydrolysis indicate that the poly IPA is of high molecular

TABLE II

ULTRAVIOLET ABSORPTION OF POLY IPA

	Poly IPA[a] pH			IPAdo-5'-p[b] pH		
	2	7	12	2	7	12
λ_{max}, nm	267	266	266	264	267	268
λ_{min}, nm	239	235	232	233	231	235
$A_{\lambda_{max}}/A_{\lambda_{min}}$	2.00	3.28	3.30	4.06	5.99	4.16
A_{250}/A_{260}	0.72	0.66	0.67	0.67	0.59	0.59
A_{280}/A_{260}	0.98	0.83	0.79	0.65	0.82	0.83
A_{290}/A_{260}	0.67	0.46	0.44	0.21	0.32	0.32
10^{-3} ε_p, λ_{max}	13.2	17.3	17.8	20.9	19.2	19.0
10^{-3} ε_p, 260nm	12.2	16.2	16.9	19.7	17.1	16.9

[a] Spectra were determined at room temperature in 0.01 M NaCl solvent and pH adjusted with 12 N HCl and 10 N NaOH (measured with glass electrode) against a water blank in a Hitachi 124 spectrophotometer.

[b] Spectra were determined and pH adjusted as with poly IPA; the extinction coefficient data taken from W.A.H. Grimm and N. J. Leonard, Biochemistry, 6, 3625 (1967).

weight. Spectra of poly IPA at pH 2, 7, and 12 were obtained and the results are presented in Table II.

DISCUSSION

A distinct bathochromic shift in the spectrum of poly IPA relative to that of monomer is seen at pH 2 and the hypochromicity of 37% at this pH is only slightly less than that of acid poly A (2). This slightly reduced hypochromicity in acid relative to poly A may be due to the orientation of the isopentenyl residues perpendicular to the plane of adenine (15) preventing such close base stacking as found in poly A. The spectral change of poly IPA between pH 7 and 2 indicates protonation of the purine N^1 and the large hypochromicity suggests formation of a helical structure in acid analogous to poly A (2), but unlike poly N^6-MeA (4) and poly N^6-HEA (5), with helix stabilization through ionic bonding between protonated IPA residues and phosphodiesters in opposite strands.

There are significant differences in the spectra of poly IPA and monomer at pH 7 and 12 suggesting that poly IPA may have some secondary structure at these pHs. Poly IPA shows only 11% hypochromicity relative to monomer at pH 7 and 25° and even though this can be increased to 22% by cooling the solution of polymer in 0.01 M NaCl - 0.01 M Na cacodylate - 5×10^{-4} M Na_2EDTA to 6°, the hypochromicity is still much less than poly A at pH 7 (2). In this solvent, poly IPA, again unlike poly A, gives two hyperchromic thermal transitions: one relatively sharp (T_m = 13°) accounting for half the total hyperchromicity and the second very broad (about 20° to 85°). The lower total hyperchromicity and the lower temperature required to include all of the transitions for poly IPA relative to poly A both suggest that base - base interaction in poly IPA is less stable than in poly A. This may be due

to the aforementioned interference of the perpendicular isopentenyl group (15) with base stacking and is also consistent with the finding (9) that ApIPA and IPApA show less base - base interaction than ApA. There is no completely satisfactory explanation for two hyperchromic thermal transitions in poly IPA at present. These are the subject of current experiments as is the possible interaction between poly IPA and polyuridylate.

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