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POLY (5-DIMETHYLAMINOCYTIDYLIC ACID)

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We are investigating the biological properties of polycytidylic acids which are substituted in the pyrimidine ring (e.g. poly(cl^5C)[1] and poly(ho^5C)[2]. These polynucleotides can be prepared by polymerising the corresponding nucleoside diphosphates with polynucleotide phosphorylase. In this paper we report the synthesis of 5-dimethylaminocytidine 5'-diphosphate (m_2n^5CDP) and its enzymic polymerisation to poly(5-dimethylaminocytidylic acid) [poly(m_2n^5C)].

5-Dimethylaminocytidine 5'-monophosphate - 5-Bromocytidine 5'-phosphoric acid[3] (1 g) was brought to pH 7 with tetrabutylammonium hydroxide, and the solution evaporated in vacuo, the last traces of water being removed at 0.1 mmHg. The gummy residue, dissolved in dry dimethylformamide (10 ml), was cooled to 0^o and dimethylamine (3 ml) was added. This reaction was kept at 60^o under a reflux condenser cooled with solid carbon dioxide/acetone for 8 hours, further portions of dimethylamine (3 ml) being added every 2 hours. The reaction vessel was then connected to a water-cooled condenser and left at 60^o overnight. Water (50 ml) was then added and hydrochloric acid to bring the pH of the solution to 2. The solution was then evaporated to dryness in vacuo, the residue dissolved in a little water and applied to a Dowex 50 column (H^+ form, 100-200 mesh, 2 x 36 cm). Elution with water gave a little ho^5CMP, followed by unreacted br^5CMP and finally m_2n^5CMP, evaporation of the last fraction gave 5-dimethylamino-cytidine 5'-monophosphoric acid (472 mg, 40%) Found C, 34.34; H, 5.60; N, 14.41; P, 8.20% Calc. for $C_{11}H_{19}N_4O_8P.H_2O$ C, 34.38; H, 5.50; N, 14.57; P, 8.06%. Ultraviolet spectrum

pH 1 λ_{max} 312 (ϵ4,800), 218 nm (ϵ9,000); pH 7 λ_{max} 294 (ϵ6,000), 224 nm (ϵ17,000); pH 12 λ_{max} 294 (ϵ7,000), 224 nm (ϵ20,000). The pKa of (m_2n^5CDP) at 21° measured spectrophotometrically was 4.14 ± 0.07.

Dephosphorylation of m_2n^5CMP - This was carried out as previously described[4] to give 5-dimethylaminocytidine which ran as a single spot on paper and silica thin layer chromatograms.

5-Dimethylaminocytidine 5'-Diphosphate was prepared in 60% yield from m_2n^5CMP by the phosphoromorpholidate method[5] and was isolated as the trisodium salt. Found C, 26.47; H, 4.46; N, 10.14% Calc. for $C_{11}H_{17}N_4O_{11}P_2Na_3$ C, 25.79; H, 3.34; N, 10.93%. Ultraviolet spectrum, pH 1 λ_{max} 312 nm (ϵ5,000); pH 7 λ_{max} 294 (ϵ6,000), pH 12 λ_{max} 294 nm (ϵ6,200).

Synthesis of Poly(5-Dimethylaminocytidylic acid) [poly(m_2n^5C)] - A solution of m_2n^5CDP (30 mg) and polynucleotide phosphorylase (Micrococcus luteus 30 U/mg) (2.5 mg) in 0.2 M Tris-chloride (pH 9.0), 6.7 mM NaEDTA, 13.3 mM $MgCl_2$, 0.02% NaN_3, 2% BSA (5 ml) was incubated at 45° overnight. After deproteinisation, the aqueous phase was desalted by dialysis for 24 hours at 5° against 0.1 M NaCl, twice against 0.001 M NaEDTA and finally against water. Lyophilisation of the product at 0° gave poly(m_2n^5C) (13 mg), which ran as a single peak on polyacrylamide gel electrophoresis and had a $s_{20,w}$ of 8-12 as determined by ultracentrifugation in an isokinetic gradient of sucrose which contained acetate at pH 7.0. The ultraviolet spectrum of poly(m_2n^5C) at 20° in 0.1 M sodium acetate pH 7.0 was λ_{max} 294 (ϵ(P)[6] 5,700), 224 nm (ϵ(P) 8,500). Spectrophotometric titration at 21° of poly(m_2n^5C) showed a sharp rise in the region of pH 4.1. Total hydrolysis of poly(m_2n^5C) by 0.1 N NaOH at 100° for 15 minutes gave a hyperchromicity in the uv spectrum of 4%. Hydrolysis of poly(m_2n^5C) (50 μM) by pancreatic RNase (0.5 μg) in 0.01 M ammonium acetate (0.2 ml, pH 7.0) at 25° gave the mononucleotide and was accompanied by a hyperchromic rise of 4% in the optical density at 272 nm; under these conditions (poly m_2n^5C) has $t_{\frac{1}{2}}$ = 2 min. and poly C has $t_{\frac{1}{2}}$ < 10 sec.

Preparation and Properties of a Poly(I) Poly(m_2n^5C) Hybrid - Equimolar quantities of poly(I) ($s_{20,w}$ = 6.64) and poly(m_2n^5C) were dissolved in 0.1 M potassium phosphate buffer pH 7.0 (2 ml) at 37° then cooled to 0°. After standing overnight the mixture was allowed to warm to room temperature and then applied to a Sepharose 4B 200 column (2 x 28 cm). Elution with water gave the hybrid poly(I) poly(m_2n^5C) in the void volume. The stoichiometry of hybridisation in 0.1 M potassium phosphate at pH 7 was determined by the method of continuous variations, monitoring the reaction at 245 nm. There was a sharp discontinuity in the curve at 50% molar concentration of poly(m_2n^5C) corresponding to the formation of a 1:1 hybrid. The melting temperature of poly(I) poly(m_2n^5C) in 0.1 M potassium phosphate pH 7.0 was 58°.

The position of substitution of the dimethylamine residue in m_2n^5C is established by NMR spectroscopy as the resonance due to H_5 is absent and the signal due to H_6 appears as a singlet at 7.6 ppm. Polymerisation of m_2n^5CDP by polynucleotide phosphorylase is slow and cannot readily be followed by phosphate release, prolonged incubation times being required for the production of poly(m_2n^5C). The polymer is more resistant to hydrolysis by pancreatic RNase than poly C and like poly C shows a sharp discontinuity around pH 4 on spectrophotometric titration, probably indicating the formation of an acid form of the polymer.

Poly(m_2n^5C) forms a 1:1 hybrid with poly(I) with a T_m of 58° in 0.1 M salt solution. This value is close to that for poly(I).poly(C) under comparable conditions and is lower than those values for hybrids of poly(I) with poly(5-halogenocytidylic acids)[7] or poly(m^5C)[8]. Thus there appears to be no simple relationship in the hybrids of poly(I) and poly (C) derivatives between the interstrand forces as measured by the T_m and any one parameter of the substituted cytidine nucleus e.g. pKa. The size of the substituent, its polarisability and its interaction with the solvent may all contribute to the interstrand stabilising forces. The activity of poly(I).poly(m_2n^5C) as an inducer of interferon is under investigation.

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REFERENCES

1. M. A. W. Eaton and D. W. Hutchinson, Biochemistry 11 (1972) 3162.
2. M. A. W. Eaton and D. W. Hutchinson, Biochim.Biophys.Acta 319 (1973) 281.
3. F. B. Howard, J. Frazier and H. T. Miles, J.Biol.Chem. 244 (1969) 1291.
4. M. Laskowski, in: Procedures in Nucleic Acid Research, Vol. 1, eds. G. L. Cantoni and D. R. Davies (Harper and Row, New York, 1966), p.154.
5. J. G. Moffatt and H. G. Khorana, J.Amer.Chem.Soc. 83 (1961) 649.
6. P. S. Chen, T. Y. Toribara and H. Warner, Anal.Chem. 28 (1956) 1756.
7. A. M. Michelson and C. Monny, Biochim.Biophys.Acta 149 (1967) 88.
8. W. Szer and D. Shugar, J.Mol.Biol. 17 (1966) 174.