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Note

Separation of acid degradation products of DNA with cation-exchange columns

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The principle of charge reduction of cation-exchange resin by a prewash which was first successfully tried in amino acid separation[1,2] has been applied to the separation of bases[3,4], nucleoside[4,6], (5′) nucleotides[5,6] or (3′) nucleotides[7] of DNA. These methods use salt-form Dowez 50 columns and citrate or formate as the elution buffer. In general, the same accessory devices, *e.g.* jackets, peristaltic pump, mixing chambers, reservoir, air-pressure regulator and a UV spectrophotometer with a continuous flow-through cell attached to a recorder are commonly used. The present method employs the salt-form of Dowex 50 but with much simpler apparatus and accomplishes one step separation of d-pTp and d-pCp or the separation and purification of these compounds when obtained from acid (2% H_2SO_4) hydrolysis of dinucleotides (d-pTpA, d-pTpG, d-pCpA and d-pCpG) or a polynucleotide (poly dA-dT) or DNA from different sources.

MATERIALS AND METHODS

Dowex 50 resin was purchased from Bio-Rad Labs., Richmond, Calif., U.S.A.) under the name of AG 50W-X4 of mesh size 400 in the hydrogen form. The procedures for the preparation of the resin, packing, prewashing and elution of the column were essentially the same as reported earlier[6]. A sample mixture was prepared by dissolving 1 mg d-pTp and 2 mg d-pCp (purchased from Collaborative Research Inc., Waltham, Mass., U.S.A.) in 0.5 ml of the elution buffer. The sample was then transferred to the top of the 30-cm column which was prewashed with 250 ml of the elution buffer. The two compounds were eluted with 14 ml of 0.1 *M* ammonium formate buffer of pH 3.2.

Acid (2% H_2SO_4) hydrolysate of highly polymerized DNA from calf thymus (purchased from Sigma, St. Louis, Mo., U.S.A.), salmon sperm or chicken blood (the last two were purchased from Calbiochem, La Jolla, Calif., U.S.A.) were prepared by refluxing 25 mg of DNA in 4 ml of 2% H_2SO_4 for 2 h. An aliquot (0.5 ml) of the hydrolysate was transferred directly to the column and eluted as described above. The synthetic dinucleotides (d-pTpA, d-pTpG, d-pCpA and d-pCpG) and a polynucleotide (poly dA-dT) (both purchased from Collaborative Research Inc.) were hydrolyzed by refluxing 5 mg of each compound in 3 ml of 2% H_2SO_4 for 2 h and 1 ml was transferred to the column. In all instances the column was prewashed with

250 ml 0.1 *M* ammonium formate buffer pH 3.2 and the diphosphate was eluted in less than 20 ml of the same buffer. The respective peaks were identified and the recoveries of the individual compounds were calculated by the procedure reported earlier[6]. The final identifications of the d-pTp and d-pCp obtained by the 2% H_2SO_4 hydrolysis of DNA were made in the following manner: The contents of the peak tubes corresponding to each compound were pooled, evaporated to dryness under vacuum and reconstituted in 0.01 *N* HCl. The UV spectra of these compounds were compared with those of the synthetic diphosphates obtained commercially. In order to rule out that the peak of the unhydrolyzed dinucleotide overlapped with that of the diphosphate, a preliminary study was made on the separation of each of the dinucleotides from the diphosphates using the above experimental conditions. A mixture of 0.9 mg of each of the dinucleotide and 1.5 mg of the diphosphate was dissolved in 0.5 ml of buffer and transferred to the column and eluted by the same procedure.

RESULTS AND DISCUSSION

Fig. 1 shows the results of the separation of a mixture of d-pTp and d-pCp using Dowex 50-X4 400 mesh column and eluted by 14 ml of 0.1 ammonium formate buffer pH 3.2. The two peaks corresponding to d-pTp and d-pCp are well separated. This procedure is also applicable to the separation of the two diphosphates when obtained by mild acid hydrolysis (2% H_2SO_4) of DNA from various sources. The results of Fig. 2 indicate that the elution profile of the two compounds obtained from 2% H_2SO_4 hydrolysate of calf thymus DNA was quite similar to that of Fig. 1. The UV spectra of the synthetic d-pTp and d-pCp were found to be identical with the isolated compounds (Figs. 3 and 4). The distribution of the two diphosphates obtained from acid hydrolysates of different DNA's reported in Table I show that DNA of calf thymus and

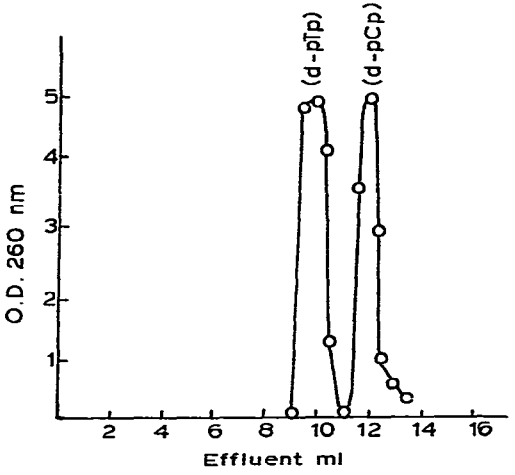

Fig. 1. Separation of diphosphates of thymidine (d-pTp) and deoxycytidine (d-pCp) using Dowex 50-X4 400 mesh resin. A 1-mg amount of d-pTp and 2 mg of d-pCp were dissolved in 0.5 ml of the elution buffer (0.1 *M* ammonium formate pH 3.2) and transferred into the surface of the 30-cm long column and eluted at the approximate rate of 1 ml/8 min at room temperature under gravity flow. The total volume of the eluate was 15 ml.

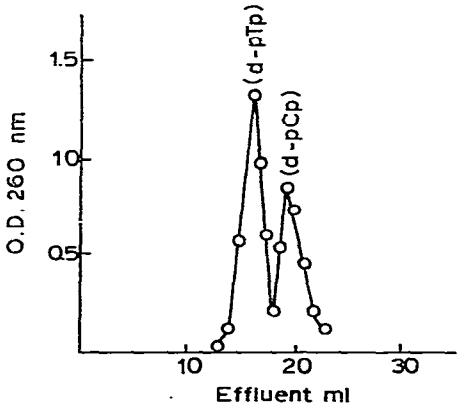

Fig. 2. As Fig. 1, except that the sample was an aliquot of a calf thymus DNA which was hydrolysed by 2% H_2SO_4.

chick blood released higher levels of d-pTp in comparison with d-pCp while the two compounds were obtained in about equal quantities from salmon sperm DNA hydrolysate. The yields of the diphosphates were between 60% for salmon sperm DNA to over 90% for calf thymus DNA.

Pyrimidine nucleoside 3'5'-diphosphates were early recognised to be among the products of moderate acid degradation of DNA[8] during which it passed through a transitory state of depurination. In the course of renewed interest in these breakdown products Dekker *et al.*[9] isolated the mixture of d-pTp and d-pCp as the barium salts from the 2% H_2SO_4 hydrolysate of herring sperm DNA and separated the two diphosphates by using Dowex-2 under acid conditions. The d-pCp was found contaminated with other degradation products and no data were given on the profiles or the yields of the diphosphates. An elimination reaction involving the phosphate esters which are beta to the aldehyde groups was formulated as the mechanism contributing to the release of the pyrimidine oligonucleotide after initial depurination[9]. A series of

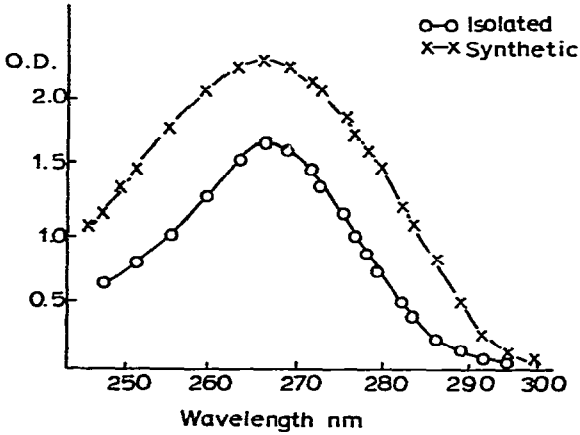

Fig. 3. A comparison of the UV spectra of synthetic d-pTp with that isolated from 2% H_2SO_4 hydrolysate of calf thymus DNA.

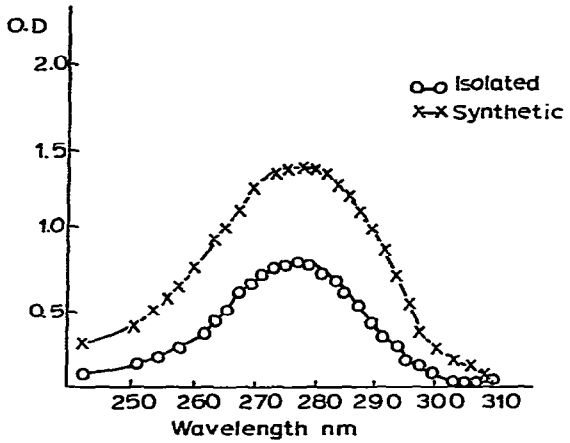

Fig. 4. A comparison of the UV spectra of synthetic d-pCp with that isolated from 2% H_2SO_4 hydrolysate of calf thymus DNA.

TABLE I

RECOVERY OF d-pTp AND d-pCp IN THE H_2SO_4 (2%) HYDROLYSATE OF DNA FROM DIFFERENT SOURCES

*Source or DNA**	*Distribution (%)*		*Yield as diphosphate (%)*
	d-pTp	*d-pCp*	
Salmon sperm	52	48	60
Calf thymus	60	40	92
Chicken blood	65	35	73

* For details regarding the procedure, see Materials and methods.

beta elimination reactions resulting in the liberation of small amounts of the diphosphates was postulated as a possible mechanism by Shapiro and Chargaff[10]. Under the conditions of the present experiments, the two diphosphates were the only major products obtained and are unlikely to have arisen solely by random fission. The results on the contrary suggest that they are probably released in an orderly fashion from those positions in the polynucleotide chain where a Pu-Py sequence is alternating.

In order to examine the significance of the above results in connection with the sequence of nucleoside residues in a polynucleotide chain, quantitative aspects of acid (2% H_2SO_4) degradation of representative dinucleotides (d-pCpA, d-pCpG, d-pTpA and d-pTpG) or a polynucleotide (poly dA-dT) were examined. The results of this study presented in Table II showed that mild acid degradation of these compounds released over 92% of the theoretical amounts of the diphosphate. These were first isolated from the reaction mixture by the same procedure as described above. The dinucleotides containing thymidine (d-pTpA and d-pTpG) were eluted before the thymidine diphosphate (d-pTp) while the cytidine containing dinucleotides (d-pCpA and d-pCpG) followed the corresponding diphosphate (d-pCp). A profile of the separation of d-pCp from d-pCpG is presented in Fig. 5. The results showed a good separation of the two compounds. The procedure reported in this paper may prove valuable in obtaining information regarding the quantitative aspect of Pu-Py alternating sequence in DNA polynucleotide.

TABLE II

RECOVERY OF d-pTp OR d-pCp IN THE H_2SO_4 (2%) HYDROLYSATE OF DI- OR POLYNUCLEOTIDES OF DNA

*Di- or polynucleotide hydrolyzed**	*Recovery in % as*	
	d-pTp	*d-pCp*
d-pCpA		95
d-pCpG		98
d-pTpA	95	
d-pTpG	92	
Poly (dA-dT)	92	

* For details regarding the procedure, see Materials and methods.

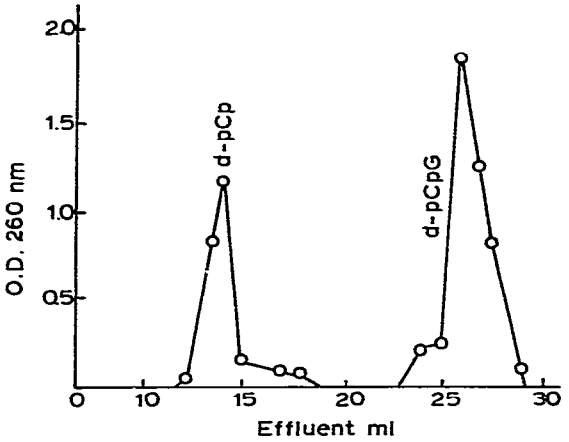

Fig. 5. As Fig. 1, except that the sample was a mixture of 1.5 mg d-pCp and 0.9 mg d-pCpG dissolved in 0.5 ml of elution buffer.

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