The Use of Fluorine-Containing Carboxylic Acids for the Large-Scale Isolation of Highly Polymerized Deoxyribonucleic Acids

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1. The precipitation of the proteins of calf-thymus nucleohistone and herring-sperm nucleoprotamine with the salts of fluorine-containing carboxylic acids has been studied. Pentadecafluoro-octanoate gave a precipitate that could be sedimented almost completely by centrifuging at 2350g for 20 min. ω -H-Hexadecafluorononanoate, ω -H-eicosafluoro-undecanoate and dodecyl sulphate gave precipitates that could only be partly sedimented under these conditions. 2. A method has been developed for the isolation of highly polymerized DNA on a large scale from calf thymus and herring sperm by the use of pentadecafluoro-octanoate. This is more convenient and less tedious than existing methods.

For the isolation of highly polymerized DNA on a large scale two sources are commonly used, herring sperm and calf thymus glands. The methods usually employed are based on that introduced by Mirsky & Pollister (1942), in which the tissue is extracted with 0.14M-sodium chloride to remove ribonucleoproteins, and the residue, containing DNA and basic proteins, is dissolved in M-sodium chloride. To obtain the DNA the protein must then be removed, and it is this stage which causes most difficulty. One procedure is to denature the protein by shaking with chloroform and pentanol as described by Sevag (1934) and Sevag, Lackman & Smolens (1938), but this is tedious because numerous treatments and much manipulation are required. A better method is to use sodium dodecyl sulphate or similar anionic detergents to precipitate the protein (Marko & Butler, 1951; Kay, Simmonds & Dounce, 1952), but in our experience this is also tedious, particularly when working on a large scale, because of the difficulty of centrifuging or filtering the voluminous precipitate from the large volume of viscous fluid. The possibility of using salts of long-chain perfluoroalkanoic acids for the removal of the protein has been investigated because it seemed possible that these would form denser and more compact precipitates.

MATERIALS AND METHODS

Fluorine-containing acids. Pentadecafluoro-octanoic acid was obtained from the Minnesota Mining and Manufacturing Co. Ltd., St Paul, Minn., U.S.A. ω -H-Hexadecafluorononanoic acid and ω -H-eicosafluoro-undecanoic acid were kindly given to this Department by Du Pont de Nemours

and Co. Inc., Wilmington, Del., U.S.A. Pentafluorobenzoic acid was obtained from Imperial Smelting Corp. Ltd., Avonmouth, Bristol. The other fluorine-containing acids were synthesized in this Department from the corresponding alcohols and were kindly supplied by Dr P. L. Coe.

The acids were used in the form of their sodium salts except ω -H-eicosafluoro-undecanoic acid, which was used as the triethanolammonium salt.

Analytical procedures. Nitrogen analyses were carried out by the indophenol blue method (Jones & Walker, 1963) and phosphorus was determined as described by Jones, Lee & Peacocke (1951). DNA was analysed for purines and pyrimidines by Wyatt's method (Wyatt, 1951; Wyatt & Cohen, 1953) and for protamine or histone by a modification of the Sakaguchi (1950) reaction, which determines the arginine content. The arginine content of herring-sperm protamine was taken to be 80% and that of calf-thymus histone to be 25%. The DNA content of solutions was determined by measuring the extinction at 260m μ , and taking the extinction of a 0.05 mg./ml. solution of DNA to be 1.0 in a 1 cm. cuvette. The fluorine content of the DNA was determined by the oxygen flask method followed by titration with thorium nitrate to an alizarin red standard (Macdonald, 1961).

Preparation of nucleoproteins. All operations were carried out at 0–4° unless otherwise stated.

Herring-sperm nucleoprotamine. Soft herring roes (100 g.) were macerated in a Towers top-drive macerator for 1 min. with 0.14M-NaCl (200 ml.) containing trisodium citrate (0.01M) and the resulting suspension was centrifuged at 2000g for 30 min. Three layers were formed, an upper layer of fibrous solid, a clear liquid and a sediment. The sediment was resuspended in 0.14M-NaCl–0.01M-citrate and the suspension centrifuged as before. This operation was repeated twice, and the supernatant liquids and the upper layer of fibrous solid (containing RNA and denatured protein) were discarded. The sediment was homogenized with an equal volume (approx. 150 ml.) of 2M-NaCl, and

then diluted once with m -NaCl (approx. 300 ml.), and poured into 6 vol. (approx. 3.6 l.) of 0.1 M -trisodium citrate. The resulting fibrous precipitate was removed on a glass rod and redissolved in an equal volume of 2 M -NaCl. The reprecipitation and redissolving were repeated twice and the material was finally dissolved in an equal volume of 2 M -NaCl (1 l.) and stored at 0°. The DNA content of the solution was 3.0 mg./ml. and the protamine content was 1.16 mg./ml.

Calf-thymus nucleohistone. The procedure was similar to that described above. The final solution of nucleohistone contained 1.94 mg. of DNA/ml. (measured by phosphorus content) and 0.475 mg. of histone/ml. The protein content of these solutions was lower than is usual for nucleoprotein solutions. This was probably due to some denaturation of the proteins during the isolation procedure.

Isolation of DNA from calf thymus. Nucleohistone from 500 g. of calf thymus glands was dissolved in m -NaCl (6 l.). To this solution was added a solution (10%, w/v) of sodium pentadecafluoro-octanoate in m -NaCl (600 ml.) and, after standing at room temperature for 18 hr., the resulting precipitate of protein was centrifuged off at 1025 g for 30 min. (MSE Major refrigerated centrifuge; 4×11 . head) and the precipitate washed with m -NaCl. A portion (500 ml.) of the combined supernatant and washings (6 l.) was taken and dialysed against distilled water for 72 hr. and freeze-dried. The bulk of the solution was treated once with chloroform and pentanol (Sevag, 1934; Sevag *et al.* 1938) and the resulting aqueous phase dialysed against distilled water for 72 hr. and freeze-dried (total yield of DNA, 8.9 g.). The protein content of the material not treated by Sevag's method was 7.2%, whereas that of the material so treated was 1.7%. The latter product had the following composition: N, 16.2%; P, 9.4% (dry wt.); the base analysis was: guanine, 0.88; cytosine, 0.80; adenine, 1.11; thymine, 1.13 moles/g. atom of phosphorus; ϵ_p , the molar extinction coefficient based on phosphorus, was 6400.

In a similar experiment on a smaller scale, the protein precipitate was centrifuged off at 4400 g for 30 min. (MSE Major refrigerated centrifuge; high-speed angle head). In this case the protein contents of the DNA were similar (Table 2) both with and without treatment with chloroform.

Large-scale isolation of DNA from herring sperm. Nucleoprotamine from 800 g. of soft herring roes was dissolved in m -NaCl (18 l.). To this solution was added a 10% solution of sodium pentadecafluoro-octanoate (400 ml.) and the suspension allowed to stand at room temperature for 18 hr. The resulting precipitate of protein was centrifuged off at 1025 g for 30 min. (MSE Major refrigerated centrifuge; 4×11 . head) and the supernatant liquid was treated once by shaking with chloroform and pentanol. The salt concentration was reduced to approx. 0.1 M by dialysing the aqueous phase for 2 days and the DNA was then precipitated with ethanol (2 vol.). The fibrous precipitate was removed, dissolved in distilled water (6 l.) and dialysed against distilled water for 4 days and freeze-dried. The yield was 33 g. The composition was: N, 11.8%; P, 7.0%; protein, 0.18% (moist material); ϵ_p , 6600. The product contained no detectable fluorine (<1.5%).

The samples of DNA were highly fibrous and gave viscous solutions in water.

RESULTS

Precipitation of the proteins of deoxyribonucleoproteins with fluorine-containing carboxylic acids. The only long-chain perfluoroalkanoic acids obtainable commercially were heptafluorobutyric acid and pentadecafluoro-octanoic acid. The other long-chain acids contained a hydrogen atom on the ω -carbon atom. Pentafluorobenzoic acid was also used.

A solution of calf-thymus nucleohistone (DNA content, 1.22 mg./ml.) in m -sodium chloride was treated with various concentrations of the salts of the fluorine-containing acids and the solutions were allowed to stand at room temperature for 18 hr. If a turbidity developed the solutions were centrifuged at 2350 g for 20 min. (MSE Minor centrifuge; 4×15 ml. head). These conditions of centrifugation were chosen because they gave an indication of the ease of removal of a precipitate on a large scale.

Table 1. *Precipitation of the histone of calf-thymus nucleohistone by fluorine-containing carboxylic acids*

The solution of the nucleohistone was mixed with the salt of the acid at the concentration indicated. The final solutions were 1 M with respect to NaCl and the DNA content was 1.22 mg./ml. Suspensions were centrifuged at 2350 g for 20 min. —, No significant increase in turbidity of the nucleoprotein solution; T, turbidity, but no significant sedimentation of a precipitate on centrifuging; +, sedimentation of a precipitate on centrifuging but supernatant still turbid; ++, sedimentation of a precipitate on centrifuging and supernatant clear.

Precipitant	Concn. of precipitant (% w/v)					
	2.9	1.7	0.9	0.4	0.2	0.1
Heptafluorobutyric acid	—	—	—	—	—	—
ω - <i>H</i> -Octafluoropentanoic acid	T	T	T	T	T	T
ω - <i>H</i> -Dodecafluoroheptanoic acid	+	+	T	T	T	T
Pentadecafluoro-octanoic acid	++	++	++	+	+	T
ω - <i>H</i> -Hexadecafluorononanoic acid	++	++	++	+	+	T
ω - <i>H</i> -Eicosafluoro-undecanoic acid	++	++	++	+	T	T
Pentafluorobenzoic acid	—	—	—	—	—	—

All of the compounds tested except heptafluorobutyrate and pentafluorobenzoate caused precipitation (Table 1); but the precipitate was not easily sedimented when ω -*H*-octafluoropentanoate and ω -*H*-dodecafluoroheptanoate were used. The amount of histone remaining in solution when pentadecafluoro-octanoate, ω -*H*-hexadecafluorononanoate and ω -*H*-eicosafuoro-undecanoate were used, was measured. For comparison a similar experiment was performed in which sodium dodecyl sulphate was used as protein precipitant. The action of pentadecafluoro-octanoate and ω -*H*-

eicosafuoro-undecanoate on herring-sperm nucleoprotamine (in *m*-sodium chloride; DNA content, 1.5 mg./ml.) was also investigated.

Fig. 1 shows that pentadecafluoro-octanoate was the most effective precipitant. Pentadecafluoro-octanoate was also considerably more effective than either ω -*H*-dodecafluoroheptanoate or ω -*H*-hexadecafluorononanoate. This marked difference may have been due to the fact that the latter two contained one hydrogen atom in their molecule. The very low efficiency of ω -*H*-eicosafuoro-undecanoate for calf-thymus histone was probably due to the very low solubility of its salts in *m*-sodium chloride. Under the conditions used sodium dodecyl sulphate was also not very effective. This was because only a low centrifugal force was used to sediment the precipitates. The results also showed that herring-sperm protamine was precipitated at much lower concentrations of fluoroalkanoic acid than was calf-thymus histone.

Comparison between the use of sodium dodecyl sulphate and sodium pentadecafluoro-octanoate for the isolation of calf-thymus DNA. A solution of calf-thymus nucleohistone (200 ml.) was mixed with 2.5% (w/v) sodium pentadecafluoro-octanoate in *m*-sodium chloride (120 ml.). A similar solution of the nucleohistone was mixed with 1.67% sodium dodecyl sulphate in *m*-sodium chloride (120 ml.). Both suspensions were allowed to stand at room temperature for 18 hr. and then centrifuged at 4400*g* for 30 min. Each supernatant liquid was divided into two equal parts; one part was treated with chloroform and pentanol in the usual way and the other part was not. The DNA was isolated from the four solutions and the compositions of the resulting products are given in Table 2. When the protein precipitate was centrifuged off at a relatively high centrifugal force, the use of chloroform and pentanol was not necessary when sodium pentadecafluoro-octanoate was used as the protein

Fig. 1. Precipitation of the proteins of nucleoproteins with salts of fluorine-containing carboxylic acids. Calf-thymus nucleohistone treated with: ○, sodium pentadecafluoro-octanoate; ×, sodium ω -*H*-hexadecafluorononanoate; □, triethanolammonium ω -*H*-eicosafuoro-undecanoate; ▲, sodium dodecyl sulphate. Herring-sperm nucleoprotamine treated with: ■, sodium pentadecafluoro-octanoate; ●, triethanolammonium ω -*H*-eicosafuoro-undecanoate. Experimental conditions are given in the text.

Table 2. *Isolation of calf-thymus DNA by the use of sodium dodecyl sulphate or sodium pentadecafluoro-octanoate*

Samples of DNA were isolated from a solution of calf-thymus nucleohistone by the procedure described in the text. Analyses were carried out on freeze-dried material (moisture content 15–20%). Yield was based on the percentage of the phosphorus of the nucleoprotein solution recovered as DNA phosphorus.

Method of isolation	Yield (%)	Composition			
		Protein (%)	N (%)	P (%)	N/P (w/w)
(1) Sodium pentadecafluoro-octanoate	87	1.0	13.2	7.9	1.67
(2) As (1), followed by chloroform-pentanol	75	1.3	13.9	8.25	1.68
(3) Sodium dodecyl sulphate	60	3.4	10.4	6.2	1.67
(4) As (3), followed by chloroform-pentanol	60	1.9	12.7	7.1	1.79

precipitant. When sodium dodecyl sulphate was used, however, a considerable improvement in the removal of protein was effected by the subsequent use of chloroform and pentanol. This was due to the much greater difficulty experienced in sedimenting the sodium dodecyl sulphate-protein precipitate.

DISCUSSION

These results show that sodium pentadecafluoro-octanoate was much more effective in removing the proteins from the nucleoproteins of herring sperm and calf thymus than was sodium dodecyl sulphate. With the former the protein precipitates were compact and easily removed by centrifuging, in marked contrast with the gelatinous voluminous precipitates produced when sodium dodecyl sulphate was used. Pentadecafluoro-octanoate was particularly effective for the isolation of herring-sperm DNA. The whole process for the isolation of 33 g. of DNA, with the exception of the time taken for the dialyses, only occupied two persons working for 2 days. This short time should offset the expense of pentadecafluoro-octanoic acid. In the method given for the large-scale isolation of herring-sperm DNA one treatment with chloroform and pentanol decreased the protein content to about 0.1%. The results with calf-thymus DNA indicated that this might not have been necessary had the protein precipitated with pentadecafluoro-octanoate been removed by high-speed centrifugation. Perfluoro-octanoate has been used by Kirby (1957) in a procedure for the isolation of DNA, in which it was

found to be moderately effective, but as in this case it was not used as a protein precipitant, but in conjunction with phenol, the method cannot be compared with that described above.

The low ϵ_r values of our products show that there was little degradation of DNA (Chargaff, 1955). If it is desired to eliminate further any modification of the secondary structure, dialysis against distilled water and freeze-drying can, of course, be avoided.

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