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The Use of Sodium Perchlorate in Deproteinization during the Preparation of Nucleic Acids

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Sodium perchlorate in high concentrations will remove from solution the detergent sodium dodecyl sulphate and protein complexed with it. This and the failure of proteins to be precipitated by ethanol from solutions containing a high concentration of sodium perchlorate can be utilized as efficient, rapid and simple deproteinization procedures during the preparation of nucleic acids.

Sodium perchlorate (1M) is used to dissociate nucleic acid–protein complexes before deproteinization by shaking microbial lysates with a chloroform–3-methylbutan-1-ol mixture in the Marmur (1961) DNA-preparation method. Both the Marmur (1961) procedure and the many variations on the phenol method of Kirby (e.g. Kirby *et al.*, 1967) utilize a two-phase system where denatured protein gathers at the interface. A great many modifications of these procedures to suit individual biological materials have been published. However, certain general observations can be made on their performance. Often a number of cycles through the two-phase system are required to remove most of the protein. Losses of nucleic acid which may occur at the interface are thus compounded. Miniaturization of the procedures frequently leads to a lowering in yield, since the loss involved in separating the two layers is not a direct function of the volumes used. Lastly these methods are time-consuming and in the case of phenol procedures relatively unpleasant.

An aqueous solution of perchlorate has been called a 'chaotropic' agent by Hamaguchi & Geiduschek (1962) and a denaturing solvent by many other authors in referring to its property of lowering the thermal 'melting' temperature of double-stranded nucleic acids.

Bauer (1972) concluded, since the addition of perchlorate to solutions of DNA changed the relative sedimentation coefficients of closed and nicked circular forms, that, as with intercalating dyes, a partial unwinding of the duplex occurred. A general pitch change rather than extensive local unwinding was considered more likely.

A homogenate of tobacco leaf in saline–EDTA (Marmur, 1961) containing 5% (w/v) sodium dodecyl sulphate was inadvertently made up to 5M-$NaClO_4$ (70%, w/v) instead of 1M-$NaClO_4$ as specified by Marmur (1961). On centrifuging it was found that the detergent formed a raft on top of the $NaClO_4$ solution. This raft contained virtually all the chlorophyll and, judged by the typical nucleic acid spectrum of the material in the solution below, most of the protein. Provided that a certain minimum detergent concentration was exceeded this was always the case. Below this concentration, protein was present in a green liquid below the raft. However, even if the latter phenomenon did occur, presumably because insufficient detergent was present to complex all the protein and adhering chlorophyll, it was found that only nucleic acid was precipitated from the liquid below the raft on addition of 2vol. of ethanol.

This situation was reconstructed for a number of common proteins in solution in 75% (w/v) $NaClO_4$. In no case could precipitation be achieved by adding 2vol. of ethanol. Indeed crystalline bovine serum albumin was freely soluble in a solution containing 25% (w/v) $NaClO_4$ and 66.7% (v/v) ethanol. This mixture might have use as an extraction medium, since it selectively dissolves proteins, is stable at very low temperatures and dissolves most common detergents including sodium dodecyl sulphate.

Applying the experience with tobacco leaves to detergent lysates of lysozyme-sensitive bacteria yields most of the RNA but little DNA precipitable by ethanol from the $NaClO_4$ solution below the raft of detergent–protein complex. If, however, instead of gently mixing the lysate with $NaClO_4$ it is mixed by vortexing, or if the lysate itself is sheared by forcing through a hypodermic needle, the yield of DNA, as judged by the recovery of acid-insoluble radioactivity from the lysate in the final preparation, is very high (70–100%). The progressive increase in DNA yield on shearing (Table 1) is consistent with the simple occlusion of high-molecular-weight DNA in the raft. A similar conclusion was reached after equilibrium buoyant-density-gradient centrifugation in CsCl of the nucleic acid preparations from the progressively sheared lysates. An increasing peak of radioactive u.v.-absorbing material at the density of *Streptomyces* DNA (1.730g/cm^3) was found. Indeed preliminary results were obtained which suggested that by gentle rocking of the lysate after addition of $NaClO_4$ very-high-molecular-weight DNA could be

Table 1. *Effect of shearing on the distribution of radioactivity in preparations of nucleic acid from [^3H]adenine-labelled lysates of Streptomyces coelicolor*

Streptomyces coelicolor ad-10 (kindly supplied by Professor D. A. Hopwood) was grown for 3 days in nutrient broth (Difco) containing 0.5% glucose, 34% (w/v) sucrose and [2-^{3}H]adenine (approx. 1 μCi/ml; 23 Ci/mmol) (The Radiochemical Centre, Amersham, Bucks., U.K.). After washing and resuspension at five times the original concentration in saline–EDTA (Marmur, 1961) containing lysozyme (Koch–Light, Colnbrook, Bucks., U.K.) at 5mg/ml the bacteria were incubated for 45min at 37°C. Lysis was achieved by addition of 25% (w/v) sodium dodecyl sulphate to a final concentration of 5%. The lysate, either untreated or sheared by forcing through a 12-gauge needle ('extent of shearing' denotes the number of passages forward and back), was made up to 75% (w/v) $NaClO_4$ by addition of 3vol. of 100% solution. Mixing was achieved by inverting the tubes three times. The mixtures were centrifuged briefly in a bench centrifuge in cellulose nitrate tubes. The bases of the tubes were punctured and each $NaClO_4$ solution was collected into 2 vol. of cold ethanol. The ethanol-insoluble material (first precipitate) was dissolved in dilute saline–citrate and a sample dried on to a glass-fibre disc (2.5cm diam.; Whatman GFC). After addition of 5ml of scintillation fluid [4g of 2,5-diphenyloxazole and 0.25g of 1,4-bis-(5-phenyloxazol-2-yl)benzene per litre] the discs were counted for radioactivity in vials in a Beckman LS-200B liquid-scintillation counter. After digestion of the remaining precipitate with ribonuclease (Koch–Light) at a final concentration of 100μg/ml for 1h solid $NaClO_4$ to about 75% (w/v) was added. A further 2vol. of cold ethanol was added and after 20min on ice the ethanol-insoluble material (second precipitate) was again sampled for radioactivity counting. Spectra of the dissolved precipitates were typical of nucleic acids, with E_{260} being about twice the E_{280} and slightly more than twice the E_{230}. Nucleic acid concentration was calculated from the relationship $E_{1cm}^{0.1\%}$ at 260nm = 23.0.

	First ethanol precipitate			Second precipitate
Extent of shearing	Total nucleic acid extracted (μg/ml of original culture)	Total radioactivity (c.p.m./ml of original culture)	Specific radioactivity (c.p.m./μg)	Radioactivity (c.p.m./ml of original culture)
0	40.0	77920	1948	769
1	53.0	101700	1919	3711
2	57.5	109380	1902	6341
5	65.5	123880	1891	12852

released in moderate yield into the $NaClO_4$ layer on centrifuging. Thus, at least to an extent, yield and high molecular weight are mutually exclusive.

Recoveries of [^{3}H]DNA (mol.wt. approx. 1×10^7) or [^{14}C]RNA (from cowpea chlorotic-mottle virus), added to tobacco leaf (*Nicotiana tabacum* var. 'White Burley') or *Arabidopsis* dry seeds and processed by the above method, were 40–70% and 10–15% for DNA in tobacco and *Arabidopsis* respectively and 70–100% for RNA in both cases. Since the grinding necessary for breaking the cell walls and seed coats will cause extensive shearing, it was considered improbable that simple occlusion in the raft was responsible for the losses of DNA but that it is more likely to be caused by some absorption phenomenon. A combination of this technique with that of Marmur (1961) was found to be most efficient for extracting DNA from seeds. Solid $NaClO_4$ (1.3g/ml) was added to a solution in dilute saline–citrate (Marmur, 1961) of the ethanol precipitate from an extract deproteinized once by shaking with the chloroform–3-methylbutan-1-ol mixture. This gives a final concentration of $NaClO_4$ of 75% (and an increase in volume of about 50%). Addition of 2vol. of ethanol to this solution results in precipitation of the nucleic acids but not the residual proteins. It has been convenient to use this procedure to isolate DNA from nucleic acid preparations after ribonuclease treatment. Both the ribonuclease and its digestion products remain in solution on addition of ethanol to the mixture made up to the high $NaClO_4$ concentration.

As an alternative to the phenol procedure, the RNA of brome-mosaic virus was isolated from material disrupted by treatment with detergent at 50°C for 2–3min. After addition of 3vol. of 100% (w/v) $NaClO_4$ and brief centrifugation at a low speed, 2vol. of ethanol was added to the $NaClO_4$ layer below the raft. A yield of RNA somewhat better than that by the phenol method was obtained. This material was infectious and gave a pattern of RNA banding on electrophoresis in polyacrylamide gels similar to that given by phenol-prepared RNA (J. Wilcockson & R. Hull, unpublished work). A virus known to contain double-stranded RNA (*Penicillium stoloniferum* virus F) similarly treated gave RNA which showed

the same gel pattern as phenol-prepared material; thus the double-strandedness is preserved (D. H. Morgan, K. F. Chater & J. Wilcockson, unpublished work). The retention of 'sensitive qualities' like infectivity and electrophoresis patterns for viral RNA and of buoyant density in CsCl for DNA confirms the conclusion of Hamaguchi & Geiduschek (1962) that there was no reaction of $NaClO_4$ with nucleic acid.

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