Fractionated Precipitation of Acid Macropolyanions by Dialysis, a Simple Method for the Estimation of DNA in Complex Biological Samples

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Estimation of DNA, Cetyltrimethylammoniumbromide, Various Objects

After efficient extraction by para-aminosalicylate, chopping, grinding and eventual sonication, the macropolyanions are transformed into their cetyltrimethylammonium salts. These have differing solubilities, strongly depending on ionic strength. The cationic detergent-macropolyanionic salts are solubilized by high salt concentration. Salt is then dialysed out, rendering the polyanions highly insoluble in a sequential fashion. The insolubilized components are determined quantitatively by monitoring turbidity, which in case of DNA is strictly proportionate to its concentration. This relation is not affected by other components. This makes DNA determination possible even in crude aqueous extracts.

The method has been applied to different objects, such as bacteria, plants, animals, soil and activated sludge. The method may prove to be especially useful in research of environmental poisons *e. g.* in rivers, lakes or clarifiers.

Introduction

Cetyltrimethylammoniumbromide has first been used by Jones as precipitating agent for polyanions [1].

The continuous precipitation of salts of macropolyanions with cetyltrimethylammoniumbromide (CTAB) has been published previously [2, 3]. By the use of this method, high yields of nucleic acids were obtained from various objects. DNA isolated in this way conserved it's biological activity [4]. So far the nucleic acids have been sequentially precipitated by continuous or discontinuous dilution of the solubilized CTA-salts on these macropolyanions to appropriately lower ionic strength. Since the essential macropolyanions precipitated at ionic strengthes differing sufficiently, separation and purification was possible in an advantageous manner.

For analytical purposes it is sufficient to follow the turbidity caused by insolubilization of CTA-polyanion salts with decreasing ionic strength. Since this has been effected by adding low salt solution, the dilution decreased the sensitivity the more, the later in the process any given anion would precipitate.

The new method using dialysis for lowering the ionic strength, keeps the sensitivity of the determination constant for all precipitating compounds. Especially the estimation of DNA by this method proved to be reliable, simple and unaffected by other compounds, *e. g.* RNA or proteins.

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Material and Methods

Apparatus

Controlled precipitation was carried out by means of an apparatus which consists of a Bausch and Lomb Spectronic 20 photometer (a) with its power suply for the lamp (b), the phototube, controlling the incident total light flux (c) and the phototube (d), measuring light output. The light is centered by a lens-system into a pear-shaped measuring cell with a volume of 3 ml. Scattered light is measured at 5 cm distance perpendicular to the incoming

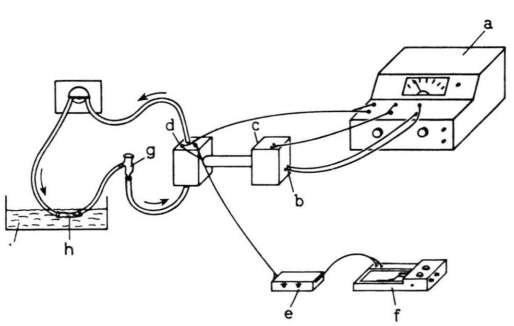

Fig. 1. Arrangement for the registration of dialysis controlled turbidity in solutions of CTA salts of macropolyanions.

beam. The measuring signal is fed into an amplifier (e) and recorded (f).

The CTA salts solution is circled at 30 ml/min through the measuring cell and the 1.5 cm long, 2.5 cm wide dialysis tube (Visking 20/32) suspended in an agitated bath containing: CTAB 1%, NaCl 0.1 M and EDTA 0.01 M at pH 8, *via* an open end glass vessel serving as a bubble trap.

Sample preparation

The material to be used was cleaned and freed of rinsing solution as far as possible and minced. It was then mixed, eventually in a blender or a homogenizer with an equal volume of a solution of *para*-aminosalicylate (PAS) 15%, EDTA 0.2 M, pH 9.

The sample was then sonicated in a B 12 sonifier (Branson Sonic Power, Danburry, Conn.) for three minutes. The insoluble parts of the mixture were removed by centrifugation in 15 min at $15000 \times g$ at 15 °C. To the clear viscous supernatant 0.3 volumes of a solution of: CTAB 4% and NaCl 2 M pH 7.2 was added while stirring. This solution has been used for the controlled DNA precipitation. Occasionally this solution was dark in colour: Then the results eventually were not reproducible. However the colour could be removed by precipitating the CTA salts adding 5 volumes of: CTAB 0.5%, EDTA 0.01 M, pH 7.2. Most of the colour is left in the supernatant. The CTA-salts were redissolved in: CTAB 1%, NaCl 1 M, EDTA 0.01 M, pH 7.2. For the estimation of DNA in plants, the tissue was chopped finely with a knife, 1/3 of the weight SiC grinding powder (500 mesh) together with one volume of: PAS 15%, EDTA 0.2 M, pH 9 was added. This mixture was homogenized in a ball mill (Hormuth, Wiesloch, Germany) for 5 min at maximum speed, the pulp was pressed through a nylon gauze and the SiC was removed by centrifugation at $5000 \times g$ in 5 min. The clear green supernatant was used for the estimation as described above.

For the calibration curve 10 mg pure DNA were dissolved in 10 ml NaCl 0.15 M, sonicated for 30 sec, 3.3 ml of a solution of: CTAB 4%, NaCl 2 M were added and the volume adjusted to 50 ml with a solution of: CTAB 1% and NaCl 1 M.

The CTA-salt of the RNA was prepared in the same way.

Results

By lowering the ionic strength in solutions of CTA-DNA salts, they will precipitate at an ionic strength equivalent about 0.7 M NaCl. This point, starting from 1 M NaCl, was reached under the conditions used, with 40 min dialysis. The CTA-DNA salt is precipitated from solution at a distinct ionic strength without any detectable amount of DNA remaining in solution. By monitoring the course of the precipitation through the light scattered by the insolubilized particles, a characteristic precipitation curve is obtained with a maximum proportionate to the DNA added (Fig. 2). Having passed its

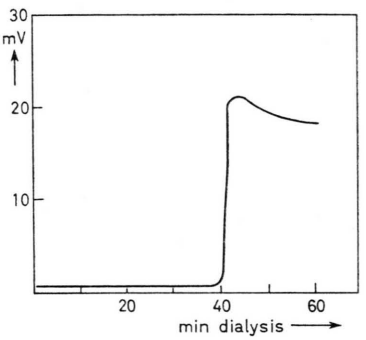

Fig. 2. Turbidity curve caused by 50 μg of trout-sperm DNA in 10 ml circulating solution. x-axis: dialysis time. y-axis: signal output in mV.

maximum, the precipitation curve drops by 8 – 10%. We suppose, that this drop signifies a decreasing number of scattering particles by formation of micelles among the insolubilized CTA-salts. The calibration curves gave exactly the same values for DNAs from different organisms, *e. g.* DNA from trout sperm, from the sponge *Geodia cydonium*, from *Holothuria tubulosa* and from *E. coli* in absence and in presence of crude extracts already containing macropolyanions. The mean deviation

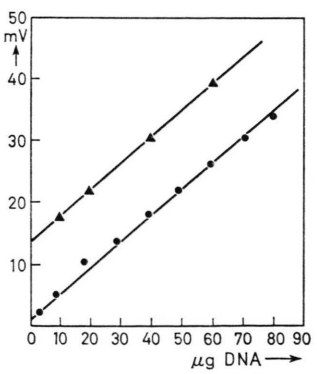

Fig. 3. DNA-calibration curves in absence (— ● —) and presence (— ▲ —) of activated sludge extract. Extrapolation yields a value of 33 μg DNA in the sludge extract from 104 mg dry material. x-axis: amount of added trout sperm DNA; y-axis: signal output in mV.

was less than 4%. In the range of ca. $10^8 - 10^5$, the molecular weight of DNA does not appreciably influence the width or the breadth of the DNA part of the precipitation curve. Thus there is no appreciable difference between DNA samples sonicated for 1, 2 or 3 min and undegraded DNA, whereas the drop of the curve following the maximum is larger with high molecular DNA. In this case the formation of threadlike aggregations can be seen.

The addition of yeast-RNA CTA-salt to the DNA standard does not interfere with the quantification of DNA. The precipitation step of DNA remains distinct. However the definition of this step can be increased considerably by stopping the dialysis with the onset of the DNA precipitation step, thus precipitating all the CTA-DNA from solution while leaving the CTA-RNA dissolved. Lowering the ionic strength further causes precipitation of CTA-RNA (Fig. 4).

Fig. 5 shows the precipitation of DNA from activated sludge. After the precipitation of acid proteins (I) the dialysis was stopped at the onset of DNA precipitation to its completion (II), which took about 10 min. Resuming dialysis caused RNA to precipitate (III).

Addition of an internal DNA standard caused a strictly proportionate rise of the y-value of the DNA step in accordance with the calibration curve.

DNA determination covering a variety of materials demonstrates the feasibility of the method described (Table I).

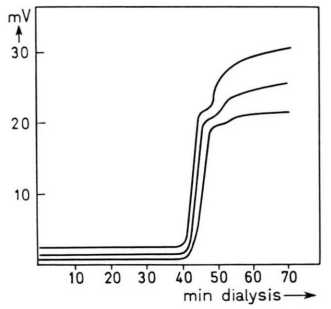

Fig. 4. The influence of RNA addition on the turbidity curve. 20, 40 and 80 μg of yeast RNA (from bottom to top curves) were added. x-axis: dialysis time in min; y-axis: signal output in mV.

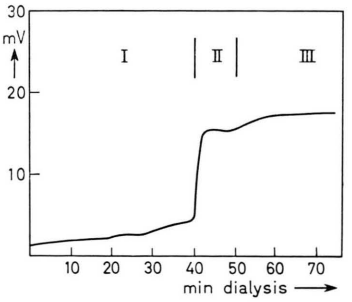

Fig. 5. Precipitation curve of macropolyanions from activated sludge. At first proteins are precipitated (I), followed by DNA (II) and RNA (III). At the onset of DNA precipitation, 40 min after start, dialysis was discontinued for 10 min to allow for complete DNA precipitation. x-axis: dialysis time in min; y-axis: signal output in mV.

Table I. DNA determination by turbidity measurement of the CTA-salt precipitation by controlled dialysis.

Material	DNA [% dry weight]	Literature value
E. coli (ATCC 113003)	3.65	3.7 – 4.2 [10]
Proteus mirabilis	4.20	
Hypnum cupressiforme (musci) gametophytes	0.01	—
Hypnum cupressiforme sporophytes	0.31	—
Equisetum palustris spores	0.14	—
5000 × *g* sediment from eutrophic lake water	0.38	—
activated sludge from a clarifier	3.23	—
garden soil	0.001	—
mouse lymphoma cells	9.7×10^{-12} g/cell	$7.9 - 9.8 \times 10^{-12}$ g/cell [11]

Literature values, available in the case of *E. coli* and mouse lymphoma cells, are in good conformance with our results.

Discussion

A method for DNA determination in a wide variety of objects, without the need for special adaption to special situations seems to be possible in a range from ca. 5 μg up to 500 μg. No efforts have been made so far to decrease this lower level which seems possible in different ways.

The method takes advantage of the very low solubility of CTA-DNA salts at low ionic strength [5]. It uses the differential solubility at moderate ionic strengthes for the different CTA-salts of macropolyanions. The discrimination power of this procedure however has not been fully used, a considerable part has been sacrificed for speed.

We are fully aware that what has been said for DNA after certain adaptions may be applied for RNA as well. Comparing the method with the com-

monly used ones basing on diphenylamine and other coloured complexes[6, 7] or on measurements of UV absorption[8, 9] it has the advantage of not being influenced by proteins, RNA and most other substances.

Interfering materials in our samples of animal, plant or microbial origin have not been met so far, however it is conceivable that non-ionic or anionic detergents or perhaps certain lipids might interfere. The method is not confined to the analytical level, it can be used on the preparative level as well, avoiding sonication.

The method seems however of special interest for the measurement of the DNA-content in activated sludge from biological clarifiers, soil or water. As the quantity of DNA is strongly correlated with the number of organisms in a sample, we think method promises to be a good possibility for correlating biological activity to the action of environmental poisons in rivers, lakes or clarifiers.

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