# 4 M GUANIDINE HYDROCHLORIDE APPLIED TO THE ISOLATION OF DNA FROM DIFFERENT SOURCES

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## 1. Introduction

Guanidine hydrochloride (GHCl) was used for isolation of RNA and DNA using repeated precipitation of nucleic acids from 4 M GHCl by ethanol [1]. Molecular sieve chromatography in GHCl has been previously used in our laboratory for isolation of high-molecular-weight calf thymus DNA [2] and *E. coli* R factor DNA [3].

In this paper the general improved procedure is described for isolation of large quantities of DNA from different sources using 4 M GHCl. Different conditions for lysis, nuclease inhibition and deproteinization using two-phase systems of organic solvents and GHCl are demonstrated. The selectivity of precipitation of DNA, RNA and polysacharide using different organic precipitants in 4 M GHCl is also discussed.

## 2. Materials and methods

GHCl was prepared from guanidine carbonate and purified by several steps of recrystallization in distilled water until the absorbance of 4 M GHCl solution at 260 and 230 nm in 1 cm cuvette was less than 0.03 and 0.08, respectively. The solution of GHCl was always buffered by 0.05 M Tris–HCl + 0.01 M EDTA pH 7.6.

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Commercial RNA from yeast (CALBIOCHEM) and dextran (Léčiva-Praha, mol. wt. 40 000) were used.

Calf thymus nuclei were prepared from quick frozen tissue (−70°C) as previously described [2] with some modifications using 0.005 M $CaCl_2$ and Tris–HCl buffer pH 7 instead of citric acid and sodium citrate. The nuclei were stored at −70°C in the medium containing 0.44 M sucrose, 0.039 M sodium glycerophosphate, 0.005 M $CaCl_2$, 0.01 M Tris pH 7.0 and 50% glycerin.

*E. coli* strain HK-26 was grown as previously described [3] and stored frozen at −70°C before use.

Tetrahymena pyriformis micronucleate strain UM 1060 (supplied by Dr Naney) was axenically grown in the medium containing 1.8% proteose peptone (Difco) 0.19% glucose, 0.09% yeast extract (Difco) and $10^{-5}$ M $FeSO_4$. The cells were harvested by centrifugation at −10°C (1000 *g* for 10 min) and immediately lysed.

### 2.1. *Isolation of DNA in 4 M GHCl solution*

Harvested viruses, bacterial cells, cells of higher organisms or cell organelles (delipidized when necessary [3]) were suspended in minimal volume of Tris buffer pH 7.6 at 0–4°C. Precooled 8 M GHCl and glycerol were added to a final concentration of 4 M and 20% respectively. The lysate was gently shaken at room temperature for 15 min for complete lysis and homogenization of the solution and then deproteinized by repeated shaking with chloroform-isoamyl alcohol [4]. DNA was precipitated by 0.75 volume of isopropanol and redissolved in 4 M GHCl. (Concentration of DNA was between 200 to 500 μg/ml.) DNA

was further deproteinized by several chloroform-isoamyl alcohol shaking steps and precipitated with isopropanol. DNA was dissolved in 0.1 × SSC (0.15 M NaCl + 0.015 M trisodium citrate) + 0.01 M EDTA, treated with RNAse and further purified [4].

The calf thymus DNA for comparative studies was isolated according to Marmur [4].

Sedimentation analysis of DNA and the sedimentation of DNA in CsCl gradient were carried out as previously described [2]. The average sedimentation coefficients $S_{20,w}$ in SSC and in alkaline medium [5] were evaluated.

The absorption melting curves of DNA were measured by the continuous method [6] in 0.1 SSC + $10^{-4}$ M EDTA or in 4 M GHCl + 0.01 M Tris and 0.01 M EDTA. The concentration of DNA in quartz-stoppered cells was 15 μg/ml. The polyphasic character of absorption melting profiles was numerically analysed as described previously [7].

The variation of pH in GHCl solutions was achieved by adding HCl and NaOH as required.

2.2. *Nuclease assays*

The incubation mixture (total volume 0.5 ml) contained 120 μg of calf thymus DNA in the 0.02 M phosphate buffer pH 7.0, 0.02 M $MgCl_2$ and 4 M GHCl. The reaction was initiated by adding of 10 μg of pancreatic DNAse I (Koch- Light) to the incubation mixture. The digest was after dialysis characterized by sedimentation analysis.

## 3. Results and discussion

4 M GHCl has been generally used as a strong denaturing agent of proteins. For isolation of DNA, it was necessary to examine its inertness to DNA and its power to denature proteins especially nucleolytic enzymes.

According to optical rotatory dispersion studies of different proteins in 4 M GHCl [8] the secondary and tertiary structure of proteins is disturbed. Our results reveal that pancreatic nuclease I has no activity in 4 M GHCl, as is indicated by $S_{20,w}$ value of purified calf thymus DNA which remained unchanged. When DNAse I was treated with 4 M GHCl (8 h at room temperature) and dialysed to phosphate buffer, its activity remained as high as that of the control. These experiments indicate that nucleases in 4 M GHCl need not be inhibited irreversibly. The partially purified calf thymus DNA obtained by one-step precipitation with alcohol from nuclear lysate in 4 M GHCl [1] has residual nuclease activity. When this DNA is incubated at 37°C in SSC for 2 hours, $S_{20,w}$ value decreases from 22.6 (that of the control) to 20.8 and alkaline $S_{20,w}$ value from 24.9 to 11 (which indicates single strand breaks). Hence the DNA of the cell lysate must be extensively purified in GHCl solution.

Chloroform–isoamyl alcohol mixture [4] when compared with different organic solvents was found to be most efficient deproteinizing agent. Some of the cell components which were not separated in the

Table 1
Selective precipitation of nucleic acids and dextran from 4 M GHCl solution by different precipitants

| Precipitant | Minimum volume percent of precipitant required for precipitation of: | | | $S_{20,w}$ = of precipitated DNA, disolved in SSC[a] | Percent of precipitated DNA |
|---|---|---|---|---|---|
| | DNA | RNA | dextran | | |
| Methanol | 290 | 220 | 120 | – | – |
| Ethanol | 120 | 180 | 120 | 24.9 | 79.9 |
| Isopropanol | 75 | 140 | 100 | 22.8 | 90.2 |
| Acetone | 80 | 150 | 100 | 20.0 | – |
| Methoxyethanol | 200 | no precipitation | | – | – |
| Ethoxyethanol[b] | 200 | no precipitation | | – | – |

[a] Calf thymus DNA isolated according to Marmur [4], $S_{20,w}$ = 22.6.
[b] DNA does not precipitate in the fibrous form.

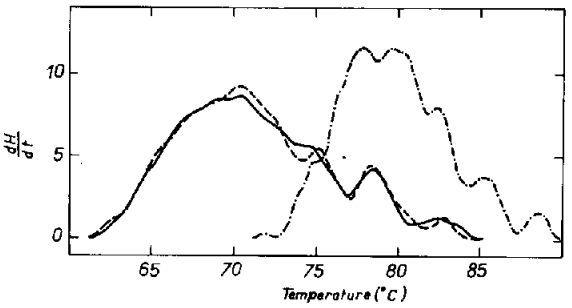


Fig.1. First derivative of the absorption melting curves of calf thymus DNA. Ordinate (dH/dt), first derivative of the smoothed absorption melting curves in percentage hyperchromicity (H) per degree. Hyperchromicity 35–36%. Approx. 80 experimental points of each integral melting curve were approximated at the confidence level $\phi(k) = 0.68$ [7]. DNA was isolated according to Marmur [4], measured in 0.1 × SSC (full line); measured in 4 M GHCl (dot-and-dash line). DNA was isolated in 4 M GHCl, measured in 0.1 × SSC (dashed line).

interphase layer may be separated from DNA by selective precipitants. Table 1 shows the selectivity of precipitation of calf thymus, DNA, RNA and dextrane in 4 M GHCl solution by different organic solvents. Isopropanol and acetone precipitate DNA before dextrane and RNA with maximal yield (about 90%) of native high-molecular-weight DNA. A small amount of RNA which is precipitated together with DNA can be removed by the action of RNAse [4].

GHCl affects the secondary structure of DNA primarily in two ways. Highly dissociable guanidinium ion stabilizes DNA helix in low concentrations (<0.5 M) while its hydrogen bond breaking ability becomes dominant in higher concentrations of GHCl [9]. The absorption melting profile of calf thymus DNA in 0.1 × SSC isolated using 4 M GHCl is the same as the melting profile of DNA prepared according to Marmur [4] in the limits of experimental error. The calf thymus DNA shows also high thermal stability under the conditions of its isolation in 4 M GHCl (fig.1). These experiments confirm earlier observations [10] that 4 M GHCl has no sequence-specific effect on the conformation of DNA. The dependence of the $T_m$ value of calf thymus DNA on the pH in 4 M GHCl (fig.2) indicates the broad range (pH 5.0–8.5) of the high thermal stability of this DNA.

Physico-chemical properties of the DNAs from different sources (table 2) (differing in G + C content and heterogeneity) isolated by the present method are in a good agreement with other isolation procedures [2,4,12]. Good yield of high-molecular-weight DNA was obtained even from Tetrahymena pyriformis which is known to contain an excess of proteins and RNA [13].

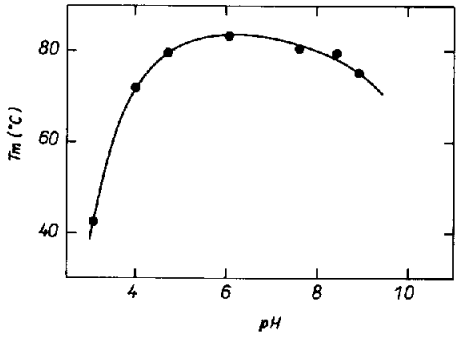


Fig.2. Dependence of the $T_m$ value of calf thymus DNA on the pH in 4 M GHCl solution.

Table 2
Physico-chemical characteristics of various DNAs isolated using 4 M GHCl

| Source of DNA | $S_{20,w}$ | Molecular weight[a] × $10^{-6}$ | $T_m$ (0.1 × SSC) | $\rho$ (g/cm³) |
|---|---|---|---|---|
| Calf thymus | 21.0 | 7.8 | 69.7 | 1.699 |
| *E. coli* | 18.7 | 5.8 | 74.8 | 1.710 |
| *E. coli*[b] | 38.0 | 33.7 | 75.0 | 1.710 |
| Tetrahymena pyriformis | 21.4 | 8.1 | 61.5 | 1.684 |

[a] Calculated from $S_{20,w}$ according to Eigner and Doty [11].
[b] Cells were lysed in the presence of 37% of glycerin.

The isolation procedure of DNAs in 4 M GHCl is characterized by several advantages. 4 M GHCl has strong ability to dissociate nucleoprotein complexes and denature proteins in the highly soluble form. The double helix structure of DNA is stable and resistant to thermal or acido-basic denaturation as well as to the mechanical breakage. The nucleolytic enzymes are inhibited and no further special conditions like low temperature or other inhibitors are required during the whole isolation procedure.

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