DETERMINATION OF THE AMOUNT OF DNA ON NITROCELLULOSE MEBRANE FILTERS

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1. Introduction

In hybridization experiments the determination of DNA present on membrane filters is indispensable for a quantitative interpretation of the results. If no radioactive DNA can be used the estimation of the DNA is generally carried out following an extraction of the filter with diluted perchloric acid (PCA). The amount of hydrolyzed DNA in the extract is then determined with the diphenylamine reagent or calculated from the extinction at 260 nm. Since we had bad experiences with the latter method, we routinely used the colour reaction.

As stated by Burton [1] a twenty minutes incubation time at 70° with 0.5 N PCA is satisfactory for most DNA extractions. Contradictory and confusing results suggested that these conditions give far too low figures in the case of filter extraction. This short report shows that optimum conditions may be realized by some simple, though important modifications. These modifications are the result of experiments in which attention has been paid to the percentage of DNA extracted and to the degradation of DNA as measured by the decrease in colour development with diphenylamine.

2. Materials and methods

2.1. *Extraction procedure*

Denatured ^{14}C-thymine labelled *E. coli* DNA (500 cpm/μ) in 10 SSC* was slowly filtered through presoaked membrane filters. The filters were washed by filtration with 1 ml 2 SSC, dried at 60° and baked *in vacuo* at 80° for 2 hr. Each filter contained about 15 μg DNA. The labelled DNA was a generous gift of Dr. S.O. Warnaar (Laboratory of Physiological Chemistry, University of Leiden, Leiden). Filter types used were: Sartorius Membrane filter type SM 11309 (0.1 μm, 23 mm) and Millipore filter type HA (0.45 μm, 23 mm).

The DNA containing filters were shaken in tightly closed vials at 70° with 1.5 ml 0.5 N, 1.1 N or 1.6 N PCA. After a fixed period of heating the vials were cooled, samples of 0.1 ml or more pipetted off, heating continued, etc.. After the last extraction period the remaining extract was pipetted off, the filters washed at least twice with 1 ml cold diluted PCA, then twice with 1 ml 2 SSC and dried finally at 60°.

The PCA extracts were made up to 1 ml with water mixed with 13 ml toluene–triton X-100 (2:1)–0.4% PPO whereafter the radioactivity was determined. The filters were counted in toluene–0.4% PPO.

* 1 SSC: standard citrate saline buffer containing 0.15 M NaCl, 0.015 M trisodium citrate, pH = 7.3.

3. Results

The measuring results of the extraction procedure are shown in fig. 1. From the experimental data the following conclusions can be derived:

a) A considerable effect of PCA concentration on the rate of extraction is evident. Up to 65% the extraction proceeds about 8 times faster when instead of 0.5 N PCA, 1.6 N PCA is used. Using 1.1 N PCA this factor amounts to about 3.

b) Under equal conditions the extraction of DNA from filters of type SM is slower than from filters of type

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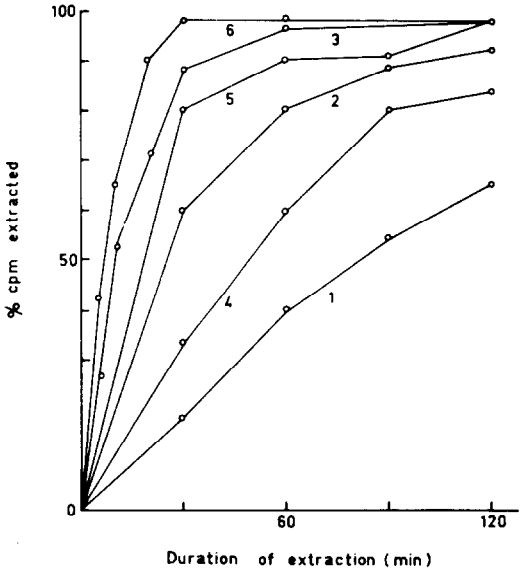

Fig. 1. The effect of PCA concentration on the extraction of DNA (70°) (1, 2, 3) Sartorius membrane filter SM 11309 0.5 N, 1.1 N and 1.6 N PCA, respectively. (4, 5, 6) Millipore filter HA 0.5 N, 1.1 N and 1.6 N PCA, respectively.

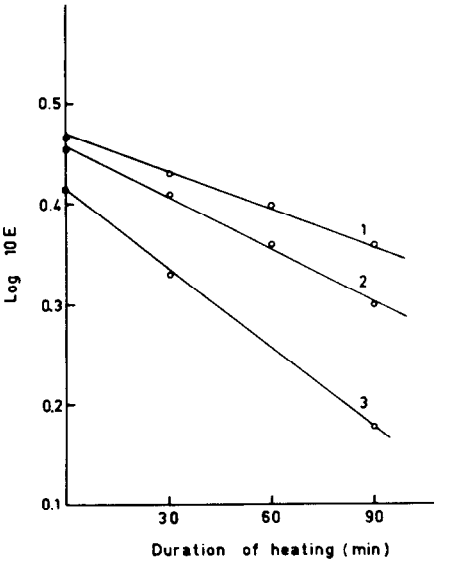

Fig. 2. The effect of PCA concentration on the degradation of DNA (70°) (1, 2, 3) 1.1 N, 1.6 N and 2.1 N PCA, respectively. The straight lines, following the equation $\log E = -kt + C$, were calculated with the method of the least squares.

HA. DNA cannot be extracted quantitatively with 0.5 N PCA within a reasonable period of time. It should be stressed that only a pyrimidine nucleotide was labelled. Therefore it provides an excellent criterion for the quantitative extraction of DNA from membrane filters.

c) After washing the filter rigorously with diluted PCA, the DNA remaining on the filter can still partly be removed with 2 SSC. It might indicate that in the extraction process an important role is played by physical adsorption. This is mainly determined by the molecular weight distribution of the polynucleotides and the adsorption properties of the filter. In this way the differences found between SM and HA filters could be explained.

Some traces of labelled material (2%) cannot be removed from the filters even after a prolonged hot acid extraction. Excluding a possible artifact we may assume the presence of nucleotides bound chemically to the filter surface by the baking process at 80°.

3.1. *Degradation of DNA*

On heating in diluted PCA the DNA moiety responsible for the colour development with diphenylamine is partly degraded. In 0.5 N PCA the degradation at 70° amounts to less than 5% per 20 min [1]. To get an idea of the influence of higher acid concentration on DNA decomposition and on colour development DNA solutions in 1.1 N, 1.6 N and 2.1 N PCA (20 μg DNA per ml) were heated for various periods of time at 70°. After rapid cooling the amount of DNA was determined according to the method of Giles and Myers [2]. The excess PCA was not not neutralized. The colour intensity was measured as the difference $E = E_{260\,nm} - E_{700\,nm}$. In fig. 2 log 10$E$ has been plotted versus the heating time. The decomposition rate was found to be correlated linearly with acid strength: 7.9%, 11.3% and 16.1% per 30 min on heating in 1.1 1.6 and 2.1 N PCA, respectively. The colour development in unheated samples was only seriously influenced by 2.1 N PCA. The extinction is then about 12% lower than for the standard procedure of Giles and Myers (1.1 N PCA). In the case of 1.6 N PCA this effect is negligible (< 3%).

4. Discussion

Knowing the effect of PCA concentration on the extraction rate and on the degradation of DNA in relation

to the diphenylamine reaction we can easily derive the optimum extraction conditions. Of course, these conditions will depend on the specific properties of the system and on one's personal taste. However, we presume that most of the DNA containing filters commonly used have similar characteristics as our SM and HA filters. Moreover, we presume that an extraction degree of 95% will be considered quite acceptable especially because well-chosen reference filters with known amounts of DNA can be used. This percentage can be attained by heating the filter at 70° with 1.6 N PCA for 1 hr in the case of SM-type filters or 30 min in the case of HA-type filters. Checking these conditions with a set of filters containing 25 μg DNA per filter we found extraction with 1.6 N PCA really preferable. When small amounts of DNA have to be detected it is possible to extract with as little as 0.7 ml diluted PCA.

We did not investigate the extraction of the filters with 2.1 N PCA since it would not be of much advantage, if any, because of the lower colour development with diphenylamine.

References

[1] K. Burton, in: Methods in Enzymology XII B (Academic Press, New York, London, 1968) p. 163.
[2] K.W. Giles and A. Myers, Nature 206 (1965) 93.