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Preliminary Notes on the Separation of Nucleic Acids and Proteins by Counter Flow Electrophoresis

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A preliminary account is given of an electrophoretic method using the difference in the migration velocities between the nucleic acids and the most rapid acid proteins and *vice versa* from cell extracts.

It is based on a counter flow of buffer against the direction of the electric migration of the nucleic acids and the acid proteins.

Technical details and some results, including the finding of depolymerase activity in the nucleic acid fraction, are reported.

A separation of proteins and nucleic acids (NA) from cell extracts could be achieved by electrophoresis on long columns stabilized by ethanolized cellulose [1]. The length of the migration path needed to effectuate a separation was 300—320 cm. The diameter of the column was 35 mm, and in a few cases 80 mm (not published). Some material contained in the protein fractions was lost during the fractionation, *e.g.* a neurotoxin from *B. pertussis*. Such long columns gave a too high dilution of the fractions, too much occasion for enzymatic activity, and furthermore work with them was too time consuming.

For these reasons we have tried to replace the long migration path by a steady flow of buffer against the direction of anodically migrating compounds. We call this procedure [2] counter flow electrophoresis (CFE).

The purpose of the present experiments is to test the method especially on freshly made extracts from *E. coli* and to study the small amounts of protein that are met with in the NA. This protein shows depolymerase activity.

METHODS

One condition for a CFE separation is that the NA are in a state permitting them to migrate anodically with a velocity of at least 3 cm/h. It does not always occur in cell extracts, especially not when they contain structures (*e.g.* not disrupted microsomes or virus). Cell extracts that have been made by the use of detergents may cause difficulties due to aggregation, induced by the preparative measures.

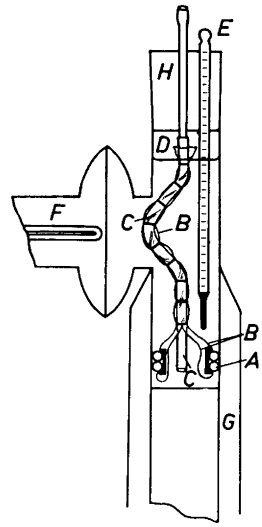

Fig. 1. A: Plastic ring and rubber bearings. B: Dialysis tubing. C: Suction tube. D: Surface of buffer solution and air bubble. E: Thermometer. F: Cooling finger in side tube. G: Cooling jacket. H: Rubber stopper.

The dimensions of the supported columns have been 450 × 35 mm and 33 × 10 mm. Ethanolized cellulose was used as support. The columns and fraction collectors were cooled with running water of + 1°C. The temperature at the top of the columns has been + 9°C during runs at 5 V/cm and 0.03 M M phosphate-0.01 M citrate of pH = 7.4. The electrolyte bathes were kept at + 4°C, and the buffer in them was continually exchanged between the bathes.

The proteins were continuously sucked off from a narrow space above the top of the columns. This space was limited by a plastic ring with rubber

Table 1. Column diameter = 10 mm. Length = 330 mm. Bed volume = 22–23 ml.

Experiment	Sample in mg: Serum albumin	NA	Sum of electrophoretic and counter flow velocities. cm/h.	Recovery in per cent. Protein calculated from the ninhydrin reaction.	NA
		DNA[1]			DNA
1	100	10	3.35	84	31
2	100	10	3.24	81	44
3	100	10	3.25	98	56
4	100	10	3.31	98	74
5	20	20	3.43	100	73
6	—	20	3.26	—	85
		RNA[2]			RNA
7	—	10	2.93	80	104
8		10	2.96	92	93

[1] From calf thymus.
[2] From yeast.

bearings tightly fitting against the ground inner glass wall and a tent of invaginated dialysis tubing (Fig. 1). This is tightly fixed by a plastic cord on the suction tube, the space in the invagination having first been filled with buffer. The suction was effected by micropumps.

In Table 1 some CFE runs are recorded on purified NA and protein. The sum of migration velocity and counter flow of course varies with the bed volume, and that can not be kept constant.

In more recent CFE runs on columns supported by plastic and in other cases by siliconed pyrex beads, where the bed volumes were only about half that of cellulose supports, the sum of velocities was about 6. In these cases the temperature was raised to about + 13°C.

Extracts of *E. coli* using 0.1 M phosphate, pH = 7.4 have been used. The cells were disrupted at −20°C at a pressure of 2.5 tons/cm (Ref. [3]). The cracked cells were extracted at 0°C for two min and immediately centrifuged at 0°C at 150 000 *g* for 30−60 min (horizontally). The pressure on the cracked cells against the bottom of the centrifuge tubes is squeezing out considerably more constituents than are diffusing out by mere extraction. Both DNA and RNA were present in the extracts in about the proportions characteristic for *E. coli* [4]. The supernatant was immediately subjected to CFE.

DISCUSSION AND RESULTS

The *NA* from CFE contains less than 0.1 mg of protein per 100 mg of NA as determined by the ninhydrin reaction after hydrolysis. Serum albumin or glycin were used as standards (Table 2).

Equimolar mixtures of ribose- and deoxyribose-mononucleotides were hydrolysed both in hydrochloric acid and in separate experiments in 88 % formic acid at 170°C. The mononucleotides gave ninhydrin reaction with identical light absorbtion as amino acids from protein. The reaction from proteins, however, was 300−400 times stronger per mg substance than that of the mononucleotides. It may be assumed that the content in the NA fractions of 0.1 % protein is not too low an estimate.

In some experiments $Na_2{}^{35}SO_4$ was included in the medium during the cultivation of *E. coli*. The NA fraction was highly labeled after dilution with inactive sodium sulfate, methionine, cysteine, cystine, and dialysis. Dilution was also made by hydrosulfate and evaporation. These preparations were carried out at + 4°C in 10 times 24 h. The activity in the CFE-protein fraction

Table 2. Column diameter = 10 mm. Length = 330 mm. Bed volume = 22−23 ml.

Experiment	Mg NA in *E. coli* extract. DNA + RNA	Sum of NA-migration and counter flow. cm/h	Protein in mg per 100 mg NA calculated from the ninhydrin reaction.
9	6.7	3.31	—
10	6.0	3.59	0.20
11	6.0	3.45	~0.1
12	6.1	3.37	~0.1

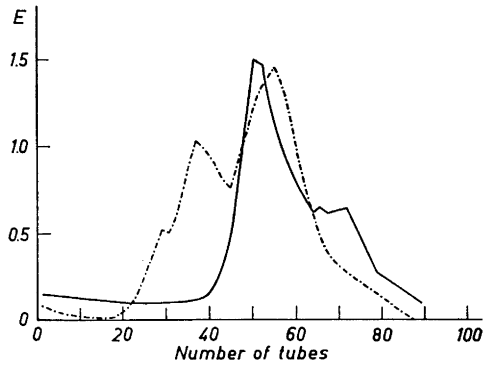

Fig. 2. DNA + serumalbumin. No counter flow. ——— Protein. Light absorbtion. Ninhydrin, 570 mμ. —·—·— DNA. Light absorbtion, 260 mμ. 4 ml per tube.

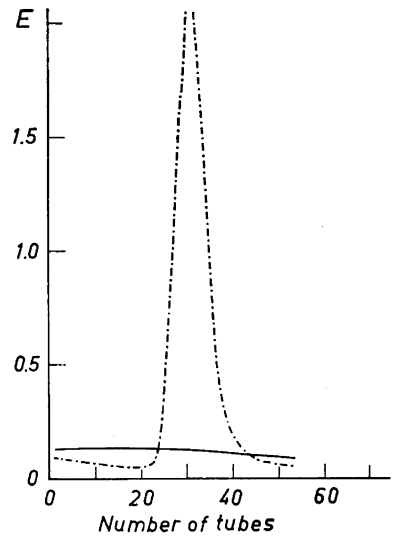

Fig. 3. DNA + serumalbumin. Counter flow. ——— Protein as the ninhydrin reaction at 570 mμ. —·—·— DNA as light absorbtion at 260 mμ. 4 ml per tube.

was about 2.2×10^6 c.p.m./mg protein. After the dilution with H_2S it dropped to 6×10^3 obviously by activation of proteolytic enzymes which have removed sulfur containing amino acids. During the same procedure the activity of the NA fraction was gradually reduced (Table 3).

The protein content in the NA fraction computed from the activities of protein- and NA-fraction has always been higher than the ninhydrin value and variable. In forthcoming experiments the isotope dilution will be made by precipitating the NA out of a mixture of adequate inactive compounds (autolysed NA fraction) (Table 3).

When, as the last step, base analysis was carried out on the assumed 2 700 μg of NA, no bases were found, although the ^{35}S activity was not correspondingly reduced, demonstrating the presence in the NA-fraction of active depolymerases that had not been removed by the CFE.

Table 3.

	Dilution with:		
	Na_2SO_4	S-amino acids	H_2S
$10^{-3} \times$ c.p.m./mg of NA	100	84	50

The reduction in the activity of the depolymerizing enzymes was not greatly less nor more than could be expected from the dilution procedure.

RNase and DNase are in *E. coli* more firmly bound to RNA and DNA than most or all other cell proteins. Considering the instability of NA-preparations a similar arrangement may possibly be expected in other cells. It remains to be seen if this arrangement is more or less general, and if it may have a biological significance.

These preliminary results indicate that the method should be supplemented by unsupported columns, and that lower temperatures are desirable.

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