Uptake of Polynucleotides by Mammalian Cells XV. Properties and Function of a DNA-Protein Complex Situated in the Outer Membrane of Ehrlich Ascites Tumor Cells

P. L. Schell

Medizinische Hochschule Hannover, Institut für Klinische Biochemie und Physiologische Chemie (Geschäftsführender Direktor: Prof. Dr. W. Lamprecht)

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DNA-Membranes, RNA-Uptake, Cells

1) DNA-protein complexes are supposed to be original constituents of the membrane of Ehrlich ascites tumor cells. These complexes can be attacked at the surface of viable cells by DNase or protease. The DNA is partially embedded in protein structures.

2) The net charge of this complex is of major importance for the RNA uptake capacity of the cells. Negatively charged DNA which is situated at the surface hinders RNA uptake. This is the explanation for the stimulation of RNA uptake by DNase or the decrease in RNA uptake after protease treatment.

3) Upon treatment of DNA-deficient complexes with homologous or heterologous DNA the original RNA uptake capacity of the cells is restored but the original conformation of the complex cannot be regained.

4) The DNase action on the complex is temperature dependent in a sigmoidal fashion. It is markedly slowed down at temperatures below 12 °C. This implies that structural changes in the complex occur at this transition temperature which make surface DNA susceptible to DNase. This effect can only be observed in original structures but not in reconstituted ones.

5) Polyanion treatment of the cells [poly(L-lysine)] which increases their RNA uptake capacity, most probably does not interact with the DNA-protein complex. Poly(L-lysine) appears to act at other membrane sites.

6) The DNA-protein complex has been investigated entirely *in situ*, *i. e.* situated in the membrane of viable cells.

Introduction

Membranes of intact mammalian cells are penetrated by ribopolynucleotides which enter the cells without being degraded. This effect apparently depends on specific membrane constituents. Previous experiments suggest that charged residues of the membrane surface are involved [1].

When Ehrlich ascites tumor cells are exposed to Pancreas DNase I, degradation of DNA situated at the surface of the cell membrane occurs [2, 3]. This effect has now been measured by determination of the removed DNA fragments and has been related to an increase in the uptake capacity of the cell for ribopolynucleotides such as poly(I), poly(C), poly(I)·poly(C), poly(U), poly(A) and poly(A)·poly(U) [4].

Experimental data indicate that DNA-protein complexes are components of the outer cellular membrane and that these complexes are involved in ribopolynucleotide uptake.

The protein-DNA complex appears to separate reversibly at temperatures of 5–25 °C. This property was used for a tentative characterisation of the conformation of the complex *in situ*. The data do favour the assumption that membrane DNA is *not* of exogenous origin.

In the course of all these experiments viable cells were used. The cells remained in an intact and viable state.

Methods and Materials

Ribopolynucleotides

Synthetic homoribopolynucleotides were obtained from Schwarz Inc. Double strands were prepared by mixing equivalent amounts of polymers in buffer (pH 7) which consisted of 0.05 M phosphate and 0.06 M NaCl. The mixture was kept at 23 °C for 5 min, and then diluted with the incubation medium [4].

Ehrlich ascites tumor cells and incubation technique [4]

The cells were harvested from NMRI mice. The incubation mixture consisted of Hanks solution (Difco) containing 0.08 M phosphate and radioactively labelled polymer. Each sample contained 1.4×10^7 cells in 0.3 ml. Variations in this volume

(0.2 – 3 ml) did not influence the uptake. The incubation temperature was 0 °C or 30 °C. Removal of cells from the medium was carried out by successive sedimentations ($80 \times g$) from 0.9% NaCl solution and several additional washings on a glass-fiber filter with 0.9% NaCl solution.

Pronase treatment of the cells [5]

The cells were suspended in Hanks solution containing pronase (5 μg/ml) and incubated at 30 °C for 15 min; the pronase was then removed by repeated sedimentations of the cells in 0.9% NaCl solution ($80 \times g$) at 25 °C.

DNase treatment of the cells [2]

The cells were suspended in Hanks solution pH 7 containing pancreas DNase (0.5 mg/ml) (E.C. 3.1.4.5.) and incubated at 30 °C for 30 min; the cells were then washed by two successive sedimentations ($80 \times g$) in 0.9% NaCl solution. The DNase used in these experiments was free from RNase contamination as determined by the method of Thatch [6]. In some experiments cells were also incubated in DNase solution (0.5 mg/ml) at 0 °C for 15 min (Fig. 3). These conditions sufficed to degrade thymus DNA within 30 sec as could be determined by the method of Kunitz [7].

Measurement of DNA fragments in the supernatant

A) Samples containing 4.5×10^7 cells were incubated in DNase solution as described above. The protein was removed from the supernatant with hot trichloroacetic acid and the DNA fragments (deoxyribose) were measured by a quantitative colorimetric assay with diphenylamin under acidic conditions [8].

B) 50 μCi [^{3}H]thymidine was injected intraperitoneally into mice which were inoculated with the tumor 6 days before. The cells were harvested 48 hours after [^{3}H]thymidine application and washed in Hanks solution until the supernatant was free from labelled materials. Samples containing 4.5×10^7cells were incubated in DNase solution and subsequently separated from this medium by centrifugation. The radioactivities of the medium and of the cells were measured. Controls: Identical experiments were carried out with Hanks solution instead of DNase solution. The viability of the cells, the constant amount of cells per volume and the absence of damaged cells were checked.

Poly (L-lysine) treatment of the cells [5]

The cells were suspended in Hanks solution containing poly(L-lysine) (0 – 5 μg/ml) and were incubated at 0 °C for 15 min. The poly(L-lysine) solution was removed by sedimentation of the cells and washing with 0.9% NaCl. The cells were still viable.

DNA treatment of the cells

The cells were suspended in a solution of ascites tumor DNA or calf thymus DNA in Hanks medium pH 7 (0.4 mg/ml), incubated and subsequently removed from the DNA medium by sedimentation ($80 \times g$). The incubation was carried out at 30 °C for 10 min, then at 20 °C for 10 min and finally at 30 °C for 10 min.

Heparin treatment of the cells

The cells were suspended in a solution of heparin in Hanks medium pH 7 (50 μg/ml), incubated at 0 °C for 10 min and subsequently removed from the heparin medium by sedimentation ($80 \times g$).

Treatment of the cells with RNase and phosphodiesterase [4]

Cells exposed to ribopolynucleotides were subsequently incubated for 15 min at 37 °C in a solution of pancreatic ribonuclease (E.C. 2.7.7.16) [4] in Hanks solution (1 mg/ml). Alternatively, the cells were treated with a solution of 20 μg snake venom diesterase (E.C. 3.1.4.1) (Boehringer) in 0.2 ml 0.1 M Tris-HCl, pH 8.

Viability tests for Ehrlich ascites tumor cells

Proliferation: Cells which had been exposed to the experimental conditions were suspended in Hanks medium (10^8 cells/ml) and were injected into mice intraperitoneally. The growth rate of tumor was compared with that of untreated controls. Dye exclusion [9]: cells were suspended in 0.3% Eosin solution. After 2 min the percentage of dyed cells (not viable) was determined.

Results

DNase treatment of Ehrlich ascites tumor cells

Ehrlich ascites tumor cells (1.4×10^7 cells/sample) were preincubated in 0.3 ml Hanks medium, pH 7.2, containing 0.5 mg/ml DNase I (30 °C, 30 min). This medium was removed by centrifugation in 0.9% NaCl solution. Afterwards the cells were exposed to homoribopolynucleotides (Table I). This ribopolynucleotide uptake was carried out in 0.3 ml of a medium which contained ribopolymers of the following radioactivity ^{3}H and concentration: 10^5 dpm/ml and 0.035 μmol/ml. The uptake capacity of the cells for the ribopolymers was stimulated by this DNase pretreatment.

Ribopolynucleotide	Compensation treatment of the cells	Ribopolynucleotide uptake					
		Uptake conditions		Uptake by the cells		Ribopolynucleotides remaining after ribonuclease (phosphodiesterase) — treatment of the cells	
		[°C, min]		[pmol/10^6 cells]		[pmol/10^6 cells]	
poly([^{3}H]U)	—	0	1	62.5	12.0 *	5.0	1.8 *
	—	0	30	274	35.5 *	17.8	3.5 *
	DNA	0	1	16.2	8.5 *	2.5	1.0 *
	DNA	0	30	40.8	32.1 *	6.1	3.6 *
	Heparin	0	1	10.0	13.2 *	0.8	0.2 *
	Heparin	0	30	37.2	38.5 *	4.0	7.5 *
poly([^{3}H]A)	—	0	1	37.6	15.7 *	35.1	10.3 *
	—	0	30	200	38.0 *	189	32.5 *
	—	30	30	350	45.0 *	316	41.1 *
	DNA	0	1	19.1	10.0 *	17.2	8.5 *
	DNA	0	30	46.5	35.0 *	40.5	33.7 *
	DNA	30	30	53.5	41.2 *	48.0	42.2 *
	Heparin	0	1	14.5	16.0 *	10.8	12.0 *
	Heparin	0	30	37.0	34.5 *	33.4	25.1 *
	Heparin	30	30	44.0	46.6 *	45.1	43.9 *
poly(A) · poly([^{3}H]U)	—	0	1	75.6	18.6 *	81.0	15.3 *
	—	0	30	250	41.5 *	235	38.8 *
	—	30	30	376	57.7 *	348	52.1 *
	DNA	0	1	21.0	19.1 *	17.0	15.6 *
	DNA	0	30	42.8	38.6 *	43.6	40.0 *
	DNA	30	30	56.0	54.1 *	51.5	52.5 *
	Heparin	0	1	22.6	24.5 *	20.1	19.8 *
	Heparin	0	30	43.2	39.0 *	45.3	41.3 *
	Heparin	30	30	61.7	56.3 *	56.5	49.7 *
poly([^{3}H]I)	—	0	6	297	54.5 *	108	37.1 *
	—	0	30	320	87.3 *	150	50.0 *
	—	30	30	150	49.8 *	134	41.5 *
	DNA	0	6	156	55.0 *	115	46.3 *
	DNA	0	30	60.3	66.5 *	40.2	48.8 *
	Heparin	0	6	39.2	42.5 *	31.5	35.0 *
	Heparin	0	30	48.5	53.7 *	35.8	44.5 *
poly([^{3}H]C)	—	0	1	60.2	20.8 *	17.5	5.3 *
	—	0	30	121	35.7 *	25.8	8.5 *
	—	30	30	163	55.3 *	31.0	12.5 *
	DNA	0	1	22.1	19.0 *	3.9	2.7 *
	DNA	0	30	39.1	34.6 *	8.4	4.0 *
	DNA	30	30	57.5	51.3 *	14.6	8.1 *
	Heparin	0	1	21.0	19.8 *	—	0.9 *
	Heparin	0	30	35.2	38.2 *	4.2	7.5 *
	Heparin	30	30	58.3	54.5 *	20.0	12.3 *
poly([^{3}H]I) · poly(C)	—	0	1	75.3	42.5 *	12.1	17.0 *
	—	0	30	100	35.0 *	19.0	12.3 *
	—	30	30	88.6	40.7 *	15.8	21.5 *
	DNA	0	1	50.8	32.6 *	11.5	15.6 *
	DNA	0	30	32.0	35.6 *	7.1	15.0 *
	DNA	30	30	48.4	34.0 *	14.3	10.2 *
	Heparin	0	1	44.1	41.0 *	10.0	18.5 *
	Heparin	0	30	36.8	32.5 *	—	—
	Heparin	30	30	49.1	38.8 *	9.2	7.5 *

Table I. Increment in ribopolynucleotide uptake by DNase pretreatment of the cells and compensation for this effect.

The cells (1.4×10^7/sample) were preincubated in 0.3 ml Hanks medium, pH 7.2, containing 0.5 mg/ml DNase I (30 °C, 30 min). This medium was removed by centrifugation in 0.9% NaCl solution. Compensation treatment: The cells were subsequently incorporated in 0.5 ml of Hanks medium, pH 7.2, which contained either thymus DNA (0.4 mg/ml) or heparin (50 μg/ml). Controls were treated with Hanks medium alone. These media were removed by centrifugation in 0.9% NaCl solution. The ribopolynucleotide uptake was carried out in 0.3 ml of a medium which contained ribopolymers of the following radioactivity ^{3}H and concentration: 10^5 dpm/ml and 0.035 μmol/ml. Polynucleotides remaining in the cell were determined by incubation of the cells with either pancreas ribonuclease [poly(U) and poly(C)] or phosphodiesterase (the other ribopolymers of this Table). Poly(U) and poly(C) are also removed by phosphodiesterase. Control: Under these conditions the polymers in solution were digested completely. The uptake is expressed in pmol mononucleotide residues or in pmol mononucleotide pairs per 10^6 cells, respectively. The data are representative for 4 triplicate experiments.

* DNase omitted.

Some polynucleotides were adsorbed to the membrane surface [poly(U) and poly(C)], and others entered the cells [poly(A) and poly(A)·poly(U)]. Poly(I) was also taken up by the cells; approximately 30% entered the cells and 70% were adsorbed at the membrane surface (Table I). Poly(I)·poly(C) was mainly adsorbed at the surface and only small amounts entered the cells (Table I). Apparently it had not been taken up as entity and duplex separation must have occured. Analogous results were obtained in experiments without DNase [4].

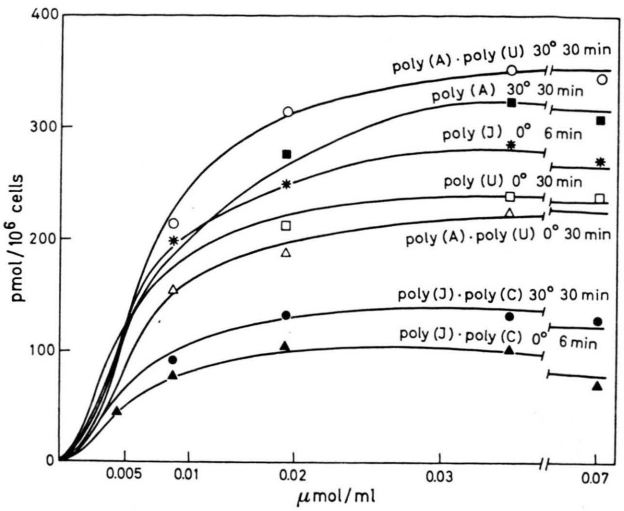
In this article the term uptake includes the irreversible binding of the ribonucleic acid to the cell surface as well as its transport into the cells. Nevertheless we were able to differentiate between these two possibilities by nuclease treatment of cells which were previously exposed to ribopolynucleotides [4] (Table I). The identity of homoribopolymers reextracted from the cells with original material can be demonstrated [4].

The overall result of all these experiments shows that the distribution of applied ribopolynucleotides in the cells is not influenced by DNase pretreatment whereas the general RNA uptake capacity is increased (Table I).

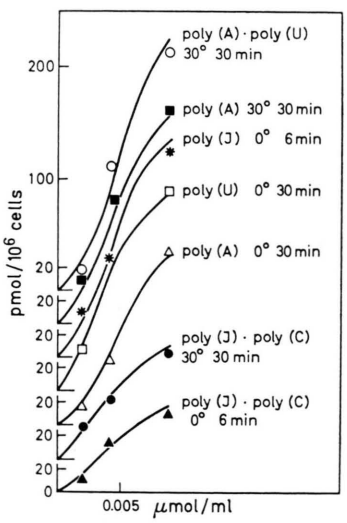
The uptake of ribopolynucleotides by cells pretreated with DNase depends on the ribopolynucleotide concentrations. For each polynucleotide a series of media was prepared which had different polynucleotide concentrations. These media were prepared by dilution of a standard polynucleotide medium with Hanks medium (Fig. 1).

This curve shows the concentration dependence of ribopolynucleotide uptake by DNase treated cells. At concentrations around 4 nmol/ml a sigmoidal deviation from an assumed saturation curve was observed. This implies a maximum of the relative effectivity of uptake at a concentration of 4 nmol/ml.

As a result of DNase-incubation DNA was removed from the membrane surface of the intact cells. Parallel batches of 10^9 cells were each incubated in 2.5 ml DNase solution (a) at 0 °C for 20 min or (b) at 25 °C for 20 min respectively. Control samples consisted of 10^9 cells in 2.5 ml Hanks solution which were incubated at 0 °C for 20 min (c) or at 25 °C for 20 min (d) respectively. After removal of the cells by centrifugation and after deproteinisation, fragments corresponding to 200 μg DNA were obtained from sample (b) in



a)



b)

Fig. 1 a and b. Concentration dependence of ribopolynucleotide uptake in Ehrlich ascites tumor cells pretreated with DNase.
For each curve (1 a) the media were prepared by dilution of a standard polynucleotide medium (0.035 μmol, 10^5 dpm/ml). The concentration of 0.07 μmol was obtained by addition of concentrated non-labelled polynucleotide to the standard medium (these uptake data were corrected). For an individual demonstration of the data points of the left hand parts of the curves and their sigmoidal shapes these parts are repeated separately (1 b). Each point is the average of corresponding values of a triplicate experiment. Each polynucleotide was investigated individually. Reference measurements of the other ribopolymers at a concentration of 0.035 μmol/ml were, however, included in these experiments.

comparison with 10 μg DNA from sample (a), 12 μg DNA from sample (c) and 25 μg DNA from sample (d). For these measurements a quantitative colorimetric assay for DNA fragments (deoxyribose) was used.

These findings correspond with the result of the following experiment. Ehrlich ascites tumor cells were labelled with [^{3}H]thymidine *in vivo*. The cells were harvested after 48 hours and washed in Hanks solution until these supernatants were free from radioactivity. Subsequently the cells (10^9 cells) were incubated in DNase solution. The radioactivity in this supernatant was measured (32000 decomp./min) and related to the radioactivity of the cells (240000 decomp./min). The corresponding values of a blank experiment (incubation in Hanks solution instead of DNase solution) were 3000 decomp./min in the supernatant and 250000 decomp./min in the cells.

Ehrlich ascites tumor cells were incubated in DNase and subsequently in 0.5 ml of Hanks medium, pH 7.2, which contained either thymus DNA (0.4 mg/ml) or heparin (50 μg/ml). Controls were treated with Hanks medium alone. These media were removed by centrifugation in 0.9% NaCl solution. Table I shows that the DNase induced increased RNA uptake capacity of the cells is abolished by DNA. Incubation of the cells in a solution of 50 μg/ml heparin in Hanks solution has an analogous effect (Table I). Apparently any polyanion with a similar anionic strength can substitute for the DNA. The $-SO_3H$ residues of heparin are definitely involved since the effect of heparin can be reversed by treatment of the cells with Ba^{2+} ions (20 μmol $BaCl_2$/ml, 0.9% NaCl, 0 °C, 10 min).

Only polyanions which are adsorbed to the cellular surface have been found to act in this membrane system. It was demonstrated that r i b o polynucleotide doublestrands [poly(A)·poly(U)] cannot replace membrane-DNA. Poly(A)·poly(U), once taken up by the cells, cannot be removed by snake venom phosphodiesterase from the cells, indicating that it has penetrated the cells [4]. This enzyme incubation is carried out under conditions similar to the DNase incubation discussed above. Ribopolynucleotides which are protected against enzyme degradation and removal, therefore, are unlikely to have occupied "empty" positions of the surface nucleoprotein.

Demonstrated in Fig. 2 are data which show the accessibility of DNA and protein to DNase and pronase respectively. DNase treatment of the cells was carried out as described above. The cells were then incubated in pronase solution which afterwards was removed by several washing steps. Final traces of proteolytic activity disappeared after incubation of the cells in Hanks solution (pH 7, 25 °C, 10 min).

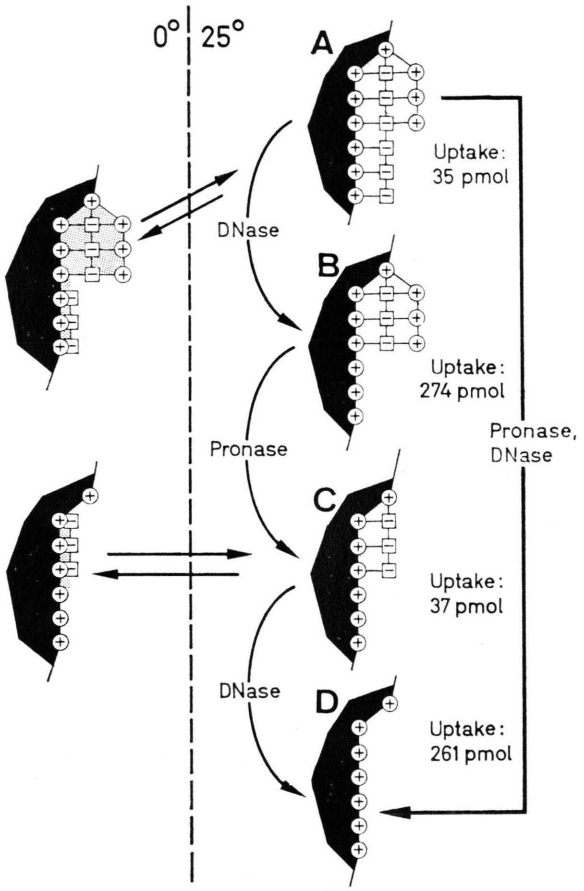


Fig. 2. Schematic representation of the DNA-protein complex at the membrane surface. —⊕—⊕—, Symbol of positively charged protein; —⊟—⊟— symbol of negatively charged DNA. The arrows symbolize enzyme incubations of the intact and viable cells. The black areas represent unspecified membrane material and the gray areas represent a tight configuration which withstands the attack of DNase. The scheme is based on data which demonstrate the accessibility of DNA and protein to DNase and pronase respectively. The modifications shown in the 0 °C section are not accessible to DNase action. DNase treatment of the cells was carried out as described in Table I. The cells were then incubated in pronase solution which afterwards was removed. These cells (C) were finally incubated with DNase to yield cells of type D. The uptake capacity of each modification is demonstrated by the uptake values of poly(U) at 0 °C, 30 min expressed in pmol/10^6 cells (data partly from Table I). Data obtained for other ribopolynucleotides: poly([^{3}H]A) A: 38, B: 200, C: 36, D: 176; poly([^{3}H]I)·poly(C) A: 35, B: 100, C: 41, D: 87 pmol/10^6 cells at 0 °C, 30 min.

By this treatment the original uptake capacity was regained. These cells (2C) were finally incubated with DNase to yield cells of type 2D which again show an increased uptake capacity. The uptake capacities of each modification were demonstrated by the uptake values of poly(U) at 0 °C, 30 min expressed in pmol/10^6 cells (data partly from Table I). Data obtained for other ribopolynucleotides; poly([^{3}H]A) A: 38, B: 200, C: 36, D: 176. Poly([^{3}H]I)·poly(C) A: 35, B: 100, C: 41, D: 87 pmol/10^6 cells at 0 °C, 30 min. This experiment was repeated with [^{3}H]thymidine labelled cells and the release of [^{3}H]DNA fragments from the membrane surface of the cells was measured. From 10^9 cells 35000 decomp./min (control: 3100 decom./min) were released during the initial DNase incubation. During the final DNase incubation 12000 decomp./min (control: 2000 decomp./min) were released. The residual cells carried 220000 decomp./min.

It is possible to convert cells of type (A) to cells of type (D) via another route. An initial protease treatment is followed by a subsequent DNase treatment.

The viability of the cells under those extreme conditions was checked by dye exclusion [9] and their proliferation behaviour in mice.

The temperature dependence of the DNA-protein complex

At 0 °C membrane DNA *in situ* is not attacked by DNase under conditions which otherwise suffice to degrade DNA at 0 °C. At 25 °C the membrane DNA is easily degraded. This was shown by the determination of DNA fragments in supernatants which were derived from a 0 °C-incubation and from a 25 °C-incubation. Fragments corresponding to 200 μg DNA were obtained from the 25 °C sample in comparison with 10 μg DNA from the 0 °C sample.

Accordingly cells exposed to DNase at 0 °C do not show any increase in RNA uptake whereas cells exposed to DNase at 25 °C do. Ehrlich ascites tumor cells were incubated in DNase solutions at the temperatures indicated in Fig. 3. Subsequently the DNase solutions were removed from the cells at 0 °C. This was followed by an incubation of the cells in poly([^{3}H]U)-solution at 0 °C for 15 min. The uptake data corresponding to each DNase preincubation are shown in Fig. 3 A.

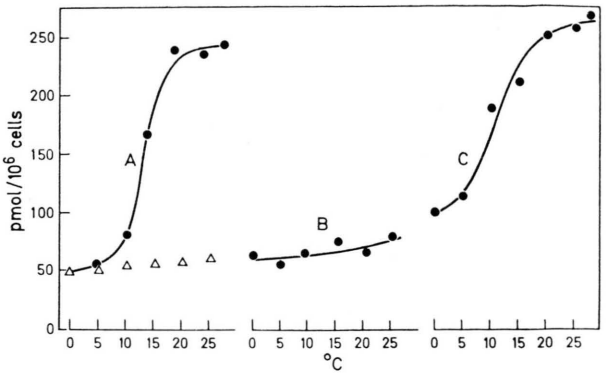


Fig. 3. Relation of poly(U) uptake and DNase pretreatment of the cells at different temperatures. (A) Cells were incubated with DNase (0.5 mg/ml) at the temperatures indicated in the diagramm and then with poly([^{3}H]U) at 0 °C as described in the legend of Table I (—●—●—). Control: The experiment was performed without DNase (—△—△—). (B) The cells were treated with DNase and with DNA as described in Table I and then washed with 0.9% NaCl solution. Afterwards the cells were incubated with DNase (0.5 mg/ml) at the temperatures indicated in the diagramm. Finally the cells were incubated with poly([^{3}H]U) at 0 °C as described in Table I. (C) The cells were treated with DNase and pronase as described in Fig. 2 C. This procedure was followed by DNase incubation at the temperatures indicated and a final poly([^{3}H]U) incubation at 0 °C.

An analogous procedure was carried out with cells which in a previous step were treated with DNase and subsequently with DNA (Fig. 3 B). Fig. 3 C shows the results of an experiment which was carried out with cells pretreated with DNase and subsequently with pronase.

The combined poly(L-lysine) and DNase treatment of the cells

DNase incubation of the cells was combined with poly(L-lysine) incubation (Table II). The DNase treatment of the cells (0.5 mg/ml DNase, 30 °C, 30 min) was followed by a treatment with poly(L-lysine) of different concentrations (0, 1, 2, 5 μg/ml, 0 °C, 10 min). Thus an overloading with poly(L-lysine) was accounted for.

The data imply that poly(L-lysine) does not interact with DNA or compete with membrane proteins for this DNA since the poly(L-lysine) stimulation does not depend on the presence of DNA.

The experiment was also carried out in the reversed fashion. The cells were treated with poly(L-lysine) and afterwards with DNase. A subsequent incubation (0 °C, 30 min) of the cells with poly([^{3}H]U) (0.035 μmol/ml and 10^5 dpm/ml)

Table II. Uptake of polynucleotides by cells pretreated with DNase and poly(L-lysine).

The cells were pretreated with DNase (0.5 mg/ml) at 30 °C for 30 min, washed once and were then treated with poly(L-lysine) (0−5 μg/ml) in Hanks solution at 0 °C for 10 min. In another experiment the cells were pretreated with poly(L-lysine) and then treated with DNase. Control: Incubations were performed without poly(L-lysine) or DNase. A subsequent incubation (0 °C, 30 min of the cells was carried out in a medium which contained ribopolymers at the following radioactivity ^3H and concentration: 0.035 μmol/ml and 10^5 dpm/ml. The uptake is expressed in pmol mononucleotide residue or in pmol mononucleotide pairs per 10^6 cells respectively.

Polynucleotide	Pretreatment	Uptake of polynucleotides by the cells at the following poly(L-lysine) concentrations during pretreatment	
		2 μg/ml poly(L-lysine) [pmol/10^6 cells]	5 μg/ml poly(L-lysine) [pmol/10^6 cells]
poly([^{3}H]A)	DNase	200	200
	poly(L-lysine)	105	150
	DNase and subsequently poly(L-lysine)	325	365
poly([^{3}H]U)	DNase	260	260
	poly(L-lysine)	120	210
	DNase and subsequently poly(L-lysine)	350	430
	poly(L-lysine) and subsequently DNase	378	445
poly(A) · poly([^{3}H]U)	DNase	257	252
	poly(L-lysine)	260	387
	DNase and subsequently poly(L-lysine)	517	630
poly([^{3}H]I) · poly(C)	DNase	110	110
	poly(L-lysine)	200	320
	DNase and subsequently poly(L-lysine)	326	465

was carried out. The uptake data are shown in Table II. Again the two stimulating effects corresponded to the sum of the single stimulations. This implies that poly(L-lysine) is not integrated with the DNA-protein complex like the scheme in Fig. 2 B where protein is thought to protect the DNA-moiety from the action of DNase.

Both results suggest that poly(L-lysine) is adsorbed onto the celluar membrane[5] at a site apart from the DNA-protein complex.

Conclusion

A DNA-protein complex at the membrane surface of Ehrlich ascites tumor cells appears to be responsible for the RNA uptake capacity of the cells. Some ideas both about the structure and a temperature dependent conformational change of this complex were derived from experiments in which the RNA uptake capacity of the cells was influenced by preincubation of the cells with enzymes.

Alternating treatment of the cells with DNase and protease result in an alternating increase and decrease of the RNA uptake capacity. Illustrated in the 25 °C section of Fig. 2 are schematic models of these experimental modifications of the DNA-protein complex.

DNase treated cells (Fig. 2 B) which have lost DNA from their surface and so have an increased capacity for RNA uptake were treated with protease to yield cells which again had the low uptake capacity for RNA (Fig. 2 C). Apparently another part of membrane DNA was uncovered by the protease. Cells with protein-DNA complexes dismantled in this fashion show the same uptake capacity as untreated cells (Fig. 2 A) which still posses their original "open" surface DNA. Cells of type (C) and cells of type (A) act analogously in still another aspect; DNase treatment brings about an increase of the RNA uptake capacity and the appearance of DNA fragments in the supernatant (C → D).

Therefore, RNA uptake effects should not be ascribed to the particular protein- or DNA moiety alone. The results rather imply a structural arrangement between the two partners which results in a mutual shielding of their respective charges. It is this net charge which is thought to be responsible for the RNA uptake effects. Besides these specific charges, their distribution and interaction an additional experimental parameter was found which could be used more directly in an elucidation of the DNA-protein complex structure.

It can be demonstrated that the DNA-protein complex *in situ* has different conformations at 0 °C and 25 °C. This effect is reversible (double arrows in Fig. 2) since cells which undergo such temperature changes are indistinguishable from those which did not. Apparently at 0 °C the membrane conformation brings about a shielding effect which prevents enzymatic degradation of membrane DNA

and we propose that protein moieties adjacent to the DNA might be directly involved. This shielding effect does not exist at temperatures around 25 °C.

Cells suspended in identical DNase solutions were incubated at defined temperatures within the temperature range of 0 – 25 °C. Subsequently the RNA uptake capacity of each sample was determined by incubation with poly([^{3}H]U) at 0 °C (Fig. 3 A). In this experiment the temperature of DNase preincubation was the only variable parameter which could influence the uptake capacity. Thus it was possible to stop and screen each temperature-induced degree of exposure of membrane DNA to DNase between 0 °C and 25 °C. Fig. 3 A shows that the relation between poly(U) uptake at 0 °C and the temperature of DNase treatment of the cells, is a sigmoidal curve. It seems the exposure of the membrane DNA to the DNase is the result of temperature dependence of the conformation which might be a conformational change. Tentatively the expression "melting" will be introduced for this process in this paper. The scheme shown in Fig. 2 and the data in the sigmoidal curve in Fig. 3 A are both based on the same cell function, namely RNA uptake.

A reaction enthalpy of the transition 0°-configuration $\rightleftarrows$ 25°-configuration might be evaluated from the curves in Fig. 3. From the data in Fig. 3 A it was determined that the value of this enthalpy is of the order of magnitude of 50 kcal (evaluation method from Riesner and Römer [10]). This procedure is based on the model proposed in Fig. 2 and on the assumption that the transition is a true or non effect.

Any temperature dependence different from that shown in Fig. 3 A most likely indicates that the membrane components have a different conformation. Fig. 3 compares DNA-protein complexes in the membranes of three classes of cells which had the same uptake capacity for homoribopolynucleotides they are described in the legend of Fig. 3. The curves (A) and (C) in Fig. 3 indicate the structural analogy of DNA-protein complexes (A) and (C) in Fig. 2. Fig. 3 B indicates a different structure; DNA appears to be more tightly bound in the reconstituted complex.

This temperature-conformation relationship, *i. e.* the thermostability of the DNA-protein complex appears to be a characteristic molecular quality. It was used to show the structural identy of different regions of the DNA-protein complex [(A) and (C) in Fig. 2]. The different conformation of DNA-protein complexes which were reconstituted artificially is a strong argument for our assumption that membrane DNA is an original constituent of the cellular surface whereas exogenous DNA can integrate into another more thermostable form and would correspond with curve 3 B.

Two other observations favour also our assumption that exogenous DNA is not part of the membrane DNA-protein complex.

(a) Only high concentrations of DNA bring about a re-constitution of the complex [4] although the general adsorption rate and relative adsorption effectivity of DNA increases with dilution [2].

(b) DNase treated cells have been incubated with DNA under annealing conditions (temperature gradients of 30 °C – 10 °C); no effect on the melting curve B in Fig. 3 could be observed.

In Fig. 2 we have proposed that the protein component of the DNA-protein complex has a high proportion of positive charge [11]. This protein could be compared with poly(L-lysine) which can be incorporated artificially in the membrane surface where it shows activities similar to those of the complex protein [5, 12]. Apparently poly(L-lysine) is incorporated, however, at sites different from the protein-DNA complex.

The uptake of single stranded and double stranded RNA species by DNase treated cells takes place without chain breaks. Such treatment of the cells might have applications in virological experiments. The ribopolynucleotides used in this investigation had chain lengths comparable to those of virions [13, 14]. On the other hand the increased uptake capacity of DNase treated cells for poly(I) · poly(C) might be of interest with respect to interferon stimulation.

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