POLYNUCLEOTIDES ADSORB ON MITOCHONDRIAL AND MODEL LIPID MEMBRANES IN THE PRESENCE OF BIVALENT CATIONS

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1. Introduction

Transfer of polynucleotides across the membrane was demonstrated for prokaryotic [1] as well as for eukaryotic cells [2]. This process involves adsorption of polynucleotides on the cell surface, penetration and release into the cell. The mechanism of crossing the hydrophobic barrier formed by protein–lipid membrane by large hydrophilic molecule is unknown. In particular, the nature of bonds which provide adsorption of polynucleotides on the membranes is unknown. Investigation of this problem with cells is difficult due to the presence of more or less expressed cell wall. Mitochondria can be used as convenient model objects to this end because their outside surface is a 'pure' protein–lipid membrane. There exist some experimental data suggesting that RNA can penetrate the mitochondrial membrane. Polynucleotides added to mitochondria stimulate protein synthesis by mitochondrial ribosomes insensitive to exogenous ribonuclease [3–6]. Mitochondria contain tRNAs which are coded by the nuclear genome and therefore have to be transferred into mitochondria from the cytoplasm [7].

We demonstrate here that polynucleotides are adsorbed by mitochondrial membranes as well as by liposomes formed either from total mitochondrial lipids or from phosphatidylcholine. The dependence of this adsorption on the presence of Mg^{2+} permits us to suggest that this interaction is due to action of bivalent cations as crosslinks between phosphate residues of polynucleotides and phosphate residues of the membranes.

2. Materials and methods

Polyuridylic acid and tRNA from *E. coli* were products of Special Bureau of Design and Technology of Biologically Active Substances (Novosibirsk, USSR). [^{14}C]Phenylalanine 200 Ci/mol (CSSR); sodium [^{3}H]borohydride 1000 Ci/mol and [^{3}H]glucose 150 Ci/mol from Amersham (England). Rat liver DNA was a gift of Dr G. M. Dymshits, [^{14}C]DNA from *E. coli* (6 Ci/mol) of Dr T. E. Vakhrusheva. Both DNA preparations were sonicated to mol. wt ~500 000. [^{14}C]RNA (8 Ci/mol) was a transcript of T7 DNA was given by Dr G. L. Dianov. Heptaadenylic acid was obtained from polyadenylic acid as in [8]. [^{3}H]Heptaadenylic acid (1.2 Ci/mol) was obtained by oxidation of the oligonucleotide with $NaIO_4$ following by reduction with NaB^3H_4 [9]. [^{14}C]Phenylalanyl-tRNA was obtained as in [10]. Mitochondria and mitoplasts were isolated from rat liver as in [11]. Concentration of protein in mitochondria was determined by biuret reaction [12].

Binding of polynucleotides to mitochondria and to mitoplasts was studied using the following mixture (1 ml): 5 mg protein of mitochondria or mitoplasts, 2 nmol [^{14}C]RNA or 1 nmol [^{14}C]DNA in the buffer contained 70 mM sucrose, 220 mM D-mannitol, 0.5 mM EDTA, 20 mM sodium succinate, 2.5 mM potassium phosphate, 25 mM magnesium chloride, 0.5 mg bovine serum albumin and 2 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (Hepes), pH 7.4. The mixture was incubated for 15 min at 25°C. The complex formed was tested by the following method: 0.1 ml aliquots of reaction mixture were

diluted 10-fold by buffer for binding, centrifugated for 2 min using an Eppendorf 5412 centrifuge, the pellets were suspended in 5% perchloric acid and radioactivities of acid-insoluble fractions were measured.

Phosphatidylcholine was isolated from hen eggs and purified on aluminium oxide as in [13]. Purity of the lipid was tested by thin-layer chromatography on silica gel. The total mitochondrial lipids were isolated as in [14]. Liposomes were prepared as in [15] in 0.002 M Tris–HCl, pH 7.5 at 1 mg/ml lipid. Prior to use, liposome suspensions were centrifuged for 10 min at 15 000 rev./min and the pellets discarded. Liposomes containing [^{3}H]glucose were prepared in the same way, but before sonication radioactive glucose was added to 100 000 cpm/ml. Liposomes were separated from free glucose by gel-filtration on Sepharose-6B.

Binding of radioactive oligo- or polynucleotides with liposomes was performed in 0.002 M Tris–HCl, pH 7.5 at 0.1 mg lipid/ml liposomes. Concentration of nucleotides varied between 1 μM and 10 μM. The mixtures were incubated for 10 min at 25°C and centrifuged for 3 h at 90 000 × g. Radioactivities and lipid amounts in the pellets were determined. The effect of polynucleotides on the turbidity of liposome suspensions was studied using a recording spectrophotometer Spekol (GDR). Polynucleotide solutions were added to liposome suspensions in 1 cm spectrophotometric cell, and turbidities were measured at 520 nm. [^{3}H]Glucose retained by liposomes after different kinds of treatment was measured by counting the radioactivity of liposome fraction by gel-filtration on Sepharose-6B.

3. Results and discussion

The centrifugation of the mixture of radioactive polynucleotides with mitochondria or mitoplasts in the presence of Mg^{2+} revealed that particles form stable complexes with the labeled polynucleotides. The complexes do not dissociate after extensive washing by buffer solution. Ultracentrifugation of such complexes in sucrose gradient (0.1–1.4 M) showed that the peaks of radioactivity associated with polynucleotides coincide with those of the optical density of mitochondria or mitoplasts. The treatment of the complexes with nucleases caused loss of radioactivity from mitochondrial suspension, suggesting that polynucleotides adsorb on the membrane surface. The formation of complexes between mitochondria and polynucleotides is an energy-independent process. Addition of a respiration inhibitor (sodium azide) or of an uncoupling agent (dinitrophenol) to the reaction mixture does not change the extent of the polynucleotide binding (table 1).

The possibility of formation of a complex of DNA or RNA with mitochondria depends on the presence of Mg^{2+} in the reaction mixture (fig.1). Increase in the concentration of univalent ions to 0.25 M leads to suppression of complex formation. Addition of NaCl up to 0.25 M to a preformed complex results in its dissociation. DNA and RNA compete in complex formation thus suggesting that the nature of the polynucleotide is not important for interaction with mitochondria (table 1). Moreover, binding of radioactive polynucleotides with mitochondria is efficient-

Table 1
Binding of radioactive polynucleotides with mitochondria

Additions	pmol nucleotide/mg mitochondrial protein	
	[^{14}C]RNA	[^{14}C]DNA
–	270	700
Sodium azide, 10^{-3} M	260	730
Dinitrophenol, 10^{-5} M	260	870
DNA, 5×10^{-6} M	96	–
Heparin, 0.04 mg/ml	80	112
NaCl, 0.25 M	22	90

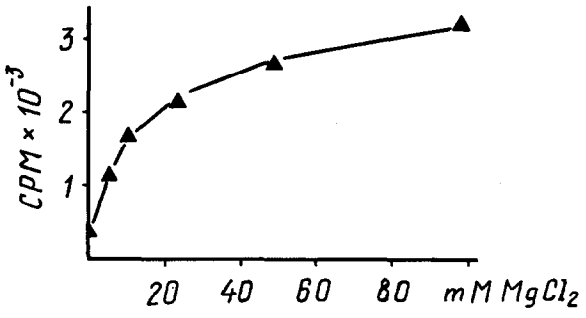

Fig.1. Efficiency of [^{14}C]DNA binding with mitochondria as a function of $MgCl_2$ concentration.

ly suppressed by an acidic polysaccharide heparin. These results lead us to a conclusion that binding of polynucleotides by mitochondria is presumably due to interaction between their anionic groups and the anionic groups of mitochondrial membrane via Mg^{2+} bridges. In order to find out what particular class of membrane components is responsible for the binding of polynucleotides, we isolated total mitochondrial lipids and studied the interaction of liposomes prepared from such lipids with [^{14}C]DNA. It was found that polynucleotides are bound by such model membranes which contain no protein. Binding is stimulated by Mg^{2+} and suppressed by Na^+ (table 2). The similarity of the behaviour of such liposomes with that of intact mitochondria suggests that it is the lipid component of mitochondrial membrane which is responsible for the binding of polynucleotides.

Polynucleotides are also bound by artificial membranes obtained from phosphatidylcholine. Oligo- and

Table 2
Binding of [^{14}C]DNA with liposomes prepared from total mitochondrial lipids

Conditions	pmol nucleotide/mg lipid
2 × 10^{-3} M Tris–HCl	780
2 × 10^{-3} M Tris–HCl 2.5 × 10^{-3} M $MgCl_2$	2600
2 × 10^{-3} M Tris–HCl 2.5 × 10^{-3} M $MgCl_2$ 0.5 M NaCl	390

polynucleotides added to the phosphatidylcholine liposomes form stable complexes in the presence of 0.01 M $MgCl_2$ as revealed by ultracentrifugation. Such complexes are not formed in the absence of Mg^{2+} (table 3). The dependence of the extent of [^{14}C]DNA binding by liposomes on the $MgCl_2$ concentration is shown in fig.2. Similar results were obtained also with $CaCl_2$. Extent of the binding of polynucleotides depends on the ratio of bi- and univalent ion concentrations (fig.2).

As seen from table 3, the formation of polynucleotide complex with liposomes depends on the polynucleotide length.

It is noteworthy that binding of long polynucleotides (DNA, RNA and polyuridylic or polycytidylic acids with mol. wt ~300 000) causes a significant increase of the turbidity of liposome suspension which is presumably due to liposome aggregation. This aggregation is reversible. Addition of 0.015 M EDTA leads to a sharp decrease of the turbidity down to the initial value.

Table 3
Binding of radioactive oligo- and polynucleotides with phosphatidylcholine liposomes

Polynucleotides	Binding (mmol nucleotide/mol lipid)		
	Without additions	10^{-2} M $MgCl_2$	10^{-2} M $MgCl_2$ 0.5 M NaCl
[^{14}C]DNA	0.14	13.2	0.5
[^{14}C]RNA	2.20	28.0	3.5
[^{14}C]Phe-tRNA	1.30	23.8	8.1
[^{3}H](pA)$_7$	0.28	2.0	0.35
[^{14}C]AMP	0.07	0.1	0.12

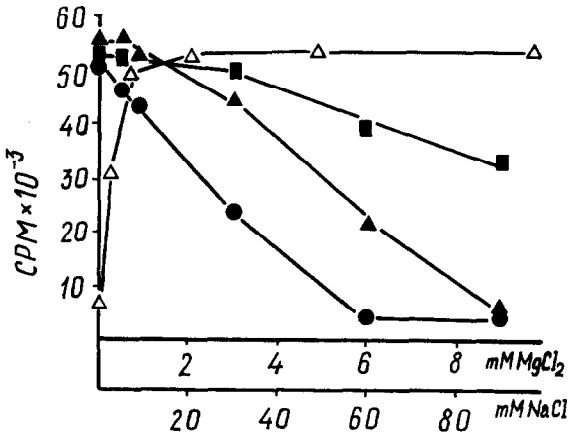

Fig.2. Dependences of the extent of [^{14}C]DNA binding by liposomes on the $MgCl_2$ concentration (△–△) and on the concentration of NaCl at predetermined concentrations of $MgCl_2$: 5 mM (●–●), 10 mM (▲–▲) and 20 mM (■–■) of $MgCl_2$.

No destruction or rearrangement of liposome membrane occurs when its complex with polynucleotides is formed. This conclusion is based on the fact that liposomes which contain radioactive glucose do not lose it during binding of DNA and subsequent dissociation of the complexes on addition of EDTA (table 4).

It is possible that the adsorption on the lipid component of the membrane is the first and necessary step in the transfer of polynucleotides through membrane. In this case the factors increasing the adsorption must increase transmembrane transfer. According to our data, adsorption of polynucleotides on membranes is mostly due to bivalent cations. Evidently, polycations should give a similar effect. Indeed, it is well known that Ca^{2+} and polycations dramatically increase the efficiency of the transformation of bacterial cells and of the transfer of nucleic acids into eukaryotic cells [16].

Table 4
Retaining of [^{3}H]glucose in liposomes at different treatments at 25°C

Conditions	Radioactivity (cpm)
–	7880
0.1 mg DNA	8300
10^{-2} M $MgCl_2$ in 5 min 1.5×10^{-2} M EDTA	6020
0.1 mg DNA, 10^{-2} M $MgCl_2$ in 5 min 1.5×10^{-2} M EDTA	7280
10 min at 65°C	790

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