Loading of Human Red Blood Cells with DNA and RNA
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Erythrocytes, Trapping, Gene Transfer, Hemolysis, SV40 DNA

Human erythrocytes were suspended in Hank's solution containing mammalian or viral DNA or RNA. After dialysis at 0 °C first against water and subsequently against Hank's solution, and a further incubation at 37 °C, the erythrocytes were found to be loaded with the nucleic acids.

The nucleic acid trapped in the erythrocytes exhibited up to 35 per cent of the external concentration.

Introduction

Human red blood cell (RBC) membranes become temporarily permeable for macromolecules and submicroscopic particles in a hypotonic surrounding1-4 or in strong electric fields5-6. Some authors have recently shown that this phenomenon can be used for trapping enzymes or drugs inside RBC. Such loaded cells, when administered (by transfusion) to patients, could provide a novel therapy for instance in cases of enzyme deficiencies. Furthermore, by fusion of loaded RBC with animal cells, a transfer of biologically active substances should be possible7-8.

In this paper we show that human RBC can also be loaded with viral or cellular genetic material. First we measured the time course, during dialysis of RBC, of the efflux of hemoglobin from RBC and the simultaneous trapping by RBC of nucleic acids. Then we showed that viral DNA or RNA of considerable molecular weight is trapped, resistant to the action of nucleases, inside of RBC.

Methods and Materials

$^3$H-labeled nucleic acids

$^3$H-cell RNA from primary African green monkey kidney (AGMK) cells: Monolayers were labeled with 70 $\mu$Ci x ml$^{-1}$ of [5,6-$^3$H]-uridine for a 3 h period. Total cell RNA was isolated according to Scherrer and Darnell9.

$^3$H-SV40 cRNA was prepared by in vitro transcription of SV40 DNA, form 1, with RNA polymerase from E. coli10, using enzyme from Boehringer Co., Tutzing. The majority of the transcript sedimented at 15S in formaldehyde sucrose gradients11, corresponding to 1.5 x 10^6 dalton or one full-length transcript.

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with Permablend III (Packard) (15 ml). Radioactivity was determined in a liquid scintillation counter.

Estimation of the size of $^{3}$H-DNA and -RNA trapped in RBC

The loaded RBC (100 $\mu$l) were lysed with NP40 detergent (20%, 5 $\mu$l). After incubation for 5 min, the lysed cells were layered on top of a linear sucrose gradient [sucrose 5 to 20% (w/v); 0.5% (w/v) SDS; 0.15 M NaCl; 0.05 M Tris-HCl; pH 7.5] and centrifuged in a Beckman rotor SW 56 at 50 000 rpm for 4 h (20 °C). Markers were $^{3}$H-SV40 DNA, form 1 (21S) and yeast tRNA (4S).

Results

Decrease of osmolarity and efflux of hemoglobin in an RBC suspension during dialysis against water

Washed RBC (100 $\mu$l cell volume) were suspended in Hank’s solution (200 $\mu$l) and dialysed at 0 °C against water in glass tubes closed with a dialysis membrane as described in “Materials and Methods”. Several batches of RBC were run in parallel, and at different periods of dialysis aliquots were drawn from the dialysis tubes. The osmolarity of these probes was measured by determination of freeze point depression (Micro-Osmometer, Fa. Knauer, Berlin).

The RBC remaining in the tube were subsequently dialysed against cold Hank’s solution (15 min) and washed three times by centrifugation with Hank’s solution (28 000 × g; 25 °C; 5 min). Aliquots (20 $\mu$l) from the RBC pellets were lysed with water (5 ml) and hemoglobin extinction (414 nm) was assayed in the 28 000 × g-supernatant. The time course of decrease of osmolarity and the efflux of hemoglobin from the RBC after dialysis is shown in Fig. 1. We found that during a 15-min dialysis (against water) the osmolarity had decreased from 290 to 60 mOsmol, and the RBC had lost 90 percent of their hemoglobin. The dotted line in Fig. 1 shows increase of osmolarity during a subsequent dialysis against Hank’s solution.

Uptake of different nucleic acids by RBC during dialysis as measured by resistance to nuclease.

In most experiments, 100 $\mu$l of RBC were suspended in 200 $\mu$l of Hank’s solution (total volume 300 $\mu$l) containing the $^{3}$H-labeled nucleic acids and were dialysed (as described in “Methods and Materials”), first against ice-cold water for various time periods and subsequently against cold Hank’s solution for 15 min. During these steps, RNA becomes trapped inside of the RBC. Uptake of RNA was measured as described in “Methods and Materials”. The efficiency of uptake was markedly increased when the loaded RBC were incubated further for a 90-min period at 37 °C. This is shown for a trapping experiment with $^{3}$H-AGMK cell RNA in Fig. 2.

Uptake efficiency can be expressed as the ratio of the concentration of $^{3}$H-RNA trapped in the pellet of washed RBC at the end of the experiment to the $^{3}$H-RNA concentration in the total dialysis volume before dialysis. Thus trapping efficiency can be defined as $^{3}$H-RNA concentration quotient × 100.

Nuclease resistance of nucleic acids trapped in RBC

To make sure that $^{3}$H-RNA is trapped inside of the RBC, loaded RBC were incubated with bovine

Fig. 1. Osmolarity and efflux of hemoglobin from RBC during dialysis. For explanation see text.

Fig. 2. Trapping of $^{3}$H-AGMK RNA (45 $\mu$g; 1.2 × $10^6$ cpm) within RBC (100 $\mu$l sediment) in a dialysis mixture (total 300 $\mu$l) with (■) and without (○) final incubation at 37 °C (90 min).
ribonuclease ($50 \mu g \times ml^{-1}$; $37 ^\circ C$). After washing twice we found that up to 90 per cent of $^3H$-RNA had been trapped nuclease-resistant. Similar results for uptake and resistance to nuclease were obtained when further nucleic acids up to a molecular weight of $25 \times 10^6$ daltons (T3 phage DNA) were trapped in RBC (Table I).

Table I. Trapping of $^3H$-DNA and -RNA in RBC.

<table>
<thead>
<tr>
<th>Nucleic acid</th>
<th>Trapping efficiency [%] with final incubation at $37 ^\circ C$</th>
<th>without incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGMK cell RNA</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td>SV40 cRNA</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td>SV40 DNA, form 1</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>SV40 DNA, form 2</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>T3 DNA</td>
<td>30</td>
<td>1</td>
</tr>
</tbody>
</table>

Influence of nucleic acid size on trapping efficiency

When the molecular size of nucleic acid was compared in the incubation mixture and in the final RBC cell mixture, it was found that there was only a slight preference for smaller molecules of RNA and DNA in the uptake by RBC (Fig. 3).

Fig. 3. Sucrose sedimentation profiles of $^3H$-AGMK RNA (left panel) and $^3H$ SV40 DNA, form 2 (right panel) before ($\bullet$) and after (O) trapping within RBC.

Discussion

During dialysis against water, RBC began to lose large amounts of their hemoglobin when the osmolarity of the solution sank below 100 mOsmol. Parallel with this efflux of hemoglobin, nucleic acids were able to permeate into the RBC; the maximum uptake began at 70 mOsmol. These findings are principally consistent with the results of similar trapping experiments with RBC and enzymes carried out by Ihler et al.\(^4\). However, we observed that for maximum nucleic acid trapping efficiency, a final incubation (at $37 ^\circ C$) of the loaded RBC under physiological osmolarity was essential. This finding is in agreement with results of Bodemann and Passow\(^1^{4}\). These authors postulated the existence, after hemolysis, of an RBC population which can regenerate their membranes only after a prolonged incubation.

A further essential experimental device for an economic loading of RBC is the presence of the nucleic acid during the gradual hemolysis in the dialysis tube. This device enables us to load the RBC with up to 35 per cent of the concentration of the nucleic acids of the RBC suspension fluid. Thus, for instance, starting with $50 \mu g$ of SV40 DNA and 100 $\mu l$ RBC sediment in a total dialysis volume of 300 $\mu l$, up to $5 \mu g$ of SV40 DNA can be trapped within RBC. This amount of viral genetic material corresponds to 600 SV40 DNA molecules, which could be transferred when one loaded RBC ($5 \times 10^{-11} ml$\(^{15}\)) is fused with a susceptible animal cell.

Thus trapping of genetic material in RBC and subsequent cell fusion with animal cells could theoretically be an equivalent of microinjection.

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