Loading of Human Red Blood Cells with DNA and RNA

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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 surrounding [1–4] or in strong electric fields [5, 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 possible [7, 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 μCi $\times$ ml^{-1} of [5,6-^{3}H]-uridine for a 3 h period. Total cell RNA was isolated according to Scherrer and Darnell [9].

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

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^{3}H-SV40-DNA form 1 and 2 were prepared by labeling of SV40 (strain 777) infected permanent AGMK cells (line TC7 [11]) with [6-^{3}H]-thymidine (40 μCi $\times$ ml^{-1}) for 12 h beginning at 60 h after the infection. The DNA was isolated according to Hirt [12] and was separated into DNA form 1 and 2 by CsCl ethidium bromide centrifugation [13].

^{3}H-T3-DNA was a gift from Dr. Rudolf Hausmann.

Human RBC

Human blood cells (blood group 0) were separated from the white cell buffy coat and suspended in Hanks solution. The RBC were used within 18 h after collection. Numbers of RBC were estimated by cell count or by sedimentation.

Loading of RBC with nucleic acid

The loading experiments were performed by dialysing a suspension of RBC in a Hank's solution containing DNA or RNA first against distilled water and subsequently against Hank's solution at 0 °C. Dialysis vessels were sterile glass tubes, diameter 20 mm, mounted in vertical position, the lower end closed with a semipermeable membrane (Viscing dialysis tubing). The glass tubes contained 300 μl of a sterile suspension of RBC (100 μl cell volume) and nucleic acid in Hank's solution. The dialysis membrane was dipped into a glass beaker containing icechilled destilled water or Hank's solution. During dialysis, the RBC were kept in suspension by a small magnetic stirring bar.

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

The loaded RBC (100 μl) were suspended in Soluene-350 (Packard) (4 ml) and incubated at 60 °C for 2 h. Then H_2O_2 (30%, 1.6 ml) was added and the mixture was further incubated at 40 °C for 30 min. Aliquots of the solution were then mixed

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 μl) were lysed with NP40 detergent (20%, 5 μl). 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 μl cell volume) were suspended in Hank's solution (200 μl) 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 μl) 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.

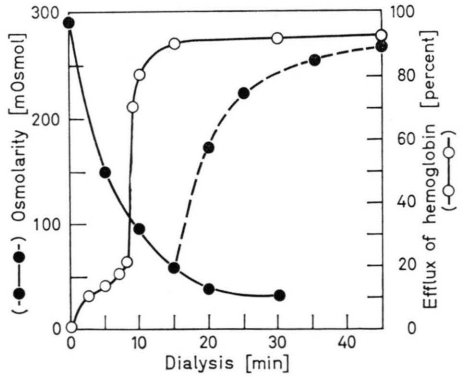

Fig. 1. Osmolarity and efflux of hemoglobin from RBC during dialysis. For explanation see text.

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

In most experiments, 100 μl of RBC were suspended in 200 μl of Hank's solution (total volume 300 μl) 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.

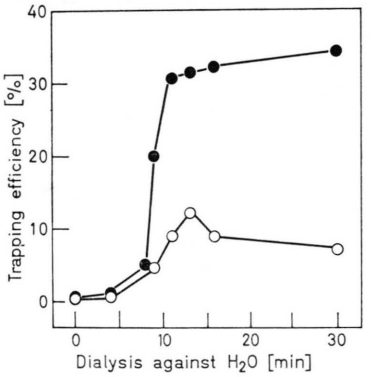

Fig. 2. Trapping of ^{3}H-AGMK RNA (45 μg; 1.2 × 10^6 cpm) within RBC (100 μl sediment) in a dialysis mixture (total 300 μl) with (●) and without (○) final incubation at 37 °C (90 min).

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

ribonuclease (50 $\mu g \times ml^{-1}$; 37 °C). After washing twice we found that up to 90 per cent of ^{3}H-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 25×10^6 daltons (T3 phage DNA) were trapped in RBC (Table I).

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

Nucleic acid	Trapping efficiency [%] with final incubation at 37 °C	without incubation
AGMK cell RNA	35	10
SV40 cRNA	35	10
SV40 DNA, form 1	30	1
SV40 DNA, form 2	30	1
T3 DNA	30	1

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).

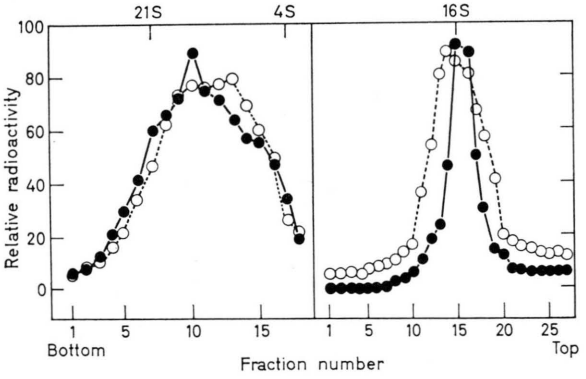

Fig. 3. Sucrose sedimentation profiles of ^{3}H-AGMK RNA (left panel) and ^{3}H SV40 DNA, form 2 (right panel) before (●) and after (○) trapping within RBC.

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 Jhler *et al.*[4]. However, we observed that for maximum nucleic acid trapping efficiency, a final incubation (at 37 °C) of the loaded RBC under physiological osmolarity was essential. This finding is in agreement with results of Bodemann and Passow[14]. 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 μg of SV40 DNA and 100 μl RBC sediment in a total dialysis volume of 300 μl, up to 5 μg 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 (5×10^{-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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