Lithium chloride purification, a rapid and efficient technique to purify RNA from DSS-treated tissues Version 2

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Abstract

Dextran sodium sulfate (DSS) is commonly used in mouse studies to induce a very reproducible colitis that effectively mimics the clinical and histological features of human inflammatory bowel disease (IBD)
patients, especially ulcerative colitis. However, the mechanisms of action of DSS remain poorly understood, and
observations by our laboratory and other groups indicate that DSS contamination of colonic tissues from DSStreated
mice potently inhibits the quantitative reverse-transcription polymerase chain reaction (qRT-PCR)
amplification of mRNA. A prior study used poly-A-mediated mRNA purification to remove DSS from RNA extracts, but we herein report a second efficient and cost-effective approach to counteract this inhibition, using lithium chloride
precipitation to entirely remove DSS from RNAs. We also explored how DSS interferes with qRT-PCR process, and
we report for the first time that DSS can alter the binding of reverse transcriptase to previously primed RNA and
specifically inhibits the enzymatic activities of reverse transcriptase and Taq polymerase in vitro. This likely explains
why DSS-treated colonic RNA is not suitable to qRT-PCR amplification without a previous purification step.
In summary, we provide a simple method to remove DSS from colonic RNAs, and we demonstrate for
the first time that DSS can inhibit the activities of both polymerase and reverse transcriptase. In order to reliably
analyze gene expression in the colonic mucosa of DSS-treated mice, the efficiency rate of qRT-PCR must be the
same between all the different experimental groups, including the water-treated control group, suggesting that
whatever the duration and the percentage of the DSS treatment, RNAs must be purified.

Citation: Emilie Viennois Lithium chloride purification, a rapid and efficient technique to purify RNA from DSS-treated tissues. **protocols.io**
dx.doi.org/10.17504/protocols.io.kk3cuyn
Published: 04 Nov 2017

Guidelines

DSS treated mice

All studies were performed in accordance with the Institutional Animal Care and Use Committee at Georgia State University (Atlanta, GA). All procedures were approved and are registered in the protocol IACUC ID: A11025, approval date 8/30/2011 to 8/30/2014. Strains, ages, and the number of animals follow the established protocol. The DSS treatment on mice were carried out in C57BL/6 mice (8 wk, 18–22 g) obtained from Jackson Laboratories (Bar Harbor, ME). Mice were group housed under a controlled temperature (25°C) and photoperiod (12:12-h light–dark cycle) and allowed unrestricted access to standard mouse chow and tap water. DSS [40,000 Da, 3% (wt/vol), ICN Biochemicals, Aurora, OH] was diluted at 3% in drinking water. After 7 days under DSS treatment, the mice were sacrificed by CO2 euthanasia. A small piece (50 mg) of proximal colon was taken for RNA extraction.

RNA extraction

Total RNA was isolated from colonic tissues using TRIzol (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Where indicated, RNA was purified via precipitation with lithium chloride. The RNA integrity was assessed by 2% agarose gel electrophoresis.

cDNA synthesis and qPCR

cDNA were synthesized using the Maxima First-Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA) according to the manufacturer's instructions. Expression of the total RNA was quantified by qPCR using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific) in a Realplex Thermal Cycler (Eppendorf, Hauppauge, NY). The qPCR primer sequences for 36B4 were 36B4-F: TCCAGGCTTTGGGCATCA and 36B4-R: CTTTATCA GCTGCACATCACTCAGA.

Polymerase assay

The polymerase enzymatic activity was measured using the EvaEZ™ Fluorometric Polymerase Activity Assay kit (Biotium, Hayward, CA) according to the manufacturer's instructions. Briefly, 0.01 units (10 mU) of recombinant Taq DNA polymerase (Thermo Scientific) were incubated with water or DSS (0.01 to 1 g/L). Dextran was used as a control. The enzymatic activity was quantified by fluorescence using the Realplex Thermal Cycler (Eppendorf). The fluorescence was read every 1 min for 60 min during the elongation step at 72°C.

Reverse transcriptase assay

The reverse transcriptase enzymatic activity was measured using the EnzChek Reverse Transcriptase Assay kit (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Briefly, 0.4 units of the reverse transcriptase, M- MuLV RT (Thermo Scientific) were incubated at 25°C with water or DSS (0.00005 g/L or 0.0005 g/L) in presence of a standardized polyA RNA template, oligodT and polymerization buffer. The enzymatic activity was quantified by fluorescence using a Synergy 2 Multi-Mode Microplate Reader (Biotek).

Surface plasmon resonance (SPR)

For SPR experiments, gold sensor chips were used (Biosensing Instrument, Tempe, AZ, USA). Briefly, the principle of this technique is the following: A first molecule is coupled to the gold sensor surface. The solution containing the second molecule (the analyte) then is flowed over the surface. This creates a mass change on the sensor surface as the two molecules interact, which is detected in real time as a deflection of the resonance angle in mDeg. In that specific experiment, the gold chip was cleaned and treated as previously described [22-24]. After placing a chip into the BI-2000 SPR (Biosensing Instrument) machine each gold biosensor chip covered with carboxydextran was activated using a mixture of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide (EDC/NHS) to form amide linkages between purified protein and the chipbound carboxydextran. Two successive injections of 2,400 units of M-MuLV Reverse Transcriptase (thermo Scientific) each were performed. The reverse transcriptase was previously purified using Slide-A-Lyser® Mini dialysis devices (ThermoScientific) and suspended in PBS. After coating the chip with reverse transcriptase, RNA, previously primed using random primers (Thermo Scientific) incubated at 42°C, or primed RNA incubated with DSS in different concentrations (0.05, 0.5, 5 g/L) were passed over the chip twice. A two-step interaction curve was obtained. The first step involved adsorption of primed RNA to the maximal level. In the second step, when the flow of primed RNA concentration returned to zero, nonspecific adsorbed primed RNA were released with the running buffer. The deviation of the resonance angle thus decreased to a plateau located at a level above the initial baseline. We assessed the laser deflection as directly correlated to the binding level. We thus used the laser deviation angle as the optimal parameter for the binding affinity. All comparisons between the different solutions of primed RNA and primed RNA with different concentrations of DSS were performed as a measure of the laser deviation in mDegrees (mDeg).

Statistical analysis

Values were expressed as means ± standard error of mean (SEM). Statistical analysis was performed using an unpaired two-tailed t-test by GraphPad Prism 5 software. $p < 0.05$ was considered statistically significant.

Materials

✓ DSS by Contributed by users

Protocol

Lithium chloride purification

Step 1.

In order to be purified from all polysaccharides including DSS, a purification using lithium chloride is performed. First, incubate the RNA with 0.1 volume of 8 M LiCl diluted in RNase-free water on ice for 2 h. (1/2)

Lithium chloride purification

Step 2.

Centrifuge at 14,000 g for 30 min at 4°C. (1/2)

Lithium chloride purification

Step 3.

Pour the supernatants out and dissolve the pellet of RNA in 200 µL of RNase-free water. (1/2)

AMOUNT

200 µl Additional info: RNase-free water

Lithium chloride purification

Step 4.

Incubate the RNA with 0.1 volume of 8 M LiCl diluted in RNase-free water on ice for 2 h. (2/2)

Lithium chloride purification

Step 5.

Centrifuge at 14,000 g for 30 min at 4°C. (2/2)

Lithium chloride purification

Step 6.

Pour the supernatants out and dissolve the pellet of RNA in 200 µL of RNase-free water. (2/2)

AMOUNT

200 µl Additional info: RNase-free water

Lithium chloride purification

Step 7.

Precipitate the RNA at − 20°C for 30 min, in 0.1 volume of 3 M sodium acetate (pH 5.2) and 2 volumes of 100% absolute ethanol.

Lithium chloride purification

Step 8.

Centrifuge the RNA at 14,000 g for 30 min at 4°C.

Lithium chloride purification

Step 9.

Pur the supernatants out and wash the pellets with 100 µL of 70% ethanol.

AMOUNT

100 µl Additional info: 70% ethanol

Lithium chloride purification

Step 10.

Centrifuge at 14,000 g for 10 min at 4°C.

Lithium chloride purification

Step 11.

Remove the supernatants and dissolve the RNA in 20– 50 µ l of RNAse-free water.

AMOUNT
50 µl Additional info: RNAse-free water