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BATCH-TO-BATCH VARIATION OF CHELEX-100 CONFOUNDS METAL-CATALYSED OXIDATION. LEACHING OF INHIBITORY COMPOUNDS FROM A BATCH OF CHELEX-100 AND THEIR REMOVAL BY A PRE-WASHING PROCEDURE

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Removal of adventitious redox-active metals from buffers by treatment with Chelex resin is a widely used procedure in free radical research. Use of a new batch of Chelex-100 resin in our laboratory coincided with a sudden inability to oxidise low-density lipoprotein with copper. We found that copper-mediated oxidation of ascorbate in water treated with the same batch of Chelex was inhibited when compared with untreated water and water treated with a different batch of the resin. Washing the Chelex removed the inhibitory effect suggesting that material was leaching from the resin. The washing procedure for Chelex-100 described is simple and can be scaled up. Oxidation of ascorbate with low concentrations of copper can be used to test the quality of batches of the resin.

KEY WORDS: Chelex, transition metals, ascorbic acid, redox reactions.

INTRODUCTION

In free radical research, Chelex resin is increasingly being used to clear buffers of contaminating adventitious redox-active metals which can be present at micromolar concentrations[1]. Chelex-100 resin contains bound to its particles the divalent metal chelator, iminodiacetic acid, which has a structure analogous to half a molecule of EDTA (ethylene diaminetetraacetic acid). But whereas EDTA is soluble, the Chelex-100 resin can be readily separated from samples. EDTA is commonly used as a chelator during the preparation of low-density lipoprotein (LDL). Prior to experiments in which we catalyse the oxidation of LDL with copper, EDTA is removed by extensive dialysis against phosphate buffered saline (PBS) or by brief size-exclusion chromatography into PBS. The addition of Chelex-100 during dialysis allows the removal of EDTA from LDL samples whilst preventing the LDL from undergoing premature and uncontrolled oxidation catalysed by the significant amounts of redox-active metals which are known to contaminate buffers such as PBS[1].

The use of a new batch of Chelex-100 in our laboratory coincided with a sudden inability to oxidise LDL with copper. We investigated this problem by comparing

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copper-catalysed oxidation of ascorbate[1] in nanopure water pre-treated with two separate batches of Chelex.

MATERIALS AND METHODS

Chelex-100 resin (100–200 mesh, sodium form) was purchased from Biorad. The batch numbers were 49419A ("batch #1") and 50187B ("batch #2").

For removal of EDTA, LDL was dialysed against PBS (containing 500 mg/L of chloramphenicol) treated with Chelex-100 resin at 4 g/L. After dialysis, LDL (at 1 mg/mL protein) was oxidised with 20 μM $CuCl_2$ (BDH) at 37°C for 24 hours.

Oxidation of ascorbate (100 μM L-ascorbic acid, Sigma), initiated by the addition of $CuCl_2$ (500 nM), was monitored for 15 min at room temperature in 10 mM phosphate buffer prepared with nanopure water or water pre-treated with the suspect batch of Chelex (#1) or with a separate batch (#2). Oxidation was assayed by measuring the decrease in absorbance at 265 nm using a Hitachi U-3210 spectrophotometer with a 3 mL quartz cell and a 1 cm light path.

For each batch a comparison was made between 50 mL of water pre-treated with 200 mg of Chelex ("unwashed") and water pre-treated with the same Chelex after a washing procedure ("washed"). This procedure involved mixing 200 mg of Chelex for 15 min in 50 mL of nanopure water. The sample was centrifuged very briefly to sediment all the resin, the washings were carefully removed and the Chelex was resuspended in the 50 mL of water. In both cases the samples of water were then mixed with Chelex overnight at 4°C. This washing procedure can be used for larger amounts of Chelex by collecting the washed Chelex with a sintered glass filter in place of centrifugation.

RESULTS

The rate of Cu(II)-mediated ascorbate oxidation was greatly retarded by the water pre-treated with the unwashed batch #1 Chelex whereas with unwashed batch #2 the inhibitory effect was much smaller (Figure 1). Washing either batch of Chelex prior to its use appeared to remove the inhibitory compound(s) (Figure 1).

The inhibitory effect was not characterised further but no difference in pH was observed between phosphate buffer prepared with water treated with either batch of Chelex.

DISCUSSION

Soluble chelator may be released from some batches of Chelex and its presence can confound metal-catalysed oxidation experiments. The technical information provided with the Chelex-100 resin does not mention the need for pre-washing the resin for batch preparations of Chelex-treated buffer. Schaich[2] has recently noted that Chelex does require extensive washing before use but has not commented on the actual leaching of chelator from it. The washing procedure outlined above is simple, takes little time and can easily be scaled-up. Batches of Chelex can be readily tested for residual leachable chelating contaminants by comparing the rate of Cu(II)-mediated ascorbate oxidation in Chelex-treated and untreated water.

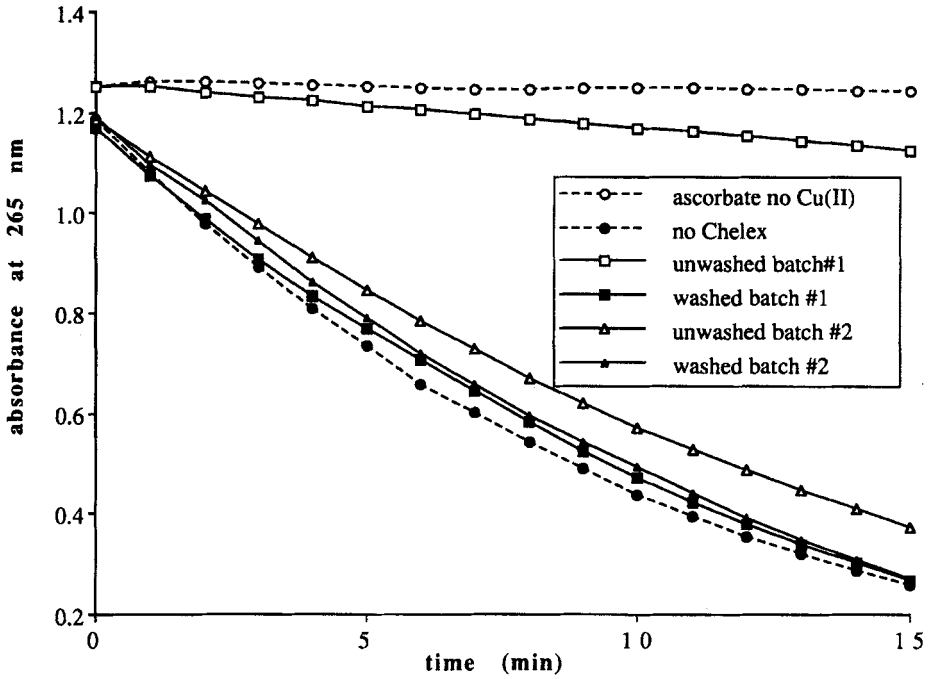

FIGURE 1 Oxidation of ascorbate (100 μM) by copper(II) chloride (500 nM) in 10 mM phosphate buffer (pH 7.4) prepared with unchelexed nanopure water (no Chelex) or nanopure water pre-treated with Chelex that was either used with ("washed") or without ("unwashed") prior washing. Consumption of ascorbate was measured as a decrease in absorbance at 265 nm (refer to Materials and methods). Results are from one of two representative experiments.

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References

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