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A method for removal of trace iron contamination from biological buffers

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Laboratory chemicals and reagents normally contain trace amounts of iron salts sufficient to catalyse free radical reactions. This iron contamination can be removed from buffers and reagents using a dialysis sac containing a high-affinity iron-binding protein like conalbumin or transferrin without altering the pH value of the fluid.

Oxygen radical; Catalytic iron; Lipid peroxidation; Iron-binding protein; Bleomycin assay

1. INTRODUCTION

Iron ions are themselves free radicals able to participate in electron transfer reactions with molecular oxygen giving rise to oxygen radicals [1].

Iron salts can also decompose a variety of organic peroxides, such as lipid hydroperoxides, to form alkoxy and peroxy radicals which propagate radical damage.

Most laboratory chemicals and reagents contain trace amounts of adventitious iron (1.6–19.4 μM) sufficient to catalyse oxygen radical formation [2,3]. Iron ions, through the formation of oxygen radicals, can cause damage to most biological molecules. Recently, the bleomycin assay was introduced to measure trace amounts of loosely bound iron in biological fluids [4,5]. One problem in using this assay is that the amounts of such iron present in human body fluids are similar to those contaminating the reagents. Although reagent-contaminating iron can be decreased using Chelex resin [6] the procedure is not always completely effective [7] and can substantially alter the pH value of solutions. Chelex resin is considerably more effective at removing copper ions, however.

It has been observed that iron bound to transferrin is ineffective in stimulating lipid peroxidation [8] and other radical reactions [9] since transferrin has a very high affinity for iron. This led us to use high-affinity iron-binding proteins to remove trace iron from buffers and reagents.

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2. MATERIALS AND METHODS

2.1. *Materials*

Conalbumin type IV was obtained from Sigma (Poole, England) and bleomycin sulphate from Bristol-Myers. All other chemicals were of the highest purity available from BDH (Poole, England).

2.2. *Preparation of conalbumin*

Suitable lengths of seamless viscose cellulose dialysis tubing were boiled in distilled water for 5 min. A sac was made by sealing one end of the tubing and filling it with a 5% (w/v) solution of conalbumin containing a few milligrams of $NaHCO_3$. The sac was sealed at the other end and placed inside the buffer solution where it was left

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for 48 h at 4°C. Providing the buffer is stored at 4°C and the sac is not punctured during pipetting it can remain in the buffer solution. As iron is withdrawn from the buffer and loaded onto the conalbumin, in the dialysis sac, the protein acts as an indicator turning salmon-pink in colour.

2.3. *Bleomycin assay for loosely bound iron*

The assay procedure for loosely bound iron has been described previously [4,5]. The reaction used here contained 0.075 units bleomycin sulphate in 0.1 M Tris buffer (pH 7.4). Samples were incubated at 37°C for 1 h and the resulting damage to DNA measured as thiobarbituric acid reactivity at 532 nm [4,5].

3. RESULTS AND DISCUSSION

The plasma protein transferrin functions as a carrier molecule for iron attaching two iron ions in the presence of bicarbonate, per molecule of protein, with high affinity. Normally, transferrin is only partly loaded with iron retaining considerable iron-binding capacity and so keeping extracellular iron levels to virtually zero. Lactoferrin, a protein similar to transferrin, is found in several body fluids including milk and is produced by phagocytic cells. Like transferrin, lactoferrin binds 2 mol Fe^{3+} per mol protein. The two proteins are immunologically distinct however, and display quite different pH-dependent iron-binding properties with transferrin releasing iron at pH values of 5.6 or less and lactoferrin holding its iron down to pH values of 4.0 [10,11]. However, it was found that conalbumin, the iron-binding protein of eggs, is equally effective in removing iron at pH 7.4 and is considerably cheaper than either transferrin or lactoferrin. All three proteins act as self-indicators for iron removal, turning pink in colour as iron is complexed to them. When buffer pH values drop to as low as 4.0 lactoferrin would have to be used in place of conalbumin.

Table 1 shows the effectiveness of a conalbumin dialysis sac in removing iron from Tris buffer (pH 7.4) spiked with $FeCl_3$. The conalbumin dialysis technique has allowed for the first time the use of a buffer in the bleomycin assay thereby giving greater sensitivity and versatility to the method [5]. The dialysis technique should also be of value in removing iron from solutions used in high-energy radiation studies, NMR, lipid peroxidation, culture media and biological assays.

Table 1

Removal of iron from Tris buffer (pH 7.4) with a dialysis sac

	Bleomycin-detectable iron	
	TBA reactivity ($A_{532\,nm}$)	Iron concentration (μM)
0.1 M Tris buffer (pH 7.4) (non-dialysed)	0.089	1.70
Control (0.1 M Tris buffer (pH 7.4) dialysed)	0.042	–
+ 1.25 μM $FeCl_3$	0.074	1.25
dialysed	0.042	0
+ 2.50 μM $FeCl_3$	0.112	2.50
dialysed	0.042	0
+ 5.00 μM $FeCl_3$	0.182	5.00
dialysed	0.042	0
+ 10.00 μM $FeCl_3$	0.320	10.00
dialysed	0.042	0

The results shown are the means of 3 separate experiments. The control dialysed Tris buffer value (0.042) has been subtracted from the absorbance values where a simple iron salt has been added to the buffer and from the non-dialysed Tris buffer value (0.089). The blank values are those of the bleomycin assay to which other reagents besides the buffer contribute iron complexes after Chelex resin treatment

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