Poster Session: Metal Ions in Biological Systems

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Comparison of the Chemical States of Chromium in Yeast and Higher Plants

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Chromium in plants has been reported to be in two very different complexes. The glucose-tolerance factor (GTF) was reported to be a 400 dalton cation [1] whereas the complex purified from higher plants (HPC) was reported to be a 3000 dalton anion [2, 3]. The purification procedures for these complexes were different. It was desired to measure the charge and relative molecular size of the chromium complexes obtained using both purification procedures on yeast and on a representative higher plant.

Chromium-51 labeled Brewers yeast (*Saccaromyces carlesbergensis*) and alfalfa (*Medicago sativa L.*) were fractionated by two published procedures for the preparation of the glucose-tolerance factor and by a procedure for the preparation of the chromium-containing complex from higher plants. One GTF procedure used a 50% n-butanol extraction followed by dialysis and chromatography on DEAE-cellulose [4] and the other used a 50% ethanol extraction followed by activated charcoal purification [5]. The HPC preparation used water extraction followed by molecular sieve chromatography on Sephadex G-25 [2]. Aqueous solutions of all three preparations from both yeast and alfalfa were chromatographed on Sephadex G-25 and the ionic charge determined by ion exchange chromatography.

The results are summarized in Table I. The GTF procedures applied to yeast produced a positively charged complex with a peak R_f of 1.71 to 1.76 and a rather diffuse peak. The GTF procedures applied to alfalfa produced a negatively charged complex with an R_f of 1.68. The HPC procedure with yeast or alfalfa gave a negatively charged complex with an R_f of 1.5 to 1.6.

TABLE I. Properties of Chromium Complexes prepared from Yeast and Alfalfa.

Preparation method	R_f on Sephadex G-25		Ionic charge	
	Yeast	Alfalfa	Yeast	Alfalfa
GTF–ethanol [5]	1.71	1.69	+	–
GTF–n-butanol [4]	1.76	1.68	+	–
HPC [2]	1.45–1.55	1.61 ± 0.04	—	—

The GTF procedures produce a smaller complex than the HPC procedure. Yeast and alfalfa complexes purified by either GTF procedure have opposite charges, the yeast complex having the charge reported for the GTF. With the HPC procedure only anionic complexes were found from either starting material.

The chromium-containing complex found in higher plants is different from the GTF even though both are alcohol soluble. A complex similar to the HPC complex can be isolated from yeast.

1 W. Mertz, *Physiol. Rev., 49,* 163 (1969).
2 C. Blincoe, *J. Sci. Food Agric., 25,* 073 (1974).
3 G. H. Starich and C. Blincoe, *J. Agric. Food Chem., 30,* 458 (1982).
4 E. W. Toepfer, W. Mertz, M. P. Polansky, E. E. Rogiuski and W. R. Wolf, *J. Agric. Food Chem., 25,* 162 (1977).
5 N. Mirsky, A. Weiss and Z. Dori, *J. Inorg. Biochem., 13,* 11 (1980).

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The Role of a Metal–Transferrin–Transferrin–Receptor Mechanism in the *in vitro* Uptake of Metals by Human Lymphoblasts (WIL-2)

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It is now clear that in addition to its role as the principal iron carrier protein in the plasma and extracellular fluid, transferrin also acts as the carrier protein for a number of toxic and non-toxic 'foreign' metals, such as gallium [1], hafnium [2], thorium [3], plutonium [4] and, probably, americium and curium [5].

Iron is known to be taken up into reticulocytes and at least some other cells through an iron–transferrin–transferrin–receptor mechanism [6], and it has been recently suggested that a similar mechanism may be concerned in the uptake of gallium by tumour cells [7]. The possibility that a metal–transferrin–transferrin–receptor mechanism may be

involved in the cellular uptake of all transferrin-transported metals is currently under investigation in this laboratory.

The transferrin binding characteristics for various 'foreign' metals are being studied and Sephadex gel-chromatographic methods are being used to measure the stability constants of the metal–transferrin complexes. Preliminary studies with iron(III) and hafnium(IV) have yielded overall stability constants of $\log \beta = \sim 30$.

In vitro uptake studies with a human lymphoblast cell line (WIL-2) have shown (Table I) that when compared to that from physiological concentrations of citrate, the uptake of iron, gallium and hafnium from serum-free medium is enhanced by transferrin. The uptake is dependent on the metal–transferrin ratio and is highest with a molar ratio of 1:1 for iron and 1:10 or 1:30 for the other metals.

TABLE I. The Uptake of Iron, Gallium and Hafnium by WIL-2 Cells *in vitro*.

Metal	Metal: TF ratio	% Uptake per 10^6 cells per 2 h	
		Transferrin	0.1 m*M* citrate
Iron	1:1	2.78 ± 0.12	0.38 ± 0.05
Gallium	1:10	0.29 ± 0.003	0.033 ± 0.002
	1:30	0.19 ± 0.005	
Hafnium	1:10	1.03 ± 0.09	0.087 ± 0.018
	1:30	1.84 ± 0.11	

Analysis of the subcellular distribution of gallium and hafnium after *in vitro* uptake indicates that, as expected from *in vivo* studies [2, 8], the incorporated metal is associated with lysosomal structures.

The uptake data for the metals studied suggest that a metal–transferrin–transferrin–receptor mechanism is involved. However, the pattern of uptake observed with iron is not identical with that of gallium or hafnium, nor probably with that of plutonium.

1 S. R. Vallabhajosula, J. F. Harwig, J. K. Siemsen and W. Wolf, *J. Nucl. Med., 21*, 650 (1980).
2 D. M. Taylor, M. Lehmann, F. Planas-Bohne and A. Seidel, *Radiat. Res.*, in the press.
3 E. Peter and M. Lehmann, *Int. J. Radiat. Biol., 40*, 445 (1981).
4 G. Boocock and D. S. Popplewell, *Nature, 208*, 282 (1965).
5 J. R. Cooper and H. S. Gowing, *Int. J. Radiat. Biol., 40*, 569 (1981).
6 J. Glass, M. T. Nunel, S. Fischer and L. M. Lavidor, *Fed. Proc., 36*, 930 (1977).
7 S. M. Larson, Z. Grunbaum and J. S. Rasey, *Int. J. Nucl. Med. Biol., 8*, 257 (1981).
8 P. A. G. Hammersley and D. M. Taylor, *Europ. J. Nucl. Med., 4*, 261 (1979).

S3

Inhibition of Lipid Peroxidation by Manganese

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The peroxidation of unsaturated lipids of biological membranes is a degenerative process usually sparked by free radicals or by singlet oxygen. $MnCl_2$ is an inhibitor of lipid peroxidation [1] but its mechanism of action is still unknown.

This report summarizes some results on the inhibitory effect of $MnCl_2$ on peroxidizing systems such as ascorbate/$FeSO_4$, NADPH/$FeCl_3$/ADP, cumene hydroperoxide (CHP), hematin and rose bengal/light. 1 m*M* $MnCl_2$ efficiently inhibited all the systems tested, with the exception of rose bengal/visible light where lipid peroxidation is not initiated by a free radical mechanism but by singlet oxygen which directly forms a hydroperoxide through an 'ene' reaction [2]. The lipid peroxidation induced by CHP was independent of iron ions since it occurred to the same extent even in the presence of 1 m*M* EDTA. Since $MnCl_2$ also strongly inhibited the CHP-induced lipid peroxidation, a competition of $MnCl_2$ for the binding sites of iron ions, which are well known promotors of lipid peroxidation, can be excluded.

Figure 1 illustrates the formation of lipid hydroperoxides (LOOH) after photoactivation in the

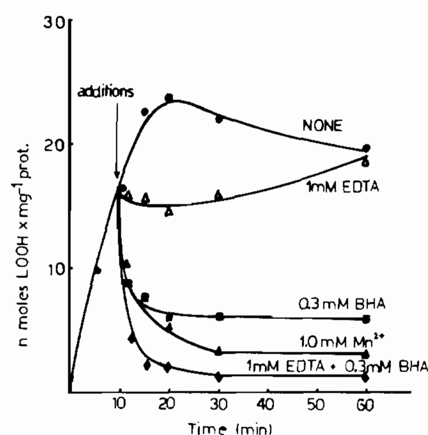

Fig. 1. Time course of LOOH formation in microsomes incubated with rose bengal. Microsomes (1 mg/ml) were incubated with 15 m*M* HEPES and 6.5 m*M* Tris pH 7.4 at 25 °C in the presence of 10 μg/ml of rose bengal and visible light. The reaction was stopped after 10 min and, at the same time, the indicated substances were added. Aliquots were withdrawn and LOOH content was estimated with KSCN [3] after extraction with $CHCl_3/CH_3OH$ (3/2, v/v).