

# Assay and characteristics of the iron binding moiety of reticulocyte endocytic vesicles

Nunez, Marco Tulio

Pinto, Ines

Glass, Jonathan

A  $^{59}\text{Fe}$  assay was designed to detect an Fe(III) binding capacity in NP-40 solubilized proteins from rabbit reticulocyte endocytic vesicles. The iron binding capacity had an apparent molecular weight as determined by gel exclusion chromatography of 450,000 daltons. The iron binding moiety coincided with the major nontransferrin iron-containing material of endocytic vesicles labeled in vivo by incubation of cells with  $^{59}\text{Fe}$ ,  $^{125}\text{I}$ -labeled transferrin. The material solubilized from vesicles with NP-40 exhibited two classes of saturable binding sites, one with an association constant for  $^{59}\text{Fe}$ -citrate of  $3.63 \times 10^9 \text{ m}^{-1}$  and with  $6.6 \times 10^{-12}$  moles of iron bound per mg protein and the other with a constant of  $3.96 \times 10^8 \text{ m}^{-1}$  and  $1.0 \times 10^{-12}$  moles of iron bound per mg protein. These affinities are sufficient to satisfy the solubility characteristics of Fe(III) at pH 5.0. Most of the  $^{59}\text{Fe}$  bound both in vivo and in vitro to the iron binding moiety could be displaced with  $^{56}\text{Fe}$  and an equivalent amount of  $^{59}\text{Fe}$  could su