Mobilization of iron from endocytic vesicles. The effects of acidification and reduction

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The factors necessary to dissociate iron from transferrin in endocytic vesicles and to mobilize the iron across the vesicle membrane were studied in a preparation of endocytic vesicles markedly enriched in transferrin-transferrin receptor complexes isolated from rabbit reticulocytes. Vesicles were prepared with essentially fully saturated transferrin by incubating the reticulocytes with the protonophore carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone prior to incubation with 59Fe,125I-transferrin with or without fluorescein isothiocyanate labeling. Initiation of acidification by the addition of ATP was sufficient to achieve dissociation of 59Fe from transferrin with a rate constant of 0.054 ± 0.06 s-1. Mobilization of 59Fe out of the vesicles required, besides ATP, the addition of a reductant with 1 mM ascorbate, allowing $\sim 60\%$ mobilization at 10 min with a rate constant of 0.0038 ± 0.0006 s-1. An NADH:ferricyanide reductase activity could be demonstrated in the vesicles with an ac