Influence of Ascorbic Acid, Sodium Citrate, and Sodium Bicarbonate on the Uptake of $^{59}$Fe-Transferrin, $^{54}$Mn-Transferrin, and $^{65}$Zn-Transferrin from Lactating Mouse Mammary Gland Cells

DIMITER A. MOUTAFCHIEV AND LJUBEN M. SIRAKOV*

Department of Biochemistry, Medical Faculty, Medical University, 2 Zdrave str., 1431 Sofia, Bulgaria

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ABSTRACT

The effects of ascorbic acid, sodium citrate, and sodium bicarbonate on $^{59}$Fe-transferrin, $^{54}$Mn-transferrin, and $^{65}$Zn-transferrin uptake by the receptors disposed of plasma membrane isolated from lactating mouse mammary gland cells have been investigated. The effect of $10^{-2}$ mol/L ascorbic acid alone and in combination with NaHCO$_3$ on the $^{59}$Fe-transferrin uptake is significant and positive. $^{54}$Mn-transferrin and $^{65}$Zn-transferrin binding to the cell receptors are influenced optimally by 0.5 mol/L sodium bicarbonate. Sodium citrate alone or in combination with other substances always has a negative effect on binding of these three metals.

It is suggested that a precise mechanism may exist with large possibilities to rearrange metal uptake and its transport from blood to milk.

Index Entries: Metal-transferrin; lactating mouse mammary gland cells; ascorbic acid; sodium citrate; sodium bicarbonate.

INTRODUCTION

Transferrin is the protein that has metal binding properties and thus transports essential microelements to different organs of mammalian

*Author to whom all correspondence and reprint requests should be addressed.
organism. Also, the transferrin molecule binds to Mn(III) (1–3) and Zn(II) (4,5). The Fe(III) binding to transferrin requires the anion binding site of protein to be occupied by bicarbonate anion (6). Citrate anion is necessary for Mn(III) binding (1) and bicarbonate for Zn(II) (7). These anions act as coligands between metals and transferrin (8).

The saturation of milk with Fe is realized by a receptor mechanism, including plasma membrane receptor on the rabbit mammary gland cell (9) and mouse (10) and Fe-transferrin. Receptors binding Mn(III) and Zn(II) without participation of transferrin have been described on the plasma membrane of the lactating mammary gland cell of mouse (11). This work has proven that MnCl₂ and ZnCl₂ successfully compete with ⁵⁹Fe(II)-ascorbate and ⁵⁹Fe(III)-oxide for the receptor sites exhibiting saturation kinetics.

The ascorbic acid acts as a reducing agent and antioxidant (12). Marx and Stiekema (13) found in subjects with Fe deficiency a 50% higher Fe absorption from ferrous ascorbate than from ferrous sulfate. The ascorbic acid alone or together with sodium citrate influences positively (but not significantly) Fe(II) and Fe(III) binding to membrane isolated from lactating mouse mammary gland (14). The role of chelate agents (sodium citrate and sodium bicarbonate) is not essential for binding, whereas the transferrin is not present in the mixture (same study).

The aim of this work is to follow the individual and joint effect of ascorbic acid, sodium bicarbonate, and sodium citrate on the transport of Fe-, Mn-, and Zn-bound transferrin to isolated cells from lactating mouse mammary gland.

**MATERIALS AND METHODS**

**Animals**

Conventional white mouse strain H were purchased from the Animal House of Bulgarian Academy of Sciences, Sofia. They were 8–12 d after delivery and were not separated from their litters up to the day of the experiment.

**Chemicals**

All chemicals used in the experiments were from Merck, Germany or Fluka, Switzerland and of analytical grade. The bovine serum albumin, fraction V, was from Koch-Light, England. The human transferrin was obtained from Serva, Germany.

**Isotopes**

⁵⁹Fe(II)-ascorbate was obtained from Akademie der Wissenschaften, Dresden (formerly DDR). ⁵⁴MnCl₂ and ⁶⁵ZnCl₂ were produced by Amersham, England.