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IRON METABOLISM IN CELLS OF TRICHOMONADS

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DISSERTATION ABSTRACT

Introduction

Iron is an element essencial for survival and replication of practically all living organisms. The cells utilize this metal in a wide range of catalytic reactions, particularly in electron transport and redox reactions depending on formation of free radicals. A negative feature of iron is a potencial toxicity of its free (not specifically bound) form manifested through the production of highly reactive hydroxyl radicals by Haber-Weiss reaction followed by auto-oxidative processes in several tissues. Organisms therefore maintain very low internal level of free iron via co-operation of three specific proteins: (1) lactoferrin, which specifically binds iron; (2) transferrin transporting iron among various tissues; (3) ferritin, which mediates intracellular iron storage.

Low level of free iron in body fluids of higher eukaryotes mediated by a specific binding of this metal also provides a defence against microbial infections. To overcome the iron-restricted environment in the host, microorganisms have evolved specific mechanisms for iron acquisition. Bacteria and fungi secrete low-molecular high-affinity iron-binding compounds called siderofores, which enable them an efficient uptake of host iron. Another mechanism is based on expression of specific surface receptors for transferrin, lactoferrin, haemoglobin, and other host-related iron-binding proteins. It has also been reported that some microorganisms are able to decrease pH and redox potencial of their enviroment which results in spontaneous release of iron from its ligands and facilitated assimilation of this metal.

Mechanisms involved in iron uptake in parasitic protozoa have not been fully elucidated so far. Although it has been shown that anaerobic protozoa display extremely high nutritional requirements for iron, receptors specific for host iron-binding proteins have been detected and partially characterized only in six species of protozoa. Genes encoding these proteins have been identified and analyzed only in case of transferrin receptor in *Trypanosoma brucei*. Neither the mechanisms involved in uptake of iron from low-molecular complexes in parasitic protozoa have been sffiviently studied so far. There is also no information about subsequent intracellular iron transport and incorporation into the cell compartments and target molecules.

Possible reason for the high demand of anaerobic protozoa for iron may reside in a dominant role played by iron-sulphur proteins in their energy metabolism, which in trichomonads takes place in hydrogenosomes, organelles responsible for ATP synthesis under anaerobic conditions.

The most important members of trichomonads are *Tritrichomonas foetus*, causative agent of veneral disease of cattle, and *Trichomonas vaginalis*, etiologic agent of sexually transmitted human trichomonosis. Considering the localization of the parasites in the urogenital and occasionally gastrointestinal tract, the following compounds could serve as potencial sources of iron for the parasites: lactoferrin present in vaginal mucus; transferrin highly abundant in uterus; and low-molecular iron complexes from intestinal content.

Due to the high nutritional requirements for iron, several potencial sources of this metal, and presence of hydrogenosomes, whose function fully rely on activity of the iron-sulphur proteins, trichomonads are highly attractive experimental model for study of iron uptake and metabolism.

Aims of the work

- to study the abilitiy of *Tritrichomonas foetus* and cells of other anaerobic protozoa to acquire iron from various sources (lactoferrin, transferrin, low-molecular iron complexes)

- to elucidate mechanisms involved in uptake of iron from lactoferrin, transferrin, and low-molecular iron complexes by cells of *Tritrichomonas foetus*
- to identify the major cell compartments and target molecules incorporating iron in *Tritrichomonas foetus* cells
- to determine whether the ability of *Tritrichomonas foetus* to acquire iron can modulate virulence of this parasite

Summary of results

1. The cells of anaerobic protozoa studied were capable to accumulate various amounts of iron from lactoferrin, transferrin, and low-molecular complexes. Experiments focused on stimulation of growth of the parasites and intracellular iron incorporation revealed that cells of *Tritrichomonas foetus* can utilize iron from all three sources whereas *G. intestinalis* and *E. histolytica* can cover their nutritional requirements exclusively by iron bound in low-molecular complexes such as Fe-nitrilotriacetate. The different rate of utilization of iron from various sources may reflect divers localization of the parasites in their hosts. Whereas *T. foetus* can colonize all regions of the reproductive tract, which are rich particularly in lactoferrin and transferrin, and has also been found in the gastrointestinal tract, the cells of *G. intestinalis* and *E. histolytica* parasitize only the gastrointestinal tract containing low-molecular complexes of iron.

2. The cells of *T. foetus* take up iron from lactoferrin via receptor-mediated endocytosis. About 1.7×10^5 binding sites specific for lactoferrin (Kd ≅ 3.6 μM) were detected on surface of *T. foetus* cell. The complex receptor-hololactoferrin is internalized by endocytosis in the first step and iron is released subsequently in acidic endosomal compartment. Apo-form of lactoferrin is then recycled. In contrast to mammalian cells, proteolytic cleavage of lactoferrin resulting in release of iron was not observed in trichomonads.

3. Iron from transferrin is taken up by the *T. foetus* cells by mechanism independent on specific surface receptors. Prior to transport, iron is extracellularly released from transferrin in an acidic environment created by trichomomads and by extracellular reduction of ferric to ferrous iron. The reduction is mediated by NADH-dependent ferrireductase activity and by secreted low-molecular extracellular reducing factors. Reduced ferrous iron is subsequently transported into the cell via mechanism based on specific membrane transporters. Iron bound to low-molecular complexes such as Fe-NTA is probably taken up via identical mechanism based on extracellular reduction of iron and its transport using a specific carrier-mediated system.

4. To study the iron incorporation into cellular compartments of *T. foetus*, the subcellular fractions were isolated from ^{59}Fe-labeled cells by means of differencial centrifugation followed by Percoll cushion separation. The hydrogenosomes represent one of the key cellular compartments with incorporated iron. These organelles are able to efficiently accumulate iron during short-term incubation with low-molecular complex Fe-NTA (360.4 pmol Fe / hour / mg protein). Atomic absorption spectrophotometry determination of steady-state level of iron after long-term cultivation in complex medium revealed unusually high iron concentration (54.4 nmol Fe / mg protein) in *T. foetus* hydrogenosomes. At least nine molecules with incorporated iron were detected by means of native gradient polyacrylamide electrophoresis followed by storage phosphoimaging analysis. Major iron-binding compound named „ band H-I“ was shown to be ferredoxin of adrenodoxin type as identified by N-terminal amino acid analysis followed by complete gene sequencing. High abundance of this protein in

hydrogenosomes suggests that in addition to participation in electron transfer machinery ferredoxin can also play an important role in processes related to iron metabolism.

5. Although the steady-state level of iron in *T. foetus* cytosol is four times lower (13.4 nmol Fe / mg protein) than that in hydrogenosomes, this compartment displays comparable rate of iron accumulation during short-term incubation with low-molecular weight complexes such as Fe-NTA (304.7 pmol Fe / hour / mg protein). Native gradient polyacrylamide electrophoresis analysis revealed LIP ("labile iron pool") as a major iron-binding compound in cytosol. It was shown that iron present in this pool was chelatable by strong chelators such as desferrioxamine. The majority of LIP consists of compounds corresponding to the relative molecular weight 5-30 kDa as determined by ultrafiltration assay. LIP is significantly more abundant in trichomonads and cells of other anaerobic protozoa than in mammalian cells. High abundance of the labile iron pool in anaerobic protozoa may reflect the absence of ferritin and it is supposable that LIP serves as an intracellular iron storage pool in anaerobic protozoa. In addition to LIP, at least five iron-binding compounds were detected in *T. foetus* cytosol with kinetics of end molecules representing final acceptors of iron.

6. The mechanisms mediating iron acquisition from the host play an important role in virulence of *Tritrichomonas foetus*. The *T. foetus* strain KV-1 displaying decreased efficiency to acquire iron from low-molecular iron complexes and transferrin exhibits moderate virulence in mouse model infections (~5% mortality rate) in comparison to LUB-1 MIP strain (~80% mortality rate), which displays higher capability to acquire iron from both sources. Increase of iron availability in the host environment by intraperitoneal injection of low-molecular iron complexes such as ferric ammonium citrate to mice models resulted in increase in virulence of KV-1 strain to the level comparable with that of LUB-1 MIP.