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# YEAST CELL BIOMASS AS A POTENTIAL SOURCE OF MAGNESIUM BIOPLEXES – A REVIEW

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The capability of yeasts for binding magnesium in the amounts exceeding its physiological demand affords an opportunity for exploiting those organisms as a natural source of a deficient bio-compound in a diet of contemporary humans. The mechanism of cation binding with yeast cells demonstrating first the character of chemisorption followed by intracellular bioaccummulation, may lead to the formation of organic linkages called "bioplexes". Magnesium ions bound with proteins in the form of the so-called "bioplexes" are very well assimilated by human and animal organisms, thus they may be an alternative to pharmacological supplementation at increasingly observed magnesium deficiency. After appropriate treatment, reducing the content of nucleic acids, cellular biomass of yeasts may not only be a valuable source of protein and vitamins but also of magnesium, especially when used in the form of protein-mineral preparations.

## INTRODUCTION

Proper balancing of dietary components is crucial to human's health. A key role of metabolic processes regulators in living organisms is ascribed to macro- and microelements. Advance in food technology results in the production of new foodstuffs on the one hand and is often found to change the quantitative and qualitative composition of essential nutrients in the processed raw materials on the other. In consequence, food additives are more commonly applied to improve the nutritive value of food so as to meet consumers' demands. Still, measures taken are not always sufficient to provide the optimal level of nutrients in a contemporary diet. At present, deficiency of one of the macroelements only, namely magnesium, has been estimated to affect from 50% to 80% of the Polish population depending on age. Often pharmacological supplementation does not produce the desired effects, mainly due to low availability of macro- and microelements from ready preparations.

Many microorganisms, including strains of *Saccharomyces* and *Candida* yeast genera, commonly accepted and acknowledged as safe, demonstrate the capability for natural binding of elements present in the environment, often in the amounts considerably exceeding their physiological demand. Metal ions adsorbed on the cell's surface may next be a subject of intracellular bioaccummulation. This way yeasts produce metal-protein complexes called metaloproteins or more generally – bioplexes. Macroelements (*e.g.* magnesium) adsorbed to proteins are available by human and animal organisms far better than their inorganic or organic salts [Vandergrift, 1991; Mardarowicz, 1997].

Cellular biomass of yeasts, rich in protein with balanced amino acid composition and good digestibility, may also become a valuable source of natural bioplexes. It opens up perspectives for a wider application of yeasts in the production of ready protein-mineral preparations or food additives enriching diets with deficient elements.

## SIGNIFICANCE OF MAGNESIUM TO THE PHYSIOLOGY OF HUMANS

Essential dietary constituents include bioelements, *e.g.* magnesium, zinc, chromium, cobalt, manganese, copper, selenium or iron, which actively participate in many metabolic pathways at a cellular level of all living organisms. Of the bioelements enumerated, a unique role is ascribed to magnesium which – as an integral component of coenzymes or prostetic groups – activates over 300 enzymes (mainly kinases) that determine the proper course of the main vital processes, including glycolysis, DNA replication, synthesis of nucleic acids, biosynthesis of proteins, and transfer through cytoplasmatic membranes [Hartwig, 2001].

This element serves as a specific regulator and activator of multiple physiological processes. In nerve-muscle junctions it demonstrates antagonistic activity against calcium ions, thus inhibits release of a neurotransmitter – acetylcholine – and consequently reduces excitability of nerves and muscles [Watson & Vaughan, 2001].

Magnesium regulates the production of cyclic adenosine monophosphate (cAMP) mediated by adenylate cyclase enzyme. This way it affects numerous physiological processes, including degradation of reserve energetic substrates, aggregation of thrombocytes, excretion and functioning of parathyroid gland hormones [Stryer, 1997].

Magnesium participates also in lipid metabolism. By affecting the activity of lipoprotein lipase it determines the

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concentration of cholesterol in blood. Reduced magnesium level in blood favours an increase in the concentration of low-density cholesterol fraction (*i.e.* LDL fraction unfavourable to health) and a concurrent decrease in the level of high-density cholesterol (HDL fraction) [Pasternak, 1999].

Magnesium cation has been reported to affect directly cardiac muscle and smooth muscular coat of blood vessels, and to counteract arrhythmia. Its high blood concentration decreases the contractility of the cardiac muscle [Watson & Vaughan, 2001; Głowania *et al.*, 2000].

$Mg^{2+}$ ions actively participate in oxidative phosphorylation which results in the formation of high-energy compounds being a source of energy to anabolic processes [Stryer, 1997].

Magnesium, as an indispensable agent activating a sodium-potassium pump ($Na^+$/ $K^+$ - ATPs), plays a key role in maintaining membraneous gradient concentration of sodium and potassium. The concentration gradient of the enumerated ions generates electric potential at both sides of cytoplasmatic membrane which is then applied for the transport of glucose, amino acids, phosphates, and other substances [Watson & Vaughan, 2001; Pasternak, 1999].

Magnesium serves also as an activator of a calcium pump ($Ca^{2+}$ - ATPs) which in turn regulates the concentration of $Ca^{2+}$ ions in cells [Walker, 1994].

Apart from a number of activating and regulatory functions, this element is also an important stabiliser of the tertiary structure of proteins and nucleic acids. In the case of DNA, it participates in the formation of nucleosomes and the consolidation of chromosome structure. It is also responsible for the stabilisation of ribosomes by participating in binding of their sub-units, thus it is crucial in the translation process [Węgleński, 1995].

Magnesium serves also other significant functions related to protein synthesis, because tRNA is able to bind amino acids only in the presence of that element [Hartwig, 2001; Walker, 1994].

In addition, $Mg^{2+}$ ions stabilize mitochondrial membranes and the mitochondria themselves [Zając, 2000; Walker, 1994], as well as the structures of polysaccharides and lipids [Rees & Stewart, 1997].

This macroelement has building functions as well. It participates in the formation and building of bones, teeth, and soft tissues. It contributes to the formation of durable amorphous form of calcium phosphate in bones [Gawęcki *et al.*, 2000].

Literature data [Gawęcki *et al.*, 2000; Skibiński, 1998; Birch, 1990; Elin, 1987; Gunther, 1981; Williams, 1970] report on a variety of other important functions of magnesium in the human organism, *i.e.* defensive activity in poisonings, support of brain functioning, relief to pain symptoms, and counteracting atherosclerosis.

At present, this element is believed to exhibit two mechanisms of activity as an enzyme co-factor. According to the first mechanism, magnesium is bound to a substrate of enzymatic reaction and becomes responsible for the formation of an active particle (an active substrate) which in this form undergoes enzymatic reaction more readily. This mechanism refers to most of enzymes whose co-factor is magnesium, *i.a.* ATP-synthetase mediating ATP synthesis in mitochondria [Heaton, 1990]. The second mechanism involves direct effects of $Mg^{2+}$ ions on an enzyme's molecule. Magnesium bound to a specified protein evokes its conformational change as a result of which the protein gains catalytic activity. An example of this mechanism is the activation of glutamate synthetase taking part in nitrogen metabolism in eukaryotic cells [Walker, 1994].

In the human organism, magnesium is the fourth macroelement, after sodium, potassium and calcium, in respect of their concentration [Watson & Vaughan, 2001]. Its content in the body of an adult man ranges from 250 to 350 mg per kg of body mass. It occurs mainly in bones where its concentration reaches *ca.* 51% of its total content in the body. It concentrates also in cell interiors (*ca.* 45%) as well as other organs and tissues (*ca.* 4%). Nearly 60% of magnesium accumulated in bones is subject to constant exchange. In blood plasma, 55% of that macroelement occur in an ionized form, 32% are bound with proteins, and another 13% form complexes with ATP [Walasek, 1998].

Demand of the human organism for magnesium is diversified depending on age, lifestyle or type of work performed. Recommended daily allowance for magnesium is 280–300 mg for women and 350–400 mg for men [Pasternak, 2000; Kunachowicz *et al.*, 1998].

A constant magnesium level in blood serum at reduced or inhibited absorption is maintained through magnesium release from the skeletal system and soft tissues. When magnesium level of blood serum falls down below 0.7 mmol/L, symptoms of hypomagnesaemia are likely to occur, which is manifested by enhanced nervous and muscular excitability and in extreme cases may lead to the state of coma. Hypomagnesaemia impairs functioning of the circulatory system, *i.e.* causes disorders in cardiac performance (arrhythmia), and an increase in arterial blood pressure which is sometimes accompanied by a disease referred to as stenocardia and spasm of coronary arteries [Walasek, 1998]. Other symptoms linked directly with insufficient supply of that ion refer to changes psychical in character, including anxiety state, depressions, and psychoses [Watson & Vaughan, 2001]. In addition, magnesium deficiency has been observed to accompany some neoplastic diseases [Rudziński, 1998].

## MAGNESIUM DEFICIENCY – CAUSES AND PROPHYLAXIS

In recent years, magnesium deficiency has been found most often diagnosed electrolyte disorder in the Polish population. It has been estimated to affect from 50% to 80% of the population depending on age group [Brzozowska, 1998].

As reported by Synowiecki [2000], considerable losses of that element are likely to occur at many stages of food production process. An example illustrating magnesium loss during raw material processing is milling of wheat flour. This technological process results in *ca.* 50% reduction of mineral components of grain, 90% of which is magnesium. During other technological processes, *e.g.* blanching or cooking of vegetables, extraction of magnesium and copper is observed, which results in 15%–70% loss of their initial content. Considerable losses of elements, especially magnesium as well as calcium and iron, are reported during preliminary processing, *i.e.* peeling or cutting off the green parts of vegetables. Elution of $Mg^{2+}$ ions has also been observed upon meat processing which consists in rinsing the meat with salt solutions.

Apart from the above-mentioned technological treatments, diminished magnesium content of food products has been observed to result from its elimination from the initial raw material due to progressing degradation of natural environment. Unfavourable changes proceeding in soil, evoked by acid rains or chemical fertilization, contribute to a diminishing level of magnesium in the soil, hence to its reduced level in plant raw material for food and feed uses [Skibiński, 1998].

It should be emphasised that magnesium is an element poorly available by human and animal organisms. It is estimated that as little as 13%–14% of magnesium is absorbed from food products. The availability of this ion is determined by the type of food products, their quality and content of antinutrients [Brzozowska, 1998; Rudziński, 1998].

In respect of magnesium content, food products can be divided into three groups. The first group includes products with a high content of this element – over 100 mg $Mg^{2+}$/100 g, *i.e.* buckweat groats, maize flour, leguminous products, nuts, cocoa, chocolate. The second group includes products with a medium content of magnesium, ranging from 25 mg to 100 mg $Mg^{2+}$/100 g, namely: cheeses, some fish (*e.g.* mackerel, cod), barley groats, whole-meal bakery products, some vegetables (*e.g.* spinach, pea), and some fruits (*e.g.* banana, blackberries). The third group includes products with a low magnesium level – below 25 mg $Mg^{2+}$/100 g – *i.e.* milk, eggs, haslets, light flours and bakery products, rice, most of fruits and vegetables [Olędzka, 1999; Kunachowicz *et al.*, 1998].

It is worth emphasising that magnesium availability from the products mentioned can be respectively increased or decreased. It is due to the presence of multiple components in food which may act either as activators or inhibitors of magnesium absorption. Compounds that decrease the availability of this ion include phytic and oxalic acids (forming hardly soluble salts with $Mg^{2+}$ ions), fluorides, sulfides, phosphates, saturated fatty acids, and heavy metals (especially lead and mercury). Compounds demonstrating beneficial effects on magnesium availability include mainly vitamin $D_3$, unsaturated fatty acids, lactose, and pyridoxine [Olędzka, 1999; Rudziński, 1998].

It seems that improper composition of a diet, *i.e.* consumption of food products with a low magnesium content and subsequent high intake of components decreasing its availability from food, constitutes a significant cause of deficiency of this element.

Magnesium availability is directly linked to the current health condition of humans. Diseases of the gastrointestinal tract and kidneys or hyperthyreosis may be the reasons of its deficiency. Deficit of this element occurs also in the diabetics and the alcoholics. A loss of $Mg^{2+}$ may also be caused by administration of some medicines, *e.g.* diuretics (ethacrynic acid) or antibiotics (gentamycin, tetracyclin) [Walker, 1994].

Contemporary lifestyle of humans, haste, continuous stress, noise, increased physical or mental activity, and alcohol consumption may reduce magnesium content in our organisms even by 50% [Skibiński, 1998].

Prophylaxis of magnesium deficiency involves the application of pharmaceutical preparations. At present, they are widely distributed on the market and most often contain inorganic (carbonates, chlorides and oxides) and organic salts of magnesium (*i.a.* lactates) [Witkowski, 1998].

Pharmacological supplementation is a very popular method to prevent deficits of bioelements. According to Świątkiewicz and Koreleski [1998], magnesium availability from those preparations is, however, insufficient and often does not exceed 15%. Those authors claim that the availability of mineral components, including magnesium, is greatly enhanced when they form linkages with proteins, hence occur in the form of the so-called "bioplexes". Also Krejpcio *et al.* [1999] emphasize that magnesium absorption from preparations of inorganic salts (chloride or sulfate) does not reach the expected level.

Vandergrift [1991] and Mardarowicz [1997] point to a better availability of elements administered in the form of protein linkages.

In recent years, the Alltech company [Kafka, 2002] has commenced the production of preparations containing deficit elements in the form of bioplexes which when applied in animal feeding were found to produce beneficial effects, *i.e.* enhanced reproduction, better growth or increased resistance to diseases and stress.

## YEASTS AS RAW MATERIAL FOR THE PRODUCTION OF PROTEIN-MINERAL PREPARATIONS

Yeasts constitute a rich source of protein with a high nutritive value. Its content in cellular biomass has been reported to reach from 45% to 75%, 80% of which is represented by albuminoid nitrogen, 12% – by nitrogen of nucleic acids, and the remaining part – by free nucleotides and other nitrogen compounds without amino acid structure [Stasińska, 1999]. Yeast protein is characterised by a high digestibility of some exogenous amino acids, especially lysine. Its content in the protein of *S. cerevisiae* and *C. utilis* accounts for 8.2 and 7.1 g/100 g protein, respectively, whereas in 100 g of standard egg white – for 6.4 g. Despite a low content of sulfuric amino acids (methionine and cysteine), yeast-originated protein demonstrates balanced amino acid composition and high nutritive value. In the case of *S. cerevisiae*, the Protein Efficiency Ratio (PER) reaches 2.0, hence it is comparable with the PER value of beef protein (*ca.* 2.3) or soybean flour (from 1.4 to 2.2) [Robinson *et al.*, 2000].

In addition, yeasts are a rich source of B-group vitamins (especially thiamine, riboflavin, pyridoxine, and niacin) and ergosterol (precursor of vitamin $D_2$) [Boze *et al.*, 1992].

Protein content of yeasts, its amino acid composition and vitamin content are determined not only by the strain but also by the medium and method of culture, hence may be regulated to a considerable extent [Giec & Skupin, 1988].

Yeasts demonstrate relatively low nutritional requirements, therefore some industrial wastes, *e.g.* vinasse, waste sulfite liquors from cellulose plants, sewage from plants of the potato industry, paraffine hydrocarbons from petroleum, as well as whey or methanol, can be applied as culture media for *e.g.* multiplication of yeast biomass [Hui & Khachatourians, 1995], which in turn enables their utilisation. Especially high content of crude protein in fodder yeasts was obtained when the culture medium was either vinasse (*ca.* 550 g of protein/kg d.m.) or waste products of petroleum processing (*ca.* 600 g of protein/kg d.m.); whereas less yeast biomass was produced on cellulose wastes (*ca.* 450 g of protein/kg d.m.).

At the multiplication of cellular biomass, high concentrations of vitamin $B_2$ and niacine may be obtained upon the application of whey or vinasse and cellulose waste products, respectively, as a source of carbon in yeast cultures [Mardarowicz, 1997; Giec & Skupin, 1988].

Genetical modification is another way of increasing protein content of yeasts and improving its nutritive value. In all yeast-originated proteins examined the amino acids that limited their nutritive value were sulfuric amino acids – sum of methionine and cysteine contents [Rozmierska *et al.*, 2001; Boze *et al.*, 1992]. Momose and Gregory [1978] obtained mutants of *Saccharomyces cerevisiae* which synthesized protein with high methionine content (4.3 g/100 g protein) substantially exceeding the level of this amino acid in the standard egg white (2.4 g/100 g protein). Wild-type strains (without mutation) demonstrated a relatively low content of methionine (1.8 g/100 g protein).

Yeasts are able to bioaccumulate elements, including magnesium, which can be permanently incorporated into cellular structures [Liu *et al.*, 2002; Blackwell *et al.*, 1995]. Yeast cells readily adsorb divalent cations on their surfaces due to the presence of phosphomannates in the cell wall and the presence of free carboxyl, hydroxyl, phosphate, and hydrosulfide groups in surface proteins [Chmiel, 1998]. The presence of $Mg^{2+}$ ions in the medium stimulates growth and extends vitality of yeasts. This element stabilizes the structures of cytoplasmatic and mitochondrial membranes, thus protecting the yeast cells against unfavourable effect of the medium, especially against high ethanol concentration, raised temperature and osmotic pressure [Walker, 1998; Walker & Maynard, 1996].

The most rapid magnesium ion biosorption by yeasts from medium is usually observed in the first phases of biomass culture, still binding with intracellular structures proceeds more slowly [Brady & Duncan, 1994]. At subsequent stages of the culture, along with progressing cell aging and decay, yeasts are able to release to the medium only magnesium ions [Walker, 1994].

When linked with enzymatic and structural proteins of yeasts, magnesium is easily available from the gastrointestinal tract of humans and animals. While occurring in the form of bioplexes, it is absorbed in the intestine in a way typical of amino acids, peptides, and proteins, but not cations. This enables avoiding competition between macroelements for the adsorption site. Therefore magnesium administered in the form of bioplexes easily penetrates the intestinal wall, contrary to magnesium occurring as hydrophylic $Mg^{2+}$ cations surrounded by a dipolar layer of water particles whose penetration through the hydrophobic cytoplasmatic membranes is hindered. Thus, much more magnesium reaches the cell interior when it appears in a complexed form compared to $Mg^{2+}$ ions. It is highly probable that elements occurring in the form of bioplexes are transported to different sites in the organism depending on proteins or amino acids they are bound with [Świątkiewicz & Koreleski, 1998; Vandergrift, 1991].

In aqueous environment, bioplexes usually escape dissociation contrary to non-organic salts. Owing to this, microelements occurring in protein complexes are considerably less susceptible to the formation of non-available compounds with other food constituents, *e.g.* phytic acid [Świątkiewicz & Koreleski, 1998]. In the case of overdosing, they produce less detrimental effects compared to these of mineral salts [Demirci & Pometto, 2000].

An additional benefit of bioplexes is the fact that they remain soluble and stable over a wide pH range of the gastrointestinal tract of humans [Vandergrift, 1991].

Cellular biomass of magnesium-enriched yeasts may not only supplement deficiency of this element in a diet but also serves as an agent with both prebiotic- and probiotic properties (especially in the ruminants). In the aqueous environment of the gastrointestinal tract, oligomannans of the yeast cell wall expand and form viscous semi-permeable mass being a substrate for lactic acid bacteria. On the other hand, some pathogens (*e.g.* these of *Salmonella* or *Clostridium* genera) are incapable of metabolizing those compounds and when immobilised in their environment, they are removed from the gastrointestinal tract [Mardarowicz, 1997; Biegańska, 1996].

Spears [1996] reports that in bioplexes the organic bonds of metals may display the character of: cation-amino acid complex, metal ion-amino acid chelates (as a result of forming a semi-polar bond), metal albuminates (chelation of metal ions with proteins), and sometimes metal-polysaccharide complexes (*e.g.* binding metal ions with extracellular exopolysaccharides).

An additional asset of yeasts to be used as a natural source of bioplexes for humans and animals is a short period of generation enabling rapid and high biomass yield.

Yeast species used for the production of protein preparations, including *Candida utilis* or *Sacccharomyces cerevisiae*, are numbered on the U.S. Food and Drug Administration list of compounds *generally recognized as safe for human consumption* (GRAS) [FDA, 2001; Robinson *et al.*, 2000]. Protein preparations have also been produced with the use of other yeast species of the *Sacccharomyces* and *Candida* genera including: *S. carlsbergensis*, *S. fragilis*, *C. tropicalis*, and *C. lipolytica* [Barnett, 2000; Stasińska, 1999; Hui & Khachatourians, 1995].

There is a number of methods for the production of yeast-based protein preparations, *i.a*. plasmolysis, thermolysis, acid-, base- or enzymatic hydrolysis, autolysis, and their multiple modifications [Kłyszejko-Stefanowicz *et al.*, 1999; Komorowska *et al.*, 1998 a, b; Komorowska & Stecka, 1998; Lahl & Braun, 1994; Origane & Sato, 1993; Kollar *et al.*, 1991].

An unquestionable drawback of yeasts, limiting their exploitation for nutritional uses, is a high content of nucleic acids occurring in protein complexes as nucleoproteins (8–15 % in yeast d.m.). The nucleic acids exert an unfavourable impact on human health as they evoke accumulation of uric acid crystals in kidneys and joints, which in turn leads to painful pathological symptoms referred to as gout. It results from a lack of uricase enzyme in the human organism, therefore daily intake of nucleic acids should be reduced to *ca.* 2 g [Rozmierska *et al.*, 2001; Parajo *et al.*, 1995 a].

An important stage in the production process of protein preparations involves the reduction of nucleic acid concentration. So far, multiple ways to decrease their content have been elaborated, thus affording the possibility of implementing higher doses of yeast-based protein preparations as an additive to food products [Stasińska, 1999; Parajo *et al.*, 1995 a, b; Martinez *et al.*, 1990; Damodaran, 1986; Damodaran & Kinsella, 1984]. This can be achieved through the

activity of natural nucleases contained in the extracts of yeast cells, at adjusted optimal temperature and pH parameters. Another way of reducing nucleic acid concentration to the level safe for human health may be incubation of cellular biomass of yeasts at increased temperature and with the addition of several-percent NaCl or KOH. It the preparations obtained, the level of those acids can be hence reduced to *ca.* 2.0–2.6 % [Martinez *et al.*, 1990]. Some authors suggest reduction of the content of nucleic acids in the extracts of cell yeasts with the use of membrane filters. As reported by Wronkowski [1978], the application of dialysis enables reduction of their content to less than 1%.

Parajo *et al.* [1995 a, b] achieved a considerable reduction of the nucleic acid content in the cellular biomass of *Candida utilis* yeasts by means of base hydrolysis, at pH 11–12 and ammonia water medium. An advantage of this method was high reactivity and selectivity of the reagent used which interacted with intracellular complexes of nucleoproteins (*i.e.* complexes of proteins and nucleic acids) without prior destruction of the cell wall. Upon the application of this method, the content of nucleic acids was reduced to the level below 2% (calculated per d.m. of yeast preparation), and the reported changes in the nutritive value of yeast preparation proteins were negligible. As reported by Komorowska *et al.* [1998 a, b], some problem encountered during isolation of intracellular components of yeasts with base hydrolysis involved the possibility of appearing lysine--alanine compounds and products of racemization and amino acid elimination in proteins. These unfavourable transformations, occasionally accompanying the base hydrolysis, may be counteracted by chemical modification of yeast proteins consisting in their succinilation or phosphorylation [Giec *et al.*, 1988; Huang *et al.*, 1987].

Problems connected with the isolation of magnesium bioplexes from the cellular biomass of yeasts have not been fully recognised so far. One of key questions refers to the durability of magnesium bioplexes at the use of standard procedures for the reduction of nucleic acid excess. Answer to this question is still being searched for by authors of this manuscript.

Up to now, the yeast cell biomass has been mainly exploited as a source of protein- or protein-vitamin preparations of microbiological origin [Stasińska, 1999].

A new direction of yeast exploitation, linked with their capability of forming bioplexes, may be their application for the production of protein-mineral preparations. Implementation of such preparations to a contemporary human diet may be a feasible alternative to pharmacological supplementation preventing deficits of some micro- and macroelements. In addition, their application as fodder additives may considerably increase animal production [Iwanska *et al.*, 1999; Lipiński, 1998; Mardarowicz, 1997; Vandergrift, 1991; Fly *et al.*, 1989].

Natural capability of yeasts for bioaccummulation of elements (*e.g.* magnesium) and their permanent incorporation into cellular structures is crucial in the production process of protein-mineral preparations. Magnesium transport to the interior of yeast cells is still explored, nevertheless the first metabolically-independent stage of this process includes binding of $Mg^{2+}$ ions with the cell wall of these fungi [Park, 2003; Birch, 2002; Saltukoglu & Slaughter, 1983].

## BINDING AND INTRACELLULAR ACCUMULATION OF MAGNESIUM IN YEASTS

The cell wall constitutes from 15% to 30% of d.m. of yeast biomass and from 25% to 50% of cell volume, and its thickness ranges from 10 to 70 nm [Brady *et al.*, 1994; Orlean, 1997]. Its composition and thickness are strain-specific, however they are determined to a great extent by growth conditions. Therefore, the cell wall restored in the protoplastization process may not be an exact copy of the original [Park, 2003; Wojtatowicz, 1994].

The cell wall of yeasts is built mainly of $\beta$-1,3 glucans (*ca.* 50% of wall weight) and mannoproteins (*ca.* 40%), followed by $\beta$-1,6 glucans (*ca.* 10 %) and chitin (*ca.* 1%) [Kapteyn *et al.*, 2000; Lipke & Ovalle, 1998; Kollar *et al.*, 1997].

Its outer layer consists of mannoproteins formed during attachment of mannan molecules to aspartic residues of proteins through N-acetylglucosamine dimers [Park *et al.*, 2003; Lipke & Ovalle, 1998]. Mannoproteins are highly-glycolysed polypeptides (saccharides constitute 50–90% of their molecular weight), hence they are often referred to as proteoglycans [Van der Vaart *et al.*, 1995].

Particular moduli of mannoproteins bind with each other, thus strengthening the entire structure of the cell wall. The mannoprotein layer formed constitutes a kind of outer defence barrier which determines the permeability of the yeast cell wall [Brady & Duncan, 1994].

The content of $Mg^{2+}$ ions adsorbed in the mono-layer of the cell wall is proportional to the total surface area of yeast cell biomass. During growth, yeast cells are usually smaller in the logarithmic phase than in the stationary phase, thus a higher cell surface area to cell volume ratio is of additional benefit to magnesium binding [Park *et al.*, 2003].

As reported by Lo *et al.* [1999], natural binding of metals with yeast cells proceeds in the form of chemisorption described by the Langmuir equation. At the first stage of this process, $Mg^{2+}$ ions are bound to the wall, mainly with negatively-charged functional groups, and may be further transported to the interior of the yeast cell [Saltukoglu & Slaughter, 1983].

Culture of some yeast species belonging to the *Saccharomyces* or *Candida* genera on media supplemented with $Mg^{2+}$ ions (in the form of non-organic or organic salts, *e.g.* chlorides, sulfates, and lactates) have led to natural and durable enrichment of cellular biomass with this element in the amounts exceeding even 3-fold the physiological demand of those fungi [Błażejak *et al.*, 2003 a, b; Tuszyński & Pasternakiewicz, 2000; Soral-Śmietana, 1999].

Magnesium uptake by yeast cells from the medium is a two-phase process. The first phase involves adsorption of magnesium to anion groups present on the surface of the cell wall, which is independent of cell metabolism and does not require energy expenditure [Gardner, 2003; Chmiel, 1998; Walker, 1994]. In this process, especially active role has been ascribed to carboxyl-, hydroxyl-, amine-, and phosphate groups occurring in the outer, mannoprotein, layer of the yeast cell wall. Removal of this component of the cell wall resulted in 30% reduction of magnesium binding by yeasts [Brady *et al.*, 1994]. Nevertheless, attempts of chemical modification of cell wall mannoproteins have been found unsuccessful in increasing the capability of those fungi for binding metal ions [Brady & Duncan, 1994].

The second phase, metabolically-independent and proceeding much slower than the chemisorption on the cell wall surface, is bioaccumulation. Usually this phase involves magnesium transport through the wall and cytoplasmatic membrane into the cell interior [Blackwell *et al.*, 1995].

Studies on the capability of baker's yeasts *S. cerevisiae* for intracellular accumulation of $Mg^{2+}$ ions [Błażejak *et al.*, 2003 b] have indicated that *ca.* 80% of the total magnesium pool permanently bound with cells during logarithmic phase of growth on experimental media were present in the cell wall of yeasts, whereas *ca.* 20% occurred in protoplasts. Taking into account nearly 3-fold increase in magnesium content of cells, compared to culture on control media, and over 30% increase in the content of crude protein, it may be assumed that in the stationary phase a part of ions previously adsorbed on the cell surface will be subject to intracellular accumulation in the form of magnesium bioplexes.

Temperature, pH, a number of yeast cells in the medium, and the presence of other ions exert a significant impact on the course of magnesium biosorption by cells [Park *et al.*, 2003; Tuszyński & Pasternakiewicz, 2000; Mowll & Gadd, 1984].

According to literature data [Brady & Duncan, 1994; Fourest & Roux, 1992], the optimal temperature of magnesium biosorption process ranges from 25 to 35°C, whereas the optimal pH from 4.0 to 8.0.

Low temperature (below 5°C) has an inhibitory effect on the process of element binding by yeasts, whereas at a temperature range of 5–25°C, the ability of cells for metal biosorption remains unchanged [White & Gadd, 1987].

The capability for binding metal ions may also be inhibited by the excessive number of yeast cells in the medium [Park *et al.*, 2003].

A similar impact on the dynamics of biosorption has also been displayed by cation concentration. As reported by Park *et al.* [2003], specific adsorption of $Cd^{2+}$ ions on the surface of yeast cells was substantially diminished along with increased concentration of cadmium ions in the medium.

A number of ions demonstrate intercorrelations during transport and adsorption into the interior of yeast cells. Uptake of *e.g.* strontium by cells from the medium reduces the possibility of simultaneous bioaccumulation of magnesium. Transport of $Sr^{2+}$ ions proceeding with enhanced activity may even lead to the partial removal of magnesium from vacuoles of yeast cells [Avery & Tobin, 1992]. Cadmium has also been reported to negatively affect the binding process of $Mg^{2+}$ ions and to reduce their number in the cell interior. Whereas magnesium has been observed to deteriorate the yeast capability for bioaccumulation of zinc [Blackwell *et al.*, 1995].

An important factor that affects magnesium uptake is yeast viability. The greater the cell yeast viability, the higher the degree of magnesium binding [Blackwell *et al.*, 1995]. The cell walls being in the logarithmic phase of growth should, therefore, bind the highest number of $Mg^{2+}$ ions, which has been confirmed in studies by Błażejak *et al.* [2002, 2003 b].

Magnesium uptake by yeast cells may also involve mechanisms designed for other elements, *e.g.* the system of $Fe^{3+}$ transport. Trivalent iron is transported by specific ligands – iron-absorptive components. They are assumed to demonstrate affinity also to magnesium and may be used by the cell for bioaccumulation of this element. Nevertheless, this process proceeds slowly and may be applied for the uptake of $Mg^{2+}$ ions only in the case of their high deficiency in the medium [Emelyanova, 2001].

Investigations carried out in recent years [Gardner, 2003; Graschopf *et al.*, 2001; Bui *et al.*, 1999; MacDiarmid & Gardner, 1998] have indicated that some transport proteins (*e.g.* Alr1p and Alr2p) participate in magnesium uptake from culture medium by yeasts. They belong to *membrane integral proteins* (MIT), which in turn are a component of cytoplasmatic membrane. The Alr proteins (aluminum-resistant permeases) demonstrate some structural resemblance to bacterial transporters of CorA (cobalt-resistant albumins) identified in *Salmonella typhimurium* which are also involved in transport of $Mg^{2+}$ ions [Liu, 2002; Hiomura & Sakurai, 2001].

The Alr proteins are characterised by high inter-resemblance in terms of their structure, molecular weight, and isoelectric point. They both are built of a single polypetide chain, and their molecular weight reaches 95.9 kDa for Alr1p and 96.7 kDa for Alr2p [MacDiarmid & Gardner, 1998]. They are localised in the cytoplasmatic membrane of yeast cells, and their expression is strictly linked with the concentration of $Mg^{2+}$ ions [Graschopf *et al.*, 2001]. According to Liu *et al.* [2002], these proteins are likely to generate an appropriate potential difference at both sides of the cytoplasmatic membrane and function as protein ionic channels. It has been demonstrated that a yeast strain with immobilised *ALR1* gene, encoding the Alr1p protein, is characterised by nearly 60% lower magnesium content in the cell, compared to a wild-type strain. Subsequent studies have confirmed that at increased concentration of $Mg^{2+}$ ions in the medium (up to *ca.* 200 mmol $Mg^{2+}$/L), a mutant strain (with immobilised *ALR1* gene) demonstrated only 25% lower content of that element in the cell, compared to the wild-type strain. Finally, it turned out that the Alr1p protein is active in the cell only when the concentration of $Mg^{2+}$ ions in the medium does not exceed 100 mmol/L. Whereas with magnesium concentration increasing in the medium, the contribution of the Alr1 protein in $Mg^{2+}$ transport diminishes. In the end, the Alr1 protein is either degraded in the vacuole or modified so that it can no longer serve as magnesium transporter. Then binding and bioaccumulation of ions proceed with other mechanisms, *e.g.* Alr2p protein transporters appearing in cells which compensate for the lack of the Alr1p proteins [Graschopf *et al.*, 2001].

Apart from Alr1p and Alr2p, other protein transporters have also been recognised, including Mrs2p and Lpe10p engaged in the process of magnesium bioaccumulation in yeasts [Gardner, 2003; Graschopf *et al.*, 2001; Gregan *et al.*, 2001]. They serve a similar function as protein transporters of MgtA and MgtB magnesium occurring in the periplasmatic space of Gram-negative bacteria [Hiromura, 2001]. Closely related Mrs2p and Lpe10p are responsible for transport of $Mg^{2+}$ ions into the inner mitochondrial membrane and maintaining their proper concentration therein [Gardner, 2003; Graschopf *et al.*, 2001; Gregan *et al.*, 2001].

Processes of intracellular accumulation of magnesium may also involve low-molecular proteins with a high content of cystein, the so-called "metalothioneins", as well as transport mechanisms of sodium and cadmium [Truchliński & Pasternak, 2002].

**SUMMARY**

Up-to-day studies into magnesium binding by yeast cells have not provided explicit answers to all questions referring to bioaccumulation of this element.

It is known that during yeast culture on media supplemented with $Mg^{2+}$ ions a rapid magnesium adsorption proceeds on the surface of the outer layer mannanoproteins of the cell wall, which is followed by much slower process of magnesium transport through the cytoplasmatic membrane into the cytosole interior [Błażejak *et al.*, 2003 b].

Neither the form of the ion (whose intracellular concentration may be 30-fold higher than in the direct surrounding of cells [Walker, 1994]) nor the yeast cell structures it appears in have been identified so far. It is hardly likely that, at low magnesium concentration, it could appear in the form of free $Mg^{2+}$ ions, since it would considerably disturb the osmotic homeostasis of a cell. It seems that the most appropriate site for accumulation of excessive $Mg^{2+}$ ions may be vacuoles, provided that magnesium will not occur there in the form of hardly-soluble phosphates, but in the form of complexes with saccharides, amino acids or proteins easily available for the needs of cellular metabolism.

In this case, isolation of cellular proteins from yeast biomass enriched in magnesium (which is usually accompanied by a decrease in the content of nucleic acids to the level safe to human health) would afford the possibility for obtaining a natural protein-mineral preparation supplementing magnesium deficiency in a diet.

Recent studies [Park *et al.*, 2003] point to a relationship between cell morphology (especially cell size and thickness of the outer mannoprotein layer) and capability for the accumulation of $Cd^{2+}$ ions by *Saccharomyces cerevisiae* yeasts.

It may be assumed therefore that the intracellular accumulation of $Mg^{2+}$ bioplexes by cell yeasts will follow the same rules.

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# BIOMASA KOMÓRKOWA DROŻDŻY JAKO POTENCJALNE ŹRÓDŁO BIOPLEKSÓW MAGNEZU

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Zdolność drożdży do wiązania magnezu w ilościach przekraczających zapotrzebowanie fizjologiczne stwarza możliwość wykorzystania tych organizmów jako naturalnego źródła deficytowego biopierwiastka w diecie współczesnego człowieka. Mechanizm wiązania kationów z komórkami drożdży mający najpierw charakter chemisorpcji, a później wewnątrzkomórkowej bioakumulacji, prowadzić może do tworzenia organicznych połączeń magnezu określanych jako biopleksy. Jony magnezu związane z białkami w postaci tzw. biopleksów są bardzo dobrze przyswajalne przez organizmy ludzi i zwierząt, a zatem mogą stanowić alternatywę dla suplementacji farmakologicznej przy coraz powszechniejszym niedoborze tego pierwiastka. W tej sytuacji biomasa komórkowa drożdży po odpowiedniej obróbce obniżającej zawartość kwasów nukleinowych może być nie tylko wartościowym źródłem białka i witamin, lecz również magnezu w postaci preparatów białkowo-mineralnych.