APPLIED ENZYME NANOREACTORS IN MEDICINE AND FOOD BIOPROCESSING

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Abstract

Enzymes nanoreactors are lipid bilayer vesicles that contain free enzymes in the trapped aqueous space. Through incorporation of some biological units (functional proteins) in vesicular walls, these systems can mimic living cell because, they can act as transfer channels for some molecules between environment and inner vesicle water soluble area (vesicle permeability) or can act as receptors for living cells.

Entrapped free enzymes can act on specific substrates, which can diffuse from environment inside of vesicles, and the enzyme reaction products can diffuse outside of vesicles where they can be used as bioactive compounds, drugs in living organisms or reagents in analytical determinations (*in vitro*).

In this paper structures of enzyme-containing vesicles will be presented and discussed, and some possible applied enzyme nanoreactors in medicine and food bioprocessing

Key words: enzyme nanoreactor, lipid bilayer vesicle, bioprocessing, nanomedicine, xenobiotic

1. Introduction

Nanotechnology is a new and fast developed domain of science, related to the structure, manufacture and use of functional systems on nanometer scale (atomic, molecular and macromolecular).

Some of the applications of nanostructure materials are in energy conversion, electronics, medicine, textiles, cosmetics, agriculture, but also in food bioprocessing.

The interest in the medical applications (*in vivo* or *in vitro*) of nanotechnology has led to a new scientific field called nanomedicine [11].

Nano delivery systems utilized small vesicles with nano diameters (20-200 nm) which are important in medicine and food because they involve absorption, incorporation or dispersion of some bioactive products. Also, the incorporated bioactive products in vesicles maybe delivered to tissues and cells or in hydrophobic or hydrophilic matrices, in protected, soluble or stable conditions.

Bilayer vesicles, structural and functionnal, sow with membrane cells or membrane cellular organelles.

Internal cellular organelles and cells are enveloped by membranes composed of proteins associated with a lipid bilayer matrix (phosphorlipids, sphingolipids, free cholesterol). Proteins represent the functional components of biological membranes and lipids represent the environment where such functional components are located [17].

Similarly to lipids, amphiphilic copolymers can self assemble into membrane biomimetic structures in aqueous media. Depending on the chemical properties, concentration, molecular structure and block length ratio, amphiphilic copolymers can aggregate into micelles, membranes, vesicles or tubes [9, 10].

Vesicles as nanoreactors or delivery compounds

Amphiphilic compounds can be used for the preparation of biomimetic bilayer structures (nano particulate vesicles) as lipid dispersions containing trapped compounds (enzymes, hormones, peptides, drugs) in aqueous space. These vesicles are analogous to liposomes, but also different, because of their external bilayer formed from amphiphilic (amphipathic) copolymers [1].

Also, vesicles are spherical aggregates containing amphiphilic molecules, disposed in two or more concentric bilayers, which has an inside aqueous area (Fig.1).

The internal bilayer content is moulded from the hydrophobic part of the amphiphilic molecules, while the external parts of the bilayer contains the hydrophilic zone of the amphiphiles, being in contact with aqueous phase (inside and outside of vesicle).

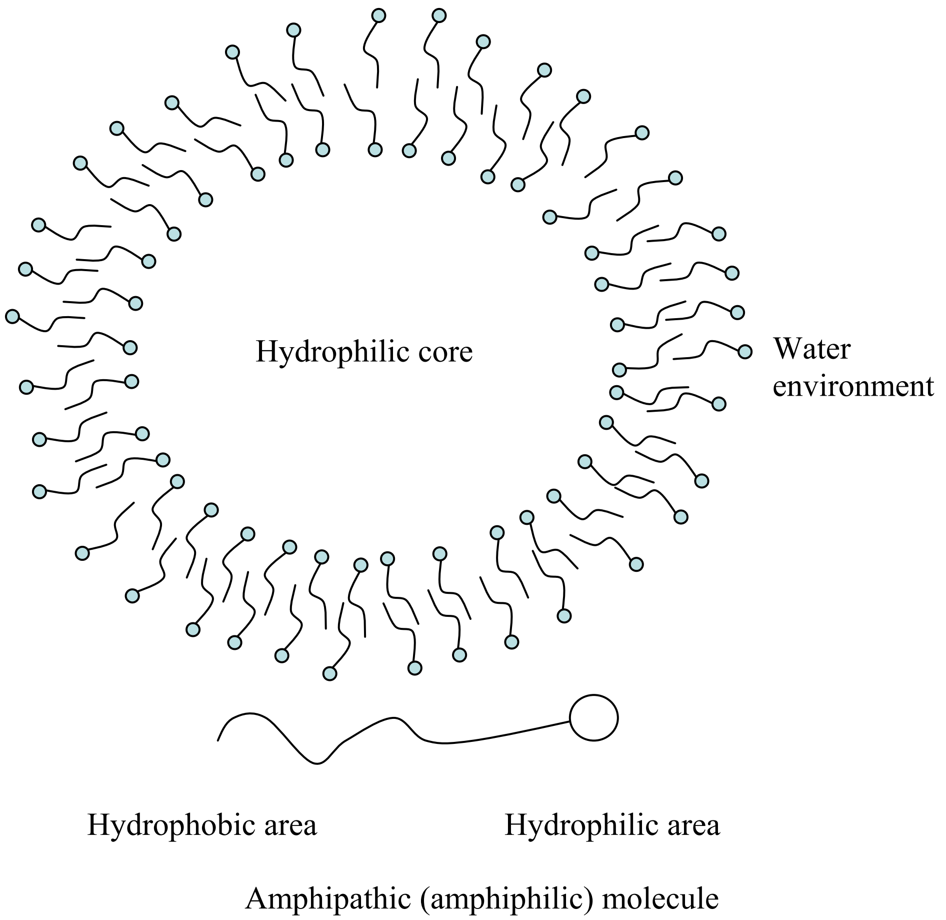

Fig.1 General structure of vesicles

Single or multi compartment closed lipid or (co)polymer bilayer structures, with hydrophilic central core, are named in this paper vesicles. They do not represent the termodynamically stable state of amphiphiles, thus may involve vesicles aggregation or fusions. Because the fluids on both sides of vesicles exert hydrostatic pressure, these adopt a big variety of equilibrium shapes in external flow [8]. Vesicles (nanocapsules) are not spontaneously formed; they require external energy to constitute. The vesicle interior (core) is an aqueous medium and may encapsulate water-soluble high molecular weight compounds (proteins, glycoproteins, drugs, hormones), which are entrapped and isolated in the vesicle [18].

Similarly to membrane, insertion of specific channel membrane proteins, in vesicular walls, can increase their permeability.

Through incorporation of biological units (functional proteins) in vesicular walls, these can act as transfer channels for molecules between core vesicles and environment (vesicle permeability) or as receptors for membrane cells (Fig.2).

These possibilities can be used for communications between synthetic and living matter with a huge impact on the development of compounds (drugs) carrier and delivery (vectors), enzyme reactions inside of vesicle (nanoreactors) or in diagnostic tools [15].

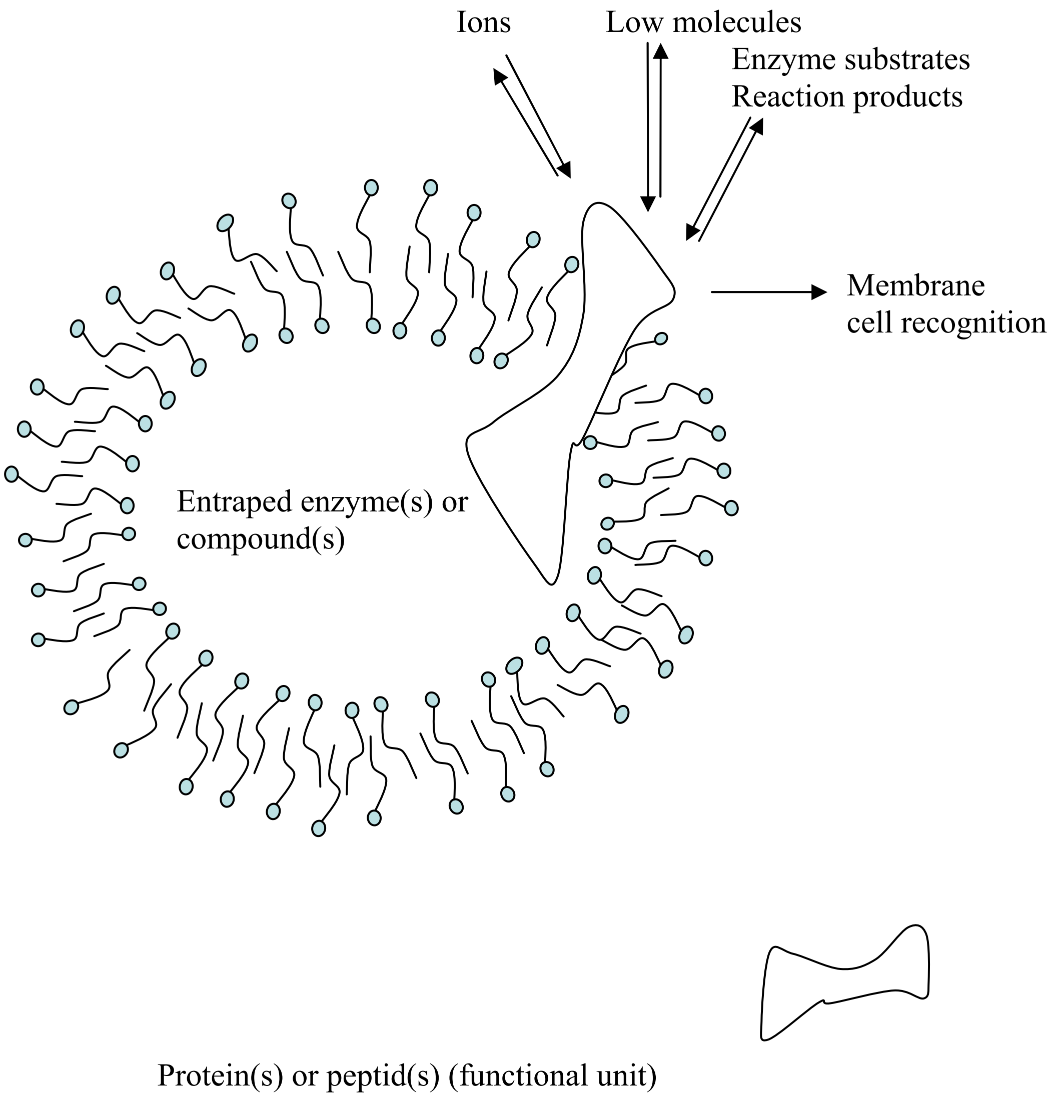

Fig.2 General structure of nanoreactors or compounds delivery vesicles

These vesicle characteristics (protein channel, binding capacity, drug delivery, labelled capacity: receptor; lectin; antigen; immunoglobulin) may led to utilization of vesicles as cells imitation.

The lipid vesicles are just carriers for the free enzymes or/and resulted compounds, which are protect from immediate contact with the medium, and their activity is conserved in time.

The entrapped enzymes can not be released from the vesicles (nanoreactors), because their large molecules, they can catalyze specific reaction by transformation of substrates, which penetrate (diffuse) through the lipid bilayers to meet the enzymes, in the internal aqueous area. The reaction products, which can by xenobiotics in living organisms, must be released from the vesicles in medium (diffusive processes), and the transformation process continues until the substrates is lost.

Vesicles can be carriers for the entrapped compounds, as well as for their transformation products, that are gradually released to a particular vesicle receptor, which can be a controlled process (time, compounds concentration and activity).

Potential enzyme-containing vesicles, used in (bio)medical applications, are: peroxidase, hexokinase, β-glucuronidase, glucose-6-phosphate-dehidrogenase, glucose oxidase, β-galactosidase, streptokinase, asparaginase alkaline phosphatase, butyryl-cholinesterase, superoxide dismutase [5, 6, 7, 12, 16, 19].

Possible applied nanotechnologies in the food chain are: agricultural production, food processing, food additives or supplements, food preservation and packaging.

Nanoparticles may appear in food in suspension or in emulsion and they may have different structures and shapes which can be [2]:

- solid nanoparticles (spherical, nonspherical or tubular homogeneous compact particles and homogeneous or heterogeneous aggregates consisting of a single or diverse particle class);
- lipid based nano delivery systems (single or bilayer lipid vesicles);
- polymer based nano delivery systems (aggregated copolymers, nanospheres, nanocapsules).

Enzyme-containing lipid vesicles with potential applications in food biotechnology are presented in more papers [4, 13, 14]:

- β-galactosidase – as food aditive for lactose intolerance treatment; in milk lactose is hydrolyzed to glucose and galactose;
- chymosin, proteinase, trypsyn and phospholipase C – for the acceleration of cheese ripening;
- neutrase and rulactine – acceleration of cheese ripening and flavour improving;
- serine protease from *Novozymes* and enzymatic extract from *Lactobacillus helveticus* – influence the cheese ripening process.

For cheese ripening process, the entrapped enzyme, from enzyme containing lipid vesicle, is gradually released, allowing catalyzed degradation and modification reactions in the cheese matrix, during the ripening period.

Nanoscience and nanotechnology have great potential in functional and nutritional foods preparation (nanofood), for delivering bioactive compounds to improve human health [3].

3. Conclusion

In this paper we have limited to an overview on vesicles with entrapped water soluble compounds (enzymes), the reactions occurring inside the vesicles, as well as the role of the reaction products in food production, preservation, packaging, or their role as additives or supplements in food.

Depending on the enzyme used and depending on the application, the nature of the vesicle amphiphile and the preparation method can be selected.

Nanotechnology promise in future results and applications for both medicine and food bioprocessing.

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