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Note

Preliminary investigation of some polysaccharides as a carrier for cell entrapment

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Abstract

Entrapment of cells within spheres of polysaccharide gel has become the most widely used technique for immobilizing living cells. Polysaccharide pectin, formed gel with calcium ions, was investigated as a precursor of spherical calcium polysaccharide gel beads. The type of pectin sample was found to be important in the formation of the beads. Partially deesterified pectin with a lower degree of esterification provided spherical beads and was chosen for immobilization of the yeast cells, *Sacchararomyces cerevisiae*, and compared to those with alginate. The effect of storage condition of the beads on the viability of the entrapped cells was also studied. After storage at 4°C or –40°C for 1 month, even lyophilization before storage, the beads with entrapped cells were sufficiently stable when compared to suspension of non-entrapped yeast cells.

Keywords: Polysaccharide; Pectin; Immobilization; Gel entrapment; Living cells; Ionotropic gelation

1. Introduction

The production of biocatalysts by ionotropic gel formation is one of the simplest, cheapest and mildest immobilization methods [1]. It has developed worldwide to become the most popular entrapment method after being first introduced by Hackel et al. [2]. The components used are nontoxic and give no cause for concern as regards food regulations so that even sensitive cell systems can be entrapped while maintaining their viability. Formation of spherical gel beads is spontaneous and results from ionic network formation [3], which is based on an ionic cross-linking of polyelectrolytes, such as alginate, carageenan, chitosan and pectin, with multivalent counter-ions. Spherical beads are produced dropwise by adding a cell suspension in a solution of a polysaccharide to water containing multivalent counter-ion salts. Most mammalian and plant cell types are suitable for immobilization by this technique.

The polysaccharide pectin is an inexpensive product isolated on a large scale from citrus peels or apple pomaces. Pectin consists mainly of linearly connected α-(1 $\rightarrow$ 4)-d-galacturonic acid residues which have carboxyl groups. The degree of esterification (DE), which is expressed as a percentage of carboxyl groups (esterified), is an important means of classifying pectin. Only low-methoxy pectin (with DE $<$50%) forms rigid gels by the action of calcium, which cross-links the galacturonic acid chains [4]. Since pectin can react with calcium ions, it is currently being investigated as a carrier material for different controlled release systems. Recently, calcium pectinate gel beads have been investigated as a sustained release drug delivery system [5].

It was the aim of the present work to extend the range of materials suitable for entrapment of living cells by ionotropic gelation. The primary focus was on an anionic water-soluble polysaccharide, pectin, as precursor to cation-polysaccharide gels in spherical form. The appearance and the

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mechanical stability of gel beads were chosen as parameters for the characterization of the gel as a matrix bead for the entrapment of yeast cells. The properties of the studied pectin matrices were compared with those obtained from calcium alginate gel beads.

2. Materials and methods

Sodium alginate (medium viscosity) and polygalacturonic acid were purchased from Sigma Chemical (MO, USA). GENUpectin type LM-104 AS-FS (with DE of 28%), type LM-101 AS (with DE of 36%), and type LM-101 AS-JS (with DE of 38%) were the generous gifts of Copenhagen Pectin (Denmark). All other chemicals were of reagent grade and were used as received. Cells of the yeast *Saccharomyces cerevisiae* were used throughout the experiment.

Sodium alginate (ALG), polygalacturonic acid (PGA), GENUpectin type LM-104 AS-FS (P28), type LM-101 AS (P36), and type LM-101 AS-JS (P38) were investigated as cell immobilization matrices. These polysaccharides were prepared as gel beads without cells by an ionotropic gelation method as follows. The aqueous solution of alginate (1% w/v) or PGA or pectin (5% w/v) was dripped through a needle (inner diameter 0.8 mm) into a stirred precipitation bath (0.3 M calcium chloride). The spherical beads were incubated in the precipitation bath for 20 min. The beads formed were then investigated for the appearance, shape, size as well as mechanical stability. The mechanical stability of the beads was observed, by stirring them in phosphate buffer pH 7.4 at 100 rpm. The bead deformability, which was chosen as a criterion of mechanical stability of the beads, was measured as a product of the force and time required to compress the gel bead to constant strain. The suitable pectin formulation was chosen for the immobilization of the yeast cells and compare to that with alginate.

A cell suspension (2.5 g dry mass) and an aqueous solution of pectin (5 g) or sodium alginate (1 g) were mixed to 100 ml with sterile water and dropped in to a 0.3 M calcium chloride solution. The beads were left to harden in calcium chloride for 20 min. All processing was carried out in a laminar air flow hood and using an aseptic technique. The beads were then collected and stored for 1 month at 4°C under static conditions in calcium chloride solution, or lyophilized before storage, or kept under frozen conditions (−40°C). The storage stability and viability of entrapped yeast cells were tested with respect to the consumption of glucose. Three hundred beads containing cells were added to 200 ml of 5% d-glucose in minimum salt medium. The experiment was performed over 24 h at 28°C while the glucose concentration in the vessels was measured polarimetrically according to USP XXIII.

3. Results and discussion

Cell entrapment in a matrix is the method most widely and universally employed, and is one which is also suitable for the immobilization of living cells [1]. By using the ionotropic gelation technique, spherical calcium pectinate gel matrices were obtained [5]. In this case, the cells are located in a polymer network (beads) which entraps the cells as in a cage but still permits the diffusion of product and substrate [6].

The type of pectin sample was found to be important in the formation of the beads. The degree of esterification of carboxyl groups with methanol, i.e. the content of methyl esters, is another important property of pectins affecting the binding of divalent cations. A degree of esterification of 30%, is the limiting value for the formation of calcium gels whereby complete deesterification of pectin to pectate (polygalacturonate) is found to be advantageous [7]. In contrast to an earlier report [8], however, spherical gel beads could not be prepared from sample with PGA. Thus, pectins used in the present work were partially deesterified pectins. The fact that polygalacturonate or pectin is the mirror image of polyglucuronate (part of alginate), except in the configuration at C_2, means that an analogous mechanism of cation binding might be anticipated. Also, the activities of calcium ions in solutions of calcium salts of oligogalacturonates and oligoguluronates exhibit a sudden decrease approximately within the same range of polymerization degree [7]. The results referred to here indicate that an identical mechanism of binding of calcium cations,

Table 1
Appearance, size and stability of gel beads

Material	Shape[a]	Size[b] (mm ± SD)	Mechanical stability[c] (10^3 g × s)		
			4 h	8 h	24 h
Calcium alginate (ALG)	Spherical	1.98 ± 0.08	6.114	6.008	5.875
Calcium pectinate (P28)	Spherical	2.14 ± 0.06	5.982	5.843	5.441
Calcium pectinate (P36)	Drop-like	2.21 ± 0.12	5.211	4.056	3.141
Calcium pectinate (P38)	Drop-like	2.25 ± 0.24	4.852	3.726	1.487
Calcium pectate (PGA)	Irregular	–	–	–	–

[a]Shape of the formed beads was investigated immediately after preparation.
[b]Mean size of the beads was calculated from 50 measurements by microscopic method.
[c]Mechanical stability of the beads was tested with respect to the bead.

the so-called egg-box model of intermolecular binding of calcium [3], is involved.

The appearance, mean size and stability in phosphate buffer of beads are given in Table 1. Fig. 1 shows a surface appearance of a bead (P28) without cells where the typical appearance of an ionotropic gel can be clearly recognized. The shape of beads with P28 and ALG was spherical while that with P36 and P38 was elliptical. The mean diameter of beads was approximately 2 mm. Furthermore, the beads with P36 and P38 decomposed with time and completely decomposed after 3 and 2 days, respectively, while those with P28 and ALG were still intact up to 7 days. The rather weak gel also allows cell leakage. Therefore, the formulation with P28 was chosen for the immobilization of the yeast cells and compared to that with alginate.

The stability of both types of gel beads, P28 and ALG, in the media did not permit the direct determination of viability, e.g. by the determination of colony-forming units [9]. Therefore, the viability of entrapped cells was tested indirectly, by means of parameters indicating the metabolic activity of living cells, i.e. glucose consumption. The result shows that no significant difference of metabolic activity of cells immobilized in P28 and ALG beads ($P > 0.05$). The

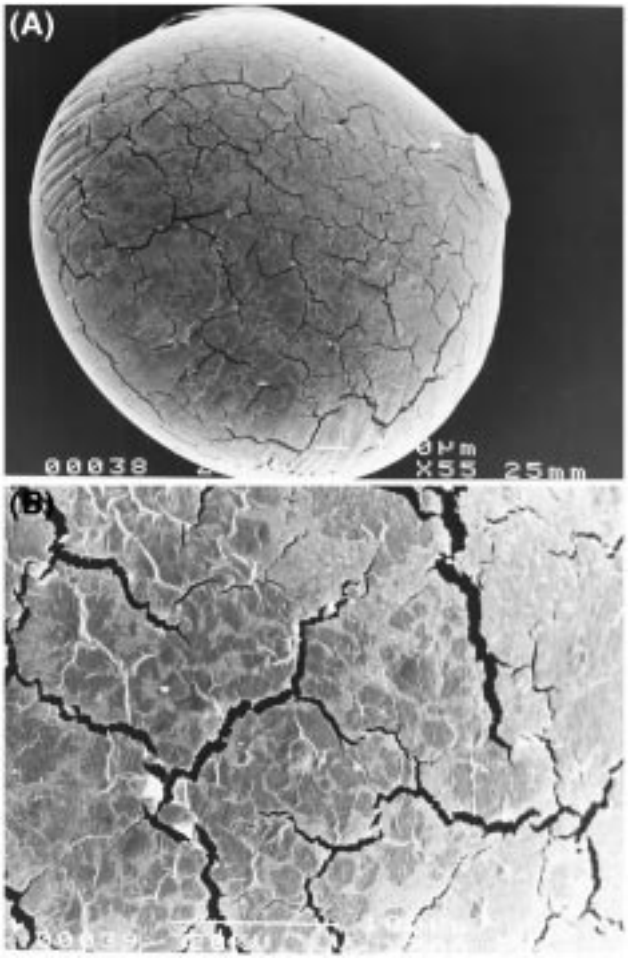

Fig. 1. Scanning electron micrographs of calcium pectinate (P28) gel bead without cells of two magnifications, ×55 (A) and ×300 (B).

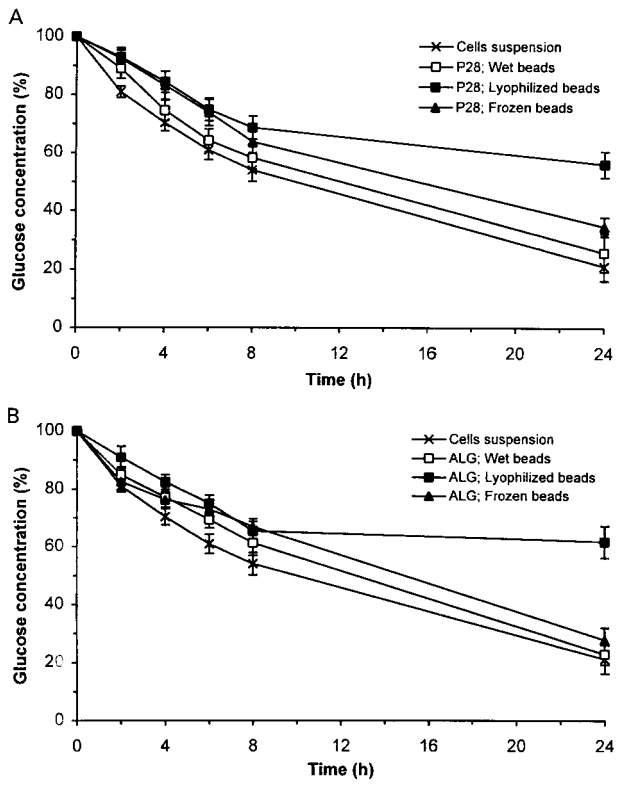

Fig. 2. Effect of storage condition of the entrapped pectin ((A); P28) and alginate ((B); ALG) beads on the glucose consumption. The concentration of pectin was 5% (w/v) and that of alginate was 1% (w/v).

metabolic activity of entrapped cells stored in the calcium chloride solution at 4°C and at −40°C do not differ from the non-entrapped cells ($P > 0.05$), in both alginate and pectin beads (Fig. 2). This indicates that the entrapped cells were viable and still active. However, the activity of immobilized cells in the lyophilized beads was decreased ($P < 0.05$). Temperature profiles during various stages of the lyophilization process are critical [10], and may affect the viability of the living cells. These results imply that pectin can be used as an alternative carrier to alginate.

In summary, this study demonstrates that pectin with DE of 28% (P28) was suitable for entrapment of living cells by gel entrapment. Calcium pectinate gel beads were successfully prepared using ionotropic gelation and appear to provide an alternative carrier to calcium alginate. Since pectin is a readily available, highly innocuous product, its use as a carrier matrix for entrapment of living cells is strongly recommended. However, additional experiments, including their stability in a packed bed reactor, would be required for a more reliable confirmation of this result.

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