EFFECTS OF ELECTROLYTE ON GELLAN, MONITORED BY DIFFERENTIAL SCANNING CALORIMETRY

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The extracellular polysaccharide, gella gum is obtained from the aerobic fermentation of *Pseudomonas elodea*. It has a tetrasaccharide repeating unit that consists of the monosaccharide building units L-rhamnose, D-glucose, and D-glucuronic acid in the molar ratios 1:2:1. The gellan molecule exists in aqueous solution as a disordered coil at high temperature and it converts reversibly to an ordered helix on cooling. The conformational transition established from different techniques (optical rotation, light scattering, viscosity, conductivity) is in agreement with a two coil to one double helix reversible transition. The conformational state of gellan gum is a sensitive function of the ionic strength, the nature of the added counter ions and temperature. By use of the differential scanning calorimetry (DSC), the conformational change of deacetylated gellan has been investigated. The introduction of cations increases the number and strength of the junction zones in the helical conformation, thus, controlling the amount of aggregation upon gelation.

From the DSC data the enthalpy ΔH and the peak temperature T_m of melting were obtained. The values of T_m usually vary for biopolymers as a function of the total ionic counter ion concentration C_T. C_T was calculated for each salt concentration and ln C_T plotted againt T_m^{-1}. The Manning polyelectrolyte theory (Manning, 1970) predicts that the slope is directly related to the enthalpy of melting by the equation

$$\Delta H = -R(\Phi_c - \Phi_h)\, \mathrm{d}\ln C_T / \mathrm{d}(1/T_m)$$

(Φ_c and Φ_h are the osmotic coefficients).

Using this relationship, ΔH was found to be 20·21 kJ/equiv. This value corresponds to the enthalpy of melting at infinite electrolyte dilution in the absence of any aggregation. The experimental exothermic enthalpies, ΔH of gellan gum solutions have been monitored as a function of external salt concentration C_s. The experimental value of 4·58 kJ/equiv. obtained in the absence of electrolyte is significantly less than the theoretical value predicted by the Manning polyelectrolyte theory. This discrepancy may be due to polymer aggregation.

Reference

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CHARACTERISATION OF COLLOIDAL GAS APHRONS (CGA-s) FOR PROTEIN RECOVERY

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Colloidal gas aphrons are microbubbles composed of a gaseous inner core surrounded by a thin soapy shell and are created by intense stirring of a solution containing surfactant. Owing to their size (from 10 to 100 μm) and structure they show colloidal behaviour, thus the main interaction forces governing this type of dispersion are caused by surface forces and electrostatic interactions.

Downstream processing, which involves the recovery, purification, separation and concentration of the products, is one of the more difficult and troublesome stages of the overall production system in biotechnological industries. Conversely, recovery steps generally represent a large part of the total capital investment in a fermentation plant. Often proteins are the target in the recovery process, especially enzymes for their use as industrial catalysts. The use of CGA-s for the separation of proteins is thought to be an attractive method for application in industry, where low cost and high efficiency, within the safest environmental conditions, are the main concerns. Other applications of the CGA-s that have been reported are:

- Removal of heavy metals from aqueous solutions (Ciriello *et al.*, 1982)
- Separation of ogranic dyes from waste water (Roy *et al.*, 1992)
- Harvesting of *Saccharomyces cerivisiae* (Save & Pangarkar, 1993).

The aim of this presentation is to show some preliminary studies undertaken for the characterisation and optimisation of the stability of the CGA-s for their further application for the separation of proteins.

Statistically designed experiments were developed in order to study the effect of different factors upon the stability of the aphrons. At the same time power consumption measurements were performed during the formation of CGA-s.

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MONOCOMPONENT ENZYMES/PECTIN METHYL ESTERASE

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Several enzymes have been cloned from *A. aculatus* by the expression cloning technique (Dalboege & Heldt-Hansen, 1994) and expressed in a host organism, either *A. niger* or *A. oryzae*. The monocomponent enzymes obtained are substantially free from interfering activities and are likely to be useful for modification purposes of for example, cell wall materials in order to obtain improved functionality. Several types of experimental enzymes are available for application trials on a small scale basis including pectin methyl esterase (PME). The kinetic and mode of action of PME has been further characterised. Pectins are widely used in the food industry — they are often modified from the natural, high methoxylated pectins to a lower level of methylation of the galacturonic acids to obtain new functionalities. PME hydrolyzes the methyl-esterified galacturonic acid residues in pectin. The enzymatically catalyzed process is a useful alternative to the chemically based modification of extracted pectin.

PME from, for example, oranges desterifies pectin blockwise, while chemical methods such as alkaline or acid treatment result in a pectin with a random distribution of the acid groups. In general, pectin esterases of fungal origin are believed to deesterify randomly as well. The distribution of the methyl groups is of importance for the functionality of the pectin product.

With the aim of characterizing the mode of action of the cloned fungal pectin esterase the distribution of the acid groups has been determined by a method described by Mort *et al.* (1993). The esterified galacturonic acids are converted to galactose by reduction with sodium borohydride. Subsequently the glycosidic linkages of the resulting galactose residues are cleaved selectively by HF solvolysis. This leads to the production of oligomers: $(Gal\ A)_n$-Gal. These oligomers represent the contiguous stretches of Gal A residues between methyl-esterified residues in the pectin. The cloned esterase was compared to an orange esterase and to alkaline treatment. By high-performance anion exchange chromatography oligomers of six galacturonic acid residues were separated and quantified. From the distribution of these oligomers it can be determined whether the acid groups in the pectin are randomly or blockwise distributed.

In conclusion it was seen that the pattern of the desterification performed by the cloned pectin esterase resembles that of an alkaline treatment. Minor differences indicate, however, that the esterification performed by the cloned enzyme is not completely random.

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EFFECT OF SUCROSE ON THERMODYNAMIC INCOMPATIBILITY OF DIFFERENT BIOPOLYMERS IN THE AQUEOUS MEDIUM

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Thermodynamic incompatibility is one of the most commonly encountered phenomenon in mixed biopolymer solutions. This phenomenon can be a controlling factor for the structure and physico-chemical properties of foods. The nature and degree of thermodynamic incompatibility depends on the interactions between all the components in the solution. By altering its composition of the aqueous medium by adding different low molecular weight substances it is possible to significantly influence the thermodynamic compatibility of the biopolymers.

Sucrose is second in importance after NaCl as a food taste additive. The necessity for studying the influence of sucrose on the thermodynamic incompatibility of biopolymers follows from the fact that sucrose is a major component of a wide variety of food. In this connection we have attempted to study the influence of sucrose on the thermodynamic incompatibility of a number of biopolymers in aqueous solutions. Three pairs of the biopolymers were chosen as the objects of our investigations, namely, sodium caseinate – ovalbumin, 11S globulin vicia faba – ovalbumin, sodium caseinate – sodium alginate.

The co-solubility of the biopolymers was investigated at different sucrose concentrations in the solution (in the range of 0 to 50% w/v). A big increase in the co-solubility of the biopolymers studied was observed as the sucrose concentration increases in the aqueous medium. It was established that the increase in co-solubility of the biopolymers occurs in accordance with an increase in the protein solubility in the aqueous medium upon sucrose addition. So it is possible to suppose that the same reason provides the basis for both an increase in co-solubility of the biopolymers and solubility of the proteins in the aqueous medium. The thermodynamic parameters of the different types of pair interactions (the second virial coefficients) were estimated using light scattering data in the binary and ternary aqueous solutions of the biopolymers without sucrose and upon addition of 25% w/v of sucrose.

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HEAT-INDUCED GELATION OF GLOBULAR PROTEIN MIXTURES

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It is now accepted that globular proteins form heat set gels under appropriate conditions of protein concentration, pH and salt. Although rheological studies on heat-induced gelation of globular proteins have been performed by several workers, there are only a few studies of the gelation kinetics. Protein gelation induced by heating is largely an irreversible process, so it is essential to understand the kinetics of the gelation. The mixed system of such globular proteins has been investigated for application particularly in the food industry. However, the gelation behaviour is not yet clarified because of the complexity of proteins. In this poster we will discuss the kinetics of heat-induced gelation of two globular protein mixtures on the basis of the gelation time for different temperatures.

The rheological measurement was performed with the Controlled Stress Rheometer CS100 (Carri-Med Co, UK) using cone-plate geometry. Bovine serum albumin (BSA) and b-Lactoglobulin (β-Lg) was mixed for different concentration ratio (10:0 to 0:10 in weight %) and dissolved in deionised water, followed by pH adjustment to 6·6. The strain and frequency were set at 1% and 1 rad/s, respectively. The storage modulus G′ and the loss modulus G″ were monitored as a function of time. The measurement was performed at different temperatures and the gelation time was defined as the time when G′ showed rapid increase because use of the so-called Winter criterion for gelation was impossible due to lack of signal. The gelation time became longer at lower temperatures. At different concentration ratios it was found that the gelation time changes drastically with the ratio of BSA/β-Lg although the change does not seem to be linear against the ratio. The results enable further insight into the co-gelation of mixed globular proteins to be developed.