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Analysis by Raman spectroscopy of the conformational structure of whey proteins constituting fouling deposits during the processing in a heat exchanger

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ABSTRACT

Whey protein fouling deposits generated on the hot wall downstream a plate heat exchanger were analyzed by micro Raman spectroscopy (MRS) carried out in the 800–1800 cm^{-1} range. Deposits were formed using a model beta-lactoglobulin (BLG) fouling solution which was made using a whey protein isolate powder (89 wt.% in BLG) and a known amount of calcium. Thermal denaturation of the fouling solution was also analyzed by MRS as well as isolated BLG aggregates obtained by microfiltration of heated solutions. Specific Raman signatures of aggregates were identified, which were not detected in the Raman spectra of denatured (i.e. unfolded BLG molecule) solutions. MRS analyses at different depths of the deposit reveal a loss of α-helix structures, as observed in denatured BLG solutions, without the detection of aggregate signatures. For the range of calcium content investigated (from 97 to 160 mg l^{-1}), no effect of calcium ions on the molecular conformation of BLG within the deposit was shown. Of great significance, results suggest that, for our set of operating conditions used, the mass distribution of the fouling deposit in a plate heat exchanger is primarily controlled by the distribution of the unfolded protein generated by the denaturation process.

1. Introduction

Fouling of heat exchangers in the dairy industry (mostly plate heat exchangers, PHEs) is still a serious issue as it reduces heat transfer efficiency, increases pressure drop and requires numerous cleaning-in-place procedures. For a temperature below 110 °C, the deposit is largely a "milk film" deposit or a type A (protein) fouling as classified by Burton (1968), consisting of 50–70 wt.% proteins, 30–40 wt.% minerals and 4–8 wt.% fat (Fickak et al., 2011). It has been largely shown in the literature that BLG plays a predominant role in milk fouling deposits; indeed BLG is the milk protein most sensitive to heating, which makes up only 10% of raw milk protein but can constitute half of the protein deposit content (Changani et al., 1997; Lalande et al., 1984, 1985).

The scientific community has examined the BLG conformational structure and its link with temperature for several decades (Mulvihill and Donovan, 1987). At room temperature and at pH 7, the secondary structure of native BLG is mainly composed of β-sheets (54%) and α-helices (17%) (Seo et al., 2010). Literature data show that an increase in temperature initiates a heat denaturation process which leads to different BLG protein conformations in addition to the native one (N), i.e. unfolded (U) and aggregated (A) state (Tolkach and Kulozik, 2007). Today it is well established that when heated in a neutral aqueous solution, globular molecules of BLG unfold with a loss of their tertiary and secondary structure. Such unfolding of the protein exposes reactive sulphydryl (–SH) groups which are normally contained within the core of protein and permits the formation of disulfide bridges with different denatured proteins present in the solution (for example casein, α-lactalbumin), resulting in the formation of aggregates (Lalande et al., 1989).

Calorimetric and spectroscopic investigations of BLG thermal denaturation, under different conditions of temperature, pH and protein concentration were carried out. Among them, one can mention those of Nonaka et al. (1993), Ikeda and Li-Chan (2004), Ngarize et al. (2004) and Seo et al. (2010). These studies conclude that a heat treatment induces changes in the BLG secondary structure. There is notably a trend toward an increase in β-sheet structures at the expense of α-helices structures with a simultaneous decrease of turn structure when heating from 70 to 90 °C. Using Raman scattering in the region of amide modes (800–1800 cm^{-1}), the mechanism of BLG thermal denaturation in the 20–100 °C temperature range could be described as a two-step process (Seo et al., 2010). The first step corresponds to the dissociation of dimers associated with the softening of the tertiary structure (observed between room temperature and 65 °C). At the end of this step (around 65 °C), the so-called "molten globule state" is obtained prior to conformational changes

Abbreviations: ANOVA, analysis of variance; BLG, beta-lactoglobulin; JEH, joule effect heater; MRS, Micro Raman spectroscopy; PHE, plate heat exchanger; RS, Raman spectroscopy; WPI, whey protein isolate.

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Nomenclature

C	native BLG concentration downstream of the pre-heating zone (g l^{-1})
C_0	native BLG concentration downstream of the PHE (g l^{-1})
D	internal diameter of the pipe within the test fouling surface (=0.0226)(m)
D_e	internal diameter of the pipe in the holding zone (=0.023) (m)
L_e	entrance length upstream of the test fouling surface (=0.43) (m)
Q	flow rate in the fouling stage (m^3 s^{-1})
Re	reynolds number of the fouling solution during the fouling step within the test fouling surface (=6.8×10^3), dimensionless
T	temperature (°C)
T_m	transition midpoint temperature for the BLG folding-unfolding transition (°C)
T_w	wall temperature in the holding zone (°C)
v	crossflow velocity within the test fouling surface (m s^{-1})
X	length of the deposit cross section related to the MRS analysis using a profile line (μm)
z	deposit thickness related to the MRS analysis on the deposit surface (μm)

Symbols

ΔT	$2 \times \Delta T$ corresponds to the temperature domain of the folding-unfolding transition (°C)
$\nu_{amide\ I}$	amide I band frequency (cm^{-1})
ν_D	frequency of the amide I band in the denatured (unfolded) state (cm^{-1})
ν_N	frequency of the amide I band in the native state (cm^{-1})
μ	dynamic viscosity of solution (Pa s)
ρ	specific mass of solution (kg m^{-3})

of the secondary structure taking place between 65 and 95 °C (Seo et al., 2010). The detailed thermal behavior of BLG at pH >6.8 and at temperatures up to 150 °C has been recently described by the review paper of de Wit (2009).

Although the connection between the thermal stability and conformational changes of BLG is well established, the role of the denaturated and aggregated species on the build-up of the fouling deposit onto a heat exchange surface is still unclear. Indeed, few studies take sides unreservedly whether BLG fouling is controlled by BLG aggregation rather than by denaturation. Trends reported in the literature are not really conclusive as regards which species is involved in protein deposits, as underlined by Bansal and Chen (2006a,b). Moreover, it is not obvious from the analysis of mechanistic models dealing with the prediction of fouling deposits in the literature to guess which species (unfolded or aggregated) shapes the fouling deposit. For example, Mahdi et al. (2009) and Jun and Puri (2007) assumed that only the aggregated protein is deposited on the wall, which is not functional for the model of de Jong et al. (1992, 1993) for which it is hypothesized that the main mechanism in fouling process is a reaction-controlled adsorption of unfolded BLG. More detailed information regarding the involvement of protein denaturation and protein aggregation as governing reaction in fouling mechanisms is given in the review paper of Bansal and Chen (2006a) to which the reader can refer.

Protein adsorption at the stainless steel interface is considered to be the initial stage of substantial milk fouling. Following protein adsorption, protein–protein interactions prevail to build-up milk fouling on stainless steel surface used for heat exchange. However, as reported by Liu and Chen (2010), this field of knowledge has been rarely investigated at the molecular level since the pioneering work of Kim and Lund (1998) who have illustrated that protein adsorption onto stainless steel could be relevant to explain the deposition mechanism during thermal processing of milk.

It is noticed that the experimental mass balance realized on the denatured protein performed between the inlet and outlet of a heat exchanger is also confusing as regards the role played by the different species (unfolded or aggregated) on fouling growth. As pointed out by Lalande et al. (1985) and Belmar-Beiny et al. (1993), only a small fraction of unfolded proteins sticked on each exchange surface. Precisely, Delplace et al. (1994, 1997) observed that only 3.6% of irreversibly unfolded BLG was involved in the deposit formation.

In view of the literature data, a controversial question arises regarding which form of BLG (unfolded or aggregated one) constitutes the first deposit layer close to the hot wall. There is also a paucity of knowledge as regards the effect of calcium ions upon the conformational structure of protein contained in the fouling deposit. Indeed no data are available in this field although it has been observed in the past (Bansal and Chen, 2006a; Guérin et al., 2007) and more recently (Petit et al., 2011) that the calcium content enhances deposition and influences the stability of the BLG thermal denaturation.

More information on the conformational structure of the whey protein molecules (especially BLG) which are present within a dairy protein deposit is needed in order to elucidate the fouling mechanisms of PHEs. The molecular structure and conformation changes of proteins can be analyzed by Raman spectroscopy (RS) which is a technique capable of providing information with respect to peptide backbone conformation as well as environment of certain side chains (Nonaka et al., 1993; Li-Chan, 1996; Chi et al., 1998; Jung et al., 2000; Ngarize et al., 2004; Ikeda and Li-Chan, 2004). Chi et al. (1998) ascertained that the protein secondary structure can be determined from the analysis of the amide I, II and III bands and the C_α–H amide bending vibration. The amide (peptide) bond of proteins has several distinct vibrational modes, of which the amide I and III bands are the most useful for the study of secondary structure (Li-Chan, 1996). Jung et al. (2000) have demonstrated the potential of two-dimensional (2D) Raman correlation spectra and its complementarity with 2D infrared correlation spectra in the amide III region (1330–1200 cm^{-1}) to investigate the secondary structure of BLG in a buffer solution at pH 6.6. Another advantage is that RS is a non destructive analytical technique capable of gaining data at high protein concentrations sufficient to cause gelation, i.e. as those encountered in a protein fouling deposit (Ikeda and Li-Chan, 2004). Ivleva et al. (2009) pointed out that Raman micro-spectroscopy (MRS) or Raman microscopy has also the advantage of providing fingerprint spectra with a spatial resolution of an optical microscope (with axial resolutions of about 5 μm in confocal mode).

Considering the dependence of the denaturation process on pH, protein concentration, thermal history, and the controversial description of the denaturation mechanism (Seo et al., 2010), a denaturation curve (represented by the temperature dependence of the amide I band frequency in the 25–100 °C temperature range)