Structure Of Whey Protein Concequence For Dairy Industry (Review)

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Abstract: Milk proteins play a range of roles which make dairy products and products containing dairy components are valuable. Theses include nutrition, physical functionality and breakdown under controlled condition to produce nutritional, functional or flavour full products. This article reviews the structure of whey protein concequence for dairy industry. [Journal of American Science. 2010;6(11):79-84]. (ISSN: 1545-1003).

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Introduction

Milk proteins play a range of roles which make dairy products and products containing dairy components are valuable. Theses include nutrition, physical functionality and breakdown under controlled condition to produce nutritional, functional or flavour full products. There is also undesirable behavior include fouling of heated surface, gelling in processing equipment during manufacture of some products. All of these behaviors related to the structure, and possible changes in structure during processing of the component milk proteins, an understanding of the structure of milk proteins, and how those structure can change under processing conditions, is therefore, an important enabling tool for the dairy processing industry.

Complete three dimensional structural information about a protein can be obtained experimentally in one of two ways: X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. There are more than 12000 sets of crystal structure coordinates for a wide variety of protein in the protein Data Bank, the so-called PBD coordinates (Berman etal .,2000), together with over 2000 sets of coordinates derived from NMR data. Not all of these sets of data are unique; many are of the same protein but with different small-molecule ligands, crystallized under different conditions of temperature and/ or pH, from different species, or with one or two amino acid residues mutated. However, each set of coordinates in the data base represents a unique structure determination. In spection of this vast data base reveals that there are relatively few families of proteins, probably about 1000, that are distinctly different in structure. Thus, most but not all, new structure can be assigned to an already known family of structures (Murzin et al., 1995, Thoronton et al., 1999) which in turn makes the determination of new structure easier. Theoretical methods of protein structure prediction can be employed. Prediction methods are still being developed and, at the present time, their success depends upon the closeness of the unknown protein sequence to that of a known structure. Generally, the closer the amino acids sequence is to that of known molecular structure, the better is the prediction. (Moult, et al., 1999).

Milk Whey Proteins

(1) α- Lactalbumin :

Two genetic variants, A and B of this protein exist. They differ by a single substitution, A having Gln. And Behaving Arg. At position 10. The B variant is the only one in the milk of European breeds; both A and B occur in Indian cattle. Some minor forms of bovine α- lactalbumin are revealed by electrophoresis. Some these contain covalently bound carbohydrate groups; the major components of bovine α- lactalbumin are devoid of carbohydrates others of minor components seem to have fewer amide groups than the major ones, and one minor α - latalbumin containing three instead of four disulfide. In total the minor components donor account for more than 10% of the - lactalbumin. The amino acid sequence of α - lactalbumin is similar to lysozymes. Indeed, bovine α - lactalbumin B and chicken egg white lysozyme have identical amino acids residues at 49 positions, and the four disulfide groups are located identically; they are at 6-120, 28-111, 61-77 and 73-91 in α-lactalbumin. It is considered that α-lactalbumin arose in evolution by gene duplication of an ancestral gene coding for lysozyme. Although the conformation of α-lactalbumin has not been defined completely by X-ray crystallography, preliminary indications are that its structure is similar to that of the well defined lysozyme. The two proteins must have very different active centers, however, since neither has the biological activity of the other; nor do they interfere with each others activity. The biological activity of α-lactalbumin is interaction with galactosyl transferase to promote the transfer of galactosefrom UDP galactose to glucose to

form lactose. The nature of the effects of LA was used to develop a model for the arrangement of binding sites for acceptor substrates and LA on glactosyl transferase. Fig (7). The acceptor binding site of galactosyl transferase is viewed as containing at leas two subsides that are capable of binding monosaccharide, one of which can bind free N-acetylglucosamine or glucose with low affinity substrate (S1) while the second (S2) binds an additional sugar in extended substrates . In the model, the binding of LA at this site in galactosyl transferase, adjacent to the monosaccharide substrate binding site, brings an eight boring site in LA close to S1 this site in LASLA ,which is proposed to be region corresponding to part of the lysozyme active site clef, forms favorable, stabilizing interactions with a monosaccharide bound to S1.

Secondary structure including four α-helices, several regions of 10 Helix and β-pleated sheet table (2)

(2) B-Lactoglobulin:

Effect of pH on the structure of β-lactoglobulin

The properties of β-lactoglobulin have been examined since the 1930 by effectively every available technique (Sawyer& Kontopidis, 2000). B-Lg from ruminant milk is polypeptide of 162 amino acids that exists as a dimmer. The pH affects the structure through a series of reversible conformational changes leading to dissociation at both high and low pH. Four out of the five cysteine residues from two disulphide bridges, 66-160 and 106-119. Leaving cys 121 as the free thiol that appears to be responsible through disulphide interchange, for the formation of aggregates upon heating (Manderson, et al., 1999). Between pH 6 and 8 there is a significant change in the reactivity of the free thiol, the disinterment of a carboxyl, now known to be Glu 89 (Qin etal., 1998) and the opening up the central, legend - binding site. The legends that bind β-Lg tend to be hydrophobic and include fatty acids, cholesterol and more weakly, hydrocarbon molecules such as toluene and pentane.

Structural Variations of native bovine β-Lg:

Both NMR and X-ray methods reveal the same general structure for bovine β-Lg, even though NMR experiments were done at pH 2 where the protein exists in monomeric form, and the X-ray diffraction experiments span the range oh pH 6-8.1, where in all cases, including those where β-Lg is liganded to fatty acid. A common dimeric structure is observed. (Jameson et al., 2002)

Denaturation of bovine β-Lg:

Various schemes of different degree of complexity have been proposed over the years for the thermal denaturation of bovine β-Lg

Although most agree that the basic steps involve (1) .The dissociation of the dimmer into monomers, (2). The loosening of the structure into a form that may be, or at least may be resemble, "molten globule state"(3) the molten state leads to the unfolding of the protein, (4). The formation of aggregates by both disulphides interchange and cross linking, and non covalent interaction (Qi et al., 1997). More recently it has been found that on early step gives rise to non-native monomer β-Lg that contains disulphide bond sin non-native configuration. The temperature, the ionic strength, the pH and the presence of legends and cations are some of the parameters determining this complex process. It is possible to use structure based approach to study a series of changes that lead to gelation or precipitation through the denatured and aggregated state, the structure must be perturbed in some way and the effects must be monitored by X-ray or crystallography or by NMR spectroscopy.

The methods of perturbing the structure are to use a denaturing agent such as urea, to raise the temperature, to modify the protein chemically or by adding legends that make the protein more or less resistant to denaturation, or to vary the pressure. All of this methods have been used, β-Lg and there are a large number of reports published over the years describing the chemical and spectroscopic effects in solution (Sawyer& Kontopidis, 2000). However, the tertiary structural details resulting from the application of these methods, where they have been applied, is recent.

Solvent unfolding:

Urea as a denaturing agent has been applied recently to study the refolding of β-Lg by NMR. The protein is first denatured or at least substantially unfolded in urea. The urea is then abruptly diluted, perhaps 20-fold to a concentration well below that required to unfold the protein. As the protein refold, the peptide N-H protons of certain residues lose their ability to exchange with solvent. Those residues, whose amide proton exchangeability is lost rapidly are those that refolded first whereas those that continue to exchange all long times after dilution of the urea are on the surface or in solvent accessible regions of the structure. With β-Lg, the protein core involving strands F, G and H and the helix form rapidly with the rest of the protein then folding around this central core. One further feature of this process is that there strong evidence of some transient structure (α helix) that is not observed in the final native structure. The evidence in this case is that (re-) folding of β-Lg is a hierarchical process (Kuwata et al., 2001b).

Effect of temperature on the structure of β-Lacto globulin

Monitoring the effects of temperature has been most efficiently carried out using spectroscopic techniques. For example, circular diachronic (CD) or Fourier transform infrared (FTIR) spectroscopy can conveniently be used to show the melting of the three turn α-helix of β-Lg at around 65 °C (Qi et al., 1997). Crystallized protein at pH 7.6 that had been heated to 60 °C, and the structure at 2.6 A° resolution was that of the native protein this resolution is substantially worse than that typically observed for β-Lg, and is a warning that the material crystallized may not be representative of the entire sample, a small part of which may have become irreversibly denature. Although studies in solution indicate that thermal effects are reversible up to 68 °C (Qi et al., 1997), the differential scanning calorimetric (DSC) technique used would not be sensitive to a significant fraction (up to 10 %) of the protein having become irreversibly denatured. However, many studies have been shown that β-Lg at natural pH gradually denatures irreversibly if held at temperatures as low as 59 °C., \As not earier, high-quality protein crystals are best obtained from pure, homogeneous material uncontaminated by other proteins or by material in non native or denatured conformations. Crystal's isomorphism with the native protein crystals will certainly grow at 50° C.

NMR studies also using deuterium exchange techniques at several temperature and pH 2 (Belloque & Smith 1998) reveal that the denaturation occurs in stages at 55 °C strand E and the B loop unfold, and the A strand becomes flexible. At 75°C the A strand and the helix unfold, but the residues in the two sheets, BCD and FGH, are surprisingly resistant to amid H/D exchange. Prolonged heating leads to the formation of a transparent gel and this does not involve disulphide bond cross-linking (Schokker, et al, 2000). At pH 7.4, similar effects are noticed but their rate is faster. Similar, but not identical observation have been made by (Edwards et al, 2002) who used a slightly different denaturation protocol and pure β-Lg B.

Effect of pressures on the structure of β-Lacto globulin

Another means of perturbing the structure is to use hydrostatic pressure and there the unfolding of the protein has been monitored by NMR (Kuwata, et al., 2001a). This show that the two B-sheet surfaces appear to unfold independently in excellent agreement with the results referred to above using temperature. The crystal structure of β-Lg at ambient pressure, after pressurization to 250 bars in a process that was not completely reversible, shows both reduced unit cell dimension and also a slight contraction of the structure, which was most marked around β- strand A.

Stabilization by bound Legends:

Legend – binding studies of β-Lg have been carried out in solution using several different spectroscopic techniques including NMR. (Muresan& de Wolf.2001). IT has emerged from their crystallographic studies that the majority of legends, e.g. palmitate, retinal and cholesterol bind within the central cavity of the protein (Sawyer & Kontopidis, 2000). The effect of this is to increase the stability of the protein to both urea and thermal denaturation in a manner that is expected when a solvent accessible internal cavity is filled (Jameson et al., 2002).

Side-chain modification:

It is clear that chemical modification of the amino side chains of β-Lg leads to changes that often result in the protein becoming more susceptible to denaturation and aggregation. (Morgan etal., 1999) Modification of Cys 121 by most reagents significantly enhanced dimmer dissociation. Thus heavy metal ions, such as mercury or gold, lead to a significant change in both the unit cell dimensions and in the structure of the dimmer interface the separation of the ant parallel β-strands I and I increase by around 1A˚- as well as small but significant changes in the structure of the monomer subunits. It is not obvious, however why reaction of Cys 121 should affect the interface because the residue is on the underside of the helix distal to the dimmer interface, and moreover, the helices themselves do not interact directly in any of the distinct crystal structure of bovine β-Lg.

Site-directed mutations:

A method somewhat akin to chemical modification as a perturbing influence is that of protein engineering (Batt, 1997). Although significant studies have been made using the natural genetic variation present in the different alleles within one species, and the variation between species, modern molecular biological techniques make site-specific mutation convenient and efficient (Sambrook & Russell,2001). For example, the two "half mixed" forms (D33G with V118, and D33 with V118A) of the A and B genetic variants promise to provide to the reason behind the different stabilities of the two forms. Similarly, C12 1S will remove the ability of β-Lg to aggregate by disulphide interchange mechanism. Both porcine β-Lg and equine β-Lg are monomers at pH 7 but the X-ray structure of the porcine (Hoedemaeker etal.,2002) and NMR structure of the equine (Kobayashi et al.,2000) protein, respectively, show a high degree of similarity to the bovine and ovine crystal and NMR structure. Structural studies combined with site directed mutagenesis will uncover many of the determinants of stability, or instability, of bovine β-Lg of importance to milk technology, the physiological role of β-Lg to both the lactating cow as donor and the calf as recipient

remains almost as mysterious now as it was more than 60 years ago. Molecular biological techniques have now provided methods to address explicitly these mysteries by examining the physiological effects on both mother and calf of cows that are unable to express β-Lg.

Immunoglobulin:

Immunoglobulin is antibodies synthesized in the response to stimulation by macromolecular antigens foreign to the animal. They are polymers of two kinds of polypeptide chains, light (L) of MW 22.400 and heavy (H). The latter are of several types, including γ (MW 52.000), α (MW 52.000-56.000), and ụ (MW 69.000). Each of the L and H chains consists of a relatively constant and highly variable sequence and appears to be coded for by two genes. IgG1 and IgG2 are each polymers of two light chains and two heavy chains of the γ type (γ1 and γ2). The chains are joined by disulfide linkages to form two antibody sites, each consisting of the variable portion of an H and L chain IgG1 and IgG2 have about 2.9% bound carbohydrate and MW of about 150.000. They differ slightly in electrophoresis mobility. IgA and IgM immunoglobulin likewise have the basic structure of two H and two L chains joined by disulfide bridges. In IgA the H chains are of the type, αand in IgM they are of they ụ type. IgA is secreted as a dimmer of two of the basic four-chain units joined by polypeptide of MW about 25.000,

called J-component, and associated with another called secretor component, Sc. This complex is called secretor IgA (SIgA) and has a MW of about 385,000. The secretor component is a protein of MW about 75,000, consisting of a single polypeptide chain with a number of internal disulfides. It has a relatively high content (10- 15 %) of bound carbohydrate consisting of N-acetylglucosamine, N-acetylgalactosamine, D-galactose, D-mannose L-fucose, and N-acetylneuraminic acid. In addition to the amount that is bound in SIgA, it occurs in the free state in milk in concentrations of 50-100 mg liter l

IgM consists of a pentamer of the basic four-chain units joined by the J-component. It has 11-12% bound carbohydrate and MW of 900.000; its diameter is about 30nm. In all cases the sites that bind antibodies consists of the variable protein of an H and L chain in juxtaposition to each other. IgG has two such sites, SIgA has four, and IgM has ten. The immunoglobulin of an animal thus consists of numerous different proteins; each antigen encountered has caused the synthesis of Ig with a different variable portion.

The immunoglobulin in milk can exert and antimicrobial action, particularly IgM, which acts as agglutinin, for instant, against some streptococci. Moreover, cow sm ilk (but not that of buffalo, goat, or sheep) contains a cry globulin (mainly consisting of IIgM) that is involved in the cold agglutination of m ilk fat globules and in the attachment of bacteria to fat globules. The immunoglobulin is among the most heat sensitive of the whey proteins.

Protease-Peptone Fraction:

Fractions of the whey proteins amounting to about 1g per kilogram of m ilk are not rendered acids-insoluble by previously heating the m ilk. .It has long been called protease-peptone. Four principal groups of components of protease-peptone are distinguishable electrophoretically. The ere components, which is probably derived from a fat globule membrane constituent, and components 5,8 fast, and 8-slow, which are derived by proteolysis of β-casein.

Other whey proteins:

A group of acid glycoproteins is retained on DEAE cellulose when blood serum or whey is passed through it at pH 4.5. Fractional elution separates a number of components. The total amount that can be obtained from bovine blood serum, colostrums, and m ilk are respectively, about 2.0, 1.0 and 0.3 g-liter $^{-1}$. One protein of this group is α acid glycoprotein, formerly called orosomucoid. It has been isolated from human serum, colostrums, and m ilk, and from bovine serum and colostrums, but it has not been detected in bovine m ilk. It consists of a polypeptide chain of 181 residues to which five heteropolysaccaride groups are linked to asparagines residues. The carbohydrate constitutes about 45% of the total m olecules. The function of this protein is not known. In any event, α $^{-}$ acid glycoprotein comprises only a small portion of the acid glycoproteins obtainable from fractionation of colostrums or m ilk on DEAE cellulose. Five other fractions have been obtained in varying states of homogeneity. A ll contain carbohydrate and phosphate and promote the growth of Bifidobacterium bifidum var. pennsylvanicus (formerly Lactobacillus bifidus). The possibility that some of these glycoproteins represent partial degradation products of caseins or membrane materials has not been elucidated.

A specific protein that binds foliate (FBP) has been isolated from m ilk. A ffinity chromatography on sepharose to which foliate has been attached is especially effective in isolating this protein. Its concentration in normal m ilk is about 8m g/kg l.

Fat globule membrane protein:

The fat globule membrane contains approximately 50% protein and accounts for about 15 of the total protein of the milk. Some of the protein constituents of the membrane are enzyme but it is not possible at present to estimate the ratio of enzymatic and non enzymatic components. The fat globule

membrane proteins are difficult to resolve analytically and to separate privatively because they interact strongly with one another and with lipids.

β2-Microglobulin:

This protein consists of a single polypeptide chain of about 100 amino acids, residues and MW of 11800. It is present in several body fluids and in membranes of various type4s of cells. Its amino acid sequence indicates homology with the constant regions of immunoglobulin light and heavy chains.

A protein that had long previously been crystallized from bovine milk and designated lactollin called bovine β² microglobulin. It is a polpeptide of 98 residues, two of which are Cys.

Direct analysis for microglobulin concentration have not been made in bovine milk; the amounts of lactollin that have been isolated from colostrums and milk are about 6 and 2 mg liter ¯\ respectively.

Lactoferrin and Transferrin

Two iron-binding proteins are found in milk. One of them, transferrin (Tf), is a common blood plasma protein; the other , lactoferrin (Lf), is secrected not only by mammary glands but also by kidney and endometrial mucosa. Both Tf and Lf appear to be large single-chain polypeptides of 600-700 residues. Reported molecular weight differ somewhat; recent work favors 75000 to 77000 for Tf, but values for Lf are not so consistent either 700 or 93000 being reported, In both proteins about 4 mol% of the residues are Cys, and both have covalently linked carbohydrate consisting of N-acetylglucosamine, mannose, galactose, and N-acetylneuraminic acid. All transferrins and lactoferrins yet worked with appear to bind 2 mol of Fe٣+ per mole. Tf and Lf differ markedly from each other on amino acid composition and in electrophoretic mobility. They can be detected readily in electrophoretic patterns by autoradiography with Fe. Electrophoreticx patterns of milk and boold preparations from individual animals reveal the occurrence of genetic variants of both proteins. No immunological cross-reaction between Tf and Lf has been demonstrated even when both are from a single species. Amino acid analysis and partial sequences of human Lf and Tf indicate some degree of homology between the two and some internal homologyof peptide segments within each; sequencing is far from complete, however, Both Tf and Lf can be determined quantitatively in a biological fluid by immunodiffusion using a specific antiserum. The concentrations and ratios of Tf and Lf in milk vary greatly among species and with stage of lactation. The concentration of Lf in colostrums is about 1250 mg/kg¯¹; in mid lactation the concentration falls to less than 100mg/kg¯¹. concentrations of Yf in milk have not been determined accurately but may be similar to those of Lf. Lactoferrin is an inhibitor of bacteria because it deprives them of iron. The concentration of Lf in bovine milk is so low, however, that it does not exert any significant antibacterial effect.

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