Biological activities of bovine glycomacropeptide

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Biological activity of bovine κ-caseino glycomacropeptide (GMP) has received much attention in recent years. Research has focused on the ability of GMP to bind cholera and *Escherichia coli* enterotoxins, inhibit bacterial and viral adhesion, suppress gastric secretions, promote bifidobacterial growth and modulate immune system responses. Of these, protection against toxins, bacteria, and viruses and modulation of the immune system are the most promising applications.

Bacterial/viral/toxin adhesion: Prebiotic: Immune system modulation

Introduction

In recent years, several lines of research have indicated that milk components, particularly those found in whey, have biological activity. Source materials for these studies are usually bovine milk components since they are most common and in largest supply. In particular, the biological activity of bovine κ-casein or κ-caseino glycomacropeptide (GMP)[†] has been studied. The introductory paragraphs recount a litany of physiological functions attributed to GMP, including:

(1) binding of cholera and *Escherichia coli* enterotoxins;
(2) inhibition of bacterial and viral adhesion;
(3) suppression of gastric secretions;
(4) promotion of bifidobacterial growth;
(5) modulation of immune system responses.

This paper summarizes literature reports of biological activities of GMP. The main focus is studies related to bovine milk GMP, and peripherally κ-casein, since that is the most abundant variety available. Bovine colostral GMP and human milk and colostral GMP, although studied in considerable detail, do not present a commercializable source for useful products. This review may provide a springboard for further research which will result in elucidation of additional health benefits of GMP and, perhaps, development of health claims for functional foods and dietary supplements.

Structure of GMP

When bovine κ-casein is treated with chymosin during cheesemaking, the protein is hydrolysed into para-κ-casein (residues 1–105), which remains with the curd, and GMP (residues 106–169), which is removed with the whey (Eigel *et al.* 1984; van Hooydonk *et al.* 1984). The latter, a glycophosphopeptide, is probably the least well known of the cheese whey proteins even though it comprises 15–20 % of the protein (Saito *et al.* 1991), as measured by total Kjeldahl nitrogen, from renneted cheese whey. Examination of amino acid sequence of GMP in Fig. 1 reveals possible reasons for its neglect (Eigel *et al.* 1984; Whitney, 1988). There are no aromatic amino acids in GMP and it is, therefore, invisible at 280 nm, the common protein detection wavelength. In addition, GMP retains a net negative charge, even at pH 3, so it is not collected on cation exchangers, nor does it move with the rest of the proteins in native polyacrylamide gel electrophoresis (PAGE). Its low molecular weight of 8000 Da makes it difficult to visualize with Coomassie Blue stain in sodium dodecyl sulphate (SDS)-PAGE. Quantification procedures for GMP require trichloroacetic acid precipitation of the other whey proteins leaving only the GMP in solution (van Hooydonk & Olieman, 1982; Sharma *et al.* 1993).

The amino acid sequences of both κ-casein and GMP and their variants have been well defined (Eigel *et al.* 1984; Whitney, 1988; Fiat & Jolles, 1989). One current GMP application is in diets for phenylketonuria patients (Nielsen & Tromholt, 1994). These individuals lack the ability to metabolize phenylalanine making GMP an amino acid source which they can tolerate. Unfortunately, the lack of other essential amino acids (arginine, cysteine, histidine, tryptophan and tyrosine) requires other protein sources in those diets.

Many researchers (Tran & Baker, 1970; Fiat *et al.* 1972; Jolles *et al.* 1973; Fournet *et al.* 1975, 1979; Doi *et al.* 1979; van Halbeek *et al.* 1980; Saito *et al.*1981; Saito & Itoh, 1992) contributed to information about the saccharide structures in GMP. In mature cow milk it has been established that five saccharides are found:

(1) monosaccharide GalNAc - O - R
(2) disaccharide Gal β1 → 3 GalNAc - O - R

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[†]Referred to in this review as glycomacropeptide but also designated caseinomacropeptide and caseinoglycopeptide in the literature.

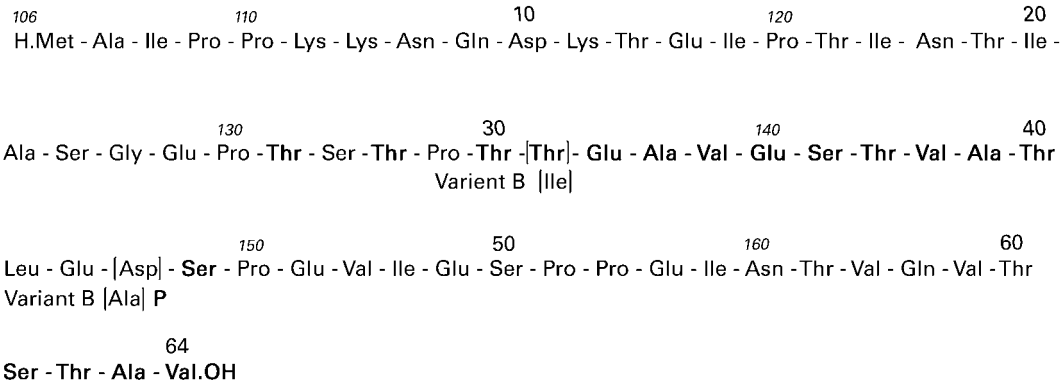

Fig. 1. Primary structure of bovine GMP variant A. (Eigel *et al.* 1984; Whitney, 1988). The enclosed amino acid residues are the sites corresponding to mutational differences in the B variant. The small italicized numbers refer to the amino acid residue sequence numbering based upon κ-casein. Sites of glycosylation and phosphorylation are boldfaced. Note that there are eleven negatively charged and three positively charged amino acid residues.

(3) trisaccharide NeuAc α2 → 3 Gal β1 → 3 GalNAc - O - R
(4) trisaccharide Gal β1 → 3 (NeuAc α2 → 6) GalNAc - O - R
(5) tetrasaccharide NeuAc α2 → 3 Gal β1 → 3 (NeuAc α2 → 6) GalNAc - O - R

where Gal = galactose, GalNAc = *N*-acetylgalactosamine, and NeuAc = sialic acid‡

Six other saccharides have been delineated in cow colostrum (Saito *et al.* 1981; Fiat & Jolles, 1989); *N*-acetylglucosamine (GlcNAc) and fucose (Fuc) have been identified as constituents in these saccharides.

It is important to draw a distinction between bovine and human GMP oligosaccharides. The latter contain no NeuAc and have Gal, GlcNAc, and Fuc as terminal sugars on their oligosaccharides (Fiat & Jolles, 1989). Of the fourteen human milk GMP oligosaccharides which have been identified, only disaccharide II is found in milk GMP from both species. There is an asialotetrasaccharide which is found in both bovine colostrum and human milk.

The point of saccharide attachment is not uniform, though it is agreed that the glycosidic linkage is through an oxygen and that R is threonine (Thr) (Eigel *et al.* 1984; Whitney, 1988; Fiat & Jolles, 1989). Jolles *et al.* (1972, 1973) have shown that Thr26 or 28 of the peptide chain (Thr131 or 133 of the κ-casein chain) bears a saccharide. There is evidence (Kanamori *et al.* 1980) that Thr30 or 31 (Thr135 or 136 of the κ-casein chain) also participate. Several reports indicate that multiple saccharide substituents may be linked on a single peptide chain (Doi *et al.* 1980; Otani *et al.* 1995). In contrast to this variability, one phosphate moiety in GMP is always at serine 44 (Ser149 of the κ-casein chain) (Fiat *et al.* 1981; Eigel *et al.* 1984; Whitney, 1988) though non-phosphorylated fractions have been found.

‡Referred to as sialic acid and *N*-acetylneuraminic acid in the literature; the former is used in this review.

Preparation of GMP

Two approaches have been taken for GMP preparation. One approach is preparation of casein or κ-casein free of whey proteins followed by hydrolysis with rennet to produce GMP. The other approach is purification of GMP directly from cheese whey made by a rennet process.

Using the former approach, Dosako *et al.* (1991) describe rennet treatment of sodium caseinate to produce a crude GMP preparation. Shammet *et al.* (1992) showed that small peptides are present. Where more-defined preparations are desired it is necessary to purify κ-casein by precipitation (McKenzie & Wake, 1961; Zittle & Custer, 1963), gel filtration (Yaguchi *et al.* 1968), or ion exchange (Hollar *et al.* 1991). Coolbear *et al.* (1996) compared these methods and determined that the GMPs resulting from the κ-caseins were virtually identical by reverse-phase chromatography and gel electrophoresis.

When the latter approach is used, the primary challenge is separation of GMP from the other whey proteins. In the laboratory, GMP is prepared by precipitation of the other whey proteins with trichloroacetic acid (Morr & Seo, 1988; Shammet *et al.* 1992) or phosphotungstic acid (Eustache, 1977*a*) followed by dialysis or ultrafiltration.

Several processes (Eustache 1977*b*; Nielsen & Tromholt, 1994) call for heating whey protein concentrate (WPC) to temperatures above 85°C in order to flocculate the whey proteins, adjusting to the isoelectric point, and separating by centrifugation or filtration. The GMP, which is heat stable, is collected in the supernatant or filtrate. Berrocal & Neeser (1993) heated WPC at pH 6, with added calcium and 25 % ethanol, to 90°C. The solution was acidified to pH 4·5 and flocculent material was removed by centrifugation leaving a supernatant containing GMP.

Ion exchange processes take advantage of the highly negative charge on GMP even at low pH where the other whey proteins are positively charged. Whey at pH 3 is contacted with a cation exchanger (Shimatini *et al.* 1993; Kawasaki & Dosako, 1994). The GMP is not adsorbed by the cation exchanger and may be concentrated and desalted by ultrafiltration. Alternatively, whey at pH less than 4 is

contacted with an anion exchanger (Kawasaki *et al.* 1994). The GMP is adsorbed on the anion exchanger while the other whey proteins are washed free. It is then possible to elute GMP from the ion exchanger. Etzel (1999) employed a copper-containing metal affinity adsorbent as well as a cation exchanger to produce GMP from whey. Until recently ion-exchange processes used very expensive hydrophilic ion exchangers such as cellulose (Shimatini *et al.* 1993; Kawasaki & Dosako, 1994; Kawasaki *et al.* 1994) or silica (Skudder, 1985) based 'resins'. Erdman & Neumann (1999) used a polystyrene weak anion exchange resin in the alkaline form to capture GMP from an acidified whey solution.

Kawasaki *et al.* (1993*a*) using gel filtration with Superose, reported that above pH 4, GMP exists as tetramers and pentamers but at pH below 4 GMP exists as monomers and dimers. Using this phenomenon, Tanimoto *et al.* (1991) carried out ultrafiltration on whey at a pH below 4 using a sufficiently large-pored membrane. The GMP, in the form of monomers and dimers, was able to pass through into the permeate. Adjustment to pH above 4 allows concentration by ultrafiltration in the form of tetramers and pentamers with a smaller-pored membrane. There is some question as to whether the observed molecular size change is due to a change in oligomerization. Minkiewicz *et al.* (1996) used reverse-phase chromatography and determined that GMP, regardless of glycosylation, is a monomer at all pH values, ionic strengths and concentrations. The large apparent molecular mass was explained by internal electrostatic and steric repulsion. Nakano & Ozimek (1998) using gel filtration with Sephadex S-200 and G-75 observed that GMP exhibits apparent molecular mass of trimers and observed no change in oligomerization between pH 7 and pH 3·5.

Biological activties

Ability to bind cholera toxin and E. coli enterotoxins

Cholera toxin produced by *Vibrio cholerae* consists of an A subunit and five B subunits. The B subunits form the attachment site which binds to oligosaccharides on cell walls. Once bound, the A subunit activates adenylate cyclase in cells which results in loss of cellular water which causes diarrhoea and possibly death (Holmgren, 1981). It has been shown that the receptor is a ganglioside G_{M1} (van Heyningen, 1974), which has the structure Gal β1 → 3 GalNAc β1 → 4 (NeuAc α2 → 3) Gal β1 → 4 Glc β1 → 1 Cer (Leeden, 1966) (where Glc = glucose and Cer = ceramide). This oligosaccharide is not identical to GMP, but other glycoproteins such as fetuin and glycophorin which have oligosaccharides similar to GMP inhibit cholera toxin (Sugii & Tsuji, 1989; Schengrund & Ringler, 1989).

Kawasaki *et al.* (1992) have shown that GMP is capable of binding cholera toxin. Normal Chinese hamster ovary (CHO)-K1 cells are spherical. In the presence of cholera toxin, CHO-K1 cells take on a spindle shape. As little as 20 p.p.m. GMP is enough to cause considerable rounding of CHO-K1 cells and 100 p.p.m. GMP results in almost completely rounded CHO-K1 cells which indicates that GMP has bound to cholera toxin. When the GMP was treated with sialidase, which hydrolyses the sialic acids, complete loss of cholera toxin inhibiting activity occurred. The peptide chain must also participate in the binding as partial loss of cholera toxin inhibiting activity occurred after treatment with proteases. Worobo *et al.* (1998) were able to narrow the activity to a peptide fraction obtained by ion-exchange chromatography.

Isoda *et al.* (1990) carried the work further to other bacterial toxins. They obtained similar inhibitions against *E. coli* heat labile enterotoxins LT-I and LT-II (associated with colonization factor antigen CFA/I and CFA/II, respectively) in the CHO-K1 model. Additionally, the ability of the GMP to protect mice against diarrhoea caused by the toxins was evaluated. Feeding 1 mg GMP per day protected 100 % of the mice against cholera toxin and LT-II, and 80 % of the mice against LT-I.

Inhibition of bacterial and viral adhesion

Many bacteria and viruses bind themselves to their hosts as a part of the colonization process. Binding to the intestine or other mucosal surfaces is achieved by adhesins, capsular material on the bacterial cell surface or hair-like fimbriae or pili which are specific for the various ceramide and ganglioside glycoconjugates which make up epithelial cell membranes (Simon, 1996). Considerable research has been done to characterize the nature of the adhesins and their receptors and it may be possible to find substances which have sufficient similarity to the receptors that they block the receptor and thereby inhibit colonization (Ofek & Sharon, 1990).

The haemagglutination assay is often used to screen for compounds which prevent bacterial or viral binding to cell receptor sites. The assay detects the ability of the bacterium or virus to bridge between erythrocytes (red blood cells) and cross-link or agglutinate the erythrocytes. If the adhesin is bound to the compound in preference to the receptor site, agglutination will not occur. Thus, haemagglutination inhibition is a sign that the compound has potential to prevent bacterial colonization.

Neeser *et al.* (1988*a*) have been investigating the mechanism by which milk components prevent dental caries. They evaluated the role of GMP in inhibiting adhesion of cariogenic bacteria (*Streptococcus mutans*, *S. sanguis*, *S. sobrinus* and *Actinomyces viscosus*) to oral surfaces. Haemagglutination by *S. mutans*, *S. sanguis* and *A. viscosus* is prevented by GMP with disaccharide II (Neeser *et al.* 1988*a*). Using saliva-covered hydroxyapatite beads, as a saliva covered tooth model, binding of bacteria in the presence of GMP was measured. GMP prevented binding of *S. sobrinus* and *S. sanguis*, but not *A. viscosus* (Neeser *et al.* 1994). A further proof of binding was obtained using GMP-gold conjugates which could be seen attached to *S. sanguis* bacteria by electron microscopy. A 23 kDa glycoprotein from *S. sanguis* which binds to buccal (cheek) epithelial cells was identified (Neeser *et al.* 1995). Trisaccharide III is on the glycoprotein. Further, *S. mutans* and *S. sobrinus* binding to salivary pellicle (the thin layer of salivary protein and glycoprotein which quickly adheres to a freshly cleaned tooth) could be prevented by GMP

(Schupbach *et al.* 1996). They believe that the mechanism by which GMP reduces dental caries is by changing the microbial composition of dental plaque from streptococci to less cariogenic *Actinomyces*. Incorporating GMP in gum or toothpaste is a method of preventing dental plaque and caries (Neeser, 1991*a*,*b*). Xylitol and GMP appear to have a synergistic effect in not only preventing caries but also remineralizing teeth (Zhang & Shapiro, 1998).

Kawasaki *et al.* (1993*b*) demonstrated that GMP inhibits haemagglutination by four strains of human influenza virus. As little as 80 p.p.m. was effective. Dosako *et al.* (1992) found 10 p.p.m. concentrations of GMP prevent Epstein–Barr virus from inducing morphological transformations in peripheral blood lymphocytes.

Bacterial adhesion offers a field ripe for further GMP application, but there are some caveats related to specificities. Neeser *et al.* (1988*b*) evaluated GMP as a haemagglutination inhibitor for CFA/I and CFA/II expressing *E. coli* which are associated with the toxins discussed above (LT-I and LT-II). N-linked glycoproteins with trisaccharide III were active, but GMP and other O-linked glycoproteins were not. Thus, GMP inhibits toxin binding but not bacterial binding. There are reports that trisaccharide III prevents haemagglutination of other *E. coli* strains (Parkkinen *et al.* 1986; Liukkonen *et al.* 1992). Also, proteins with O-linked trisaccharide III and tetrasaccharide IV have modest activity in binding of *Mycoplasma gallisepicum* (Glasgow & Hill, 1980) and *M. pneumoniae* (Loomes *et al.* 1984) which are associated with an autoimmune disorder. There should be evaluations of GMP in these applications.

Suppression of gastric secretions

A Russian group led by Stan and Chernikov (Vasilevskaya *et al.* 1977) showed that GMP inhibits gastric secretions and slows stomach contractions in dogs. When dogs were intravenously injected with 10–15 mg of bovine GMP, their gastric secretions became less acidic and motions at the gastric fundus and the duodenum were reduced (Stan & Chernikov, 1982). Rat derived GMP had the same effect (Vasilevskaya *et al.* 1977). Further experiments showed that a pepsin digest of GMP produced two active fractions which were separable with Sephadex (Chernikov & Stan, 1982; Stan *et al.* 1983). The stronger of these was a 700–2000 Da peptide fraction (Stan & Chernikov, 1979). Activity of the two fractions was resistant to proteolysis with pepsin, trypsin and chymotrypsin. It was also established that GMP peptides could reduce gastric acid secretions by half and blood serum gastrin levels by 8 % (Aleinik *et al.* 1986).

Further experiments were carried out in France at the INRA laboratories. Guilloteau *et al.* (1987) found that intravenous injection of GMP afforded no inhibition of gastric secretions or changes of digestive hormone blood plasma levels in preruminant calves. However, they did find that feeding GMP at levels similar to those experienced in normal feeding resulted in inhibition of gastric secretion during the first and second hour after feeding (Guilloteau *et al.* 1994). Feeding at five times the normal feeding level resulted in no effect. Beucher *et al.* (1994*b*) found that feeding one GMP fraction, stimulated the intestinal hormone cholecystokinin which, in turn, regulates gastrointestinal functions. Non-glycosylated GMP had no effect on the basal cholecystokinin level and B variant (which varies from the A variant by two amino acids) glycosylated GMP had only a slight stimulating effect in the rat (Beucher *et al.* 1994*a*). The A variant with a terminal sialic acid (Yvon *et al.* 1994) exhibited the largest stimulation which indicates that both the peptide chain and the carbohydrate structure are important for stimulating gastric secretions.

Stan *et al.* (1983) proposed that it is necessary for the GMP molecule to enter the blood in order to cause gastric acid inhibition. Yvon *et al.* (1994) demonstrated that GMP acts by triggering receptors on the intestinal mucosa.

Promotion of bifidobacterial growth

Bifidobacteria predominate in the lower intestine and are thought to inhibit pathogenic bacterial growth and thereby afford protection from gastrointestinal diseases (Faure *et al.* 1984). Gyorgy *et al.* (1954*a*) found the first evidence of a bifidobacterial growth promoting factor in human colostrum and human milk. The activity in human milk was about half that of colostrum on a dry weight basis. They also found that bovine colostrum has about one-tenth of the activity and bovine milk has one-hundredth of the activity of human colostrum on a dry weight basis (Gyorgy et al. 1954*b*). In a search to identify the bifidus factor, Gyorgy *et al.* (1974) found that GlcNAc and oligosaccharides with terminal GlcNAc promote bifidobacterial growth. Sialidase treatment of α_1-acid glycoprotein resulted in an increase in bifidobacterial growth and they proposed that this is due to cleavage of the terminal NeuAc exposing a GlcNAc.

Since that time the elusive bifidus factor has been sought in bovine milk in general and GMP in particular. Kehagias *et al.* (1977) found some bifidobacterial growth promoting activity in a fraction obtained from a sulphuric acid treatment of whole casein. The preparation was ill defined. They had evidence that it was perhaps κ-casein derived but not similar to GMP. Bezkorovainy *et al.* (1979) found glycopeptide from a bovine milk casein chymotryptic digest to have one-tenth the growth-promoting activity of human milk solids. Azuma *et al.* (1985) compared human and bovine GMPs and found that acidity generation with the latter was one-third as large as the acidity generation with the former.

The situation appears to be quite complex with conflicting data which are not favourable to bovine GMP as a specific bifidus growth promoter. Poch & Bezkorovainy (1988) evaluated several promoter candidates, including bovine casein digest and bovine milk whey, on eight bifidus species. Each of the candidates promoted growth of one or more bifidus species. In further work they showed that any growth-promoting activity of κ-casein goes with the para-κ-casein rather than the GMP when rennet-treated κ-casein is fractionated (Poch & Bezkorovainy, 1991). Petschow & Talbott (1991) reported that growth promoting activity for some bifidus species is present in cow milk ultrafiltration permeate as well as retentate. This would rule GMP out because, as noted

above, oligomerization prevents GMP from passing through an ultrafiltration membrane except at a very low pH. Research by Proulx *et al.* (1992) would lead one to believe that the amino acid portion is critical for bifidus growth but (patented) work (Idota, 1996; Yakabe *et al.* 1994) would favour saccharides containing sialic acid.

Modulation of immune system responses

Splenocyte (spleen lymphocyte) proliferation is a step in the inflammatory response. Inhibition of splenocyte proliferation can be used to demonstrate suppression of an immune response such as an allergic reaction. Research by Otani *et al.* (1992) demonstrated that casein inhibits mouse splenocyte proliferation induced by the mitogen *Salmonella typhimurium* lipopolysaccharide (LPS). Inhibitory activity was due to κ-casein, which upon rennet hydrolysis, results in inhibitory activity being found in the GMP fraction. Para-κ-casein had no inhibitory activity. Upon sialidase digestion, GMP lost its inhibitory activity, indicating that sialic acid is critical to the phenomenon (Otani & Monnai, 1993). Inhibitory activity was reduced after GMP digestion with chymotrypsin but inhibitory activity increased after GMP digestion with trypsin or pronase so the peptide chain must also participate. Inhibition of splenocyte proliferation by GMP was also observed against concanavalin A (Con A), phytohaemagglutinin-P (PHA) and pokeweed mitogen in addition to LPS (Otani *et al.* 1992; Otani & Hata, 1995).

Otani *et al.* (1995) were also able to separate GMP into seven distinct fractions with up to five sialic acid groups containing one or two of the di-, tri- and tetrasaccharides shown above. The fractions inhibited both mouse splenocyte and rabbit Peyer's patch cell proliferation as follows:

(1) three fractions inhibited LPS-induced proliferation;
(2) five fractions, with activity in proportion to the number of sialic acids present, inhibited PHA-induced proliferation;
(3) none of the fractions inhibited Con A-induced proliferation.

Because of reduced inhibitory activity after chymotrypsin digestion, the researchers suggest that the Ser-149 phosphate plays a part in GMP binding to the mitogen receptor.

The investigation then turned to the mechanism by which GMP inhibits mitogens from inducing splenocyte proliferation. Cells were incubated with and without GMP. The cells were then immunostained with anti-κ-casein antibody. Only the cells incubated with GMP retained the anti-κ-casein antibody thus demonstrating that GMP adheres directly to the cell surface (Otani & Monnai, 1995). Monnai & Otani (1997) found that when cells were incubated with GMP, one of the cytokines in the interleukin-1 (IL-1) family, IL-1ra in particular, is synthesized. The IL-1ra blocks the action of IL-1 by binding to IL-1 receptors. Since IL-1 cannot bind to its receptors, it cannot trigger splenocyte proliferation and, in turn, an inflammatory response. In addition to the inhibition of LPS-induced binding of IL-1, they also showed that GMP binds to CD4 + T cells and suppresses PHA-stimulated expression of interleukin-2 (IL-2) receptor and inflammatory response (Otani *et al.* 1996) and whereas GMP binds to CD4+T cells, it does not bind to CD8 + T cells.

Yun *et al.* (1996) studied the effect of GMP on immunoglobulins produced by LPS-stimulated splenocytes. They found that only the IgA concentration was increased by GMP and that only the population of surface IgA-positive cells was increased by GMP. Snow Brand Milk Products (1996) was granted a patent on the use of bovine GMP for accelerating human B lymphocyte growth. In a culture test they showed that GMP accelerates proliferation of normal human B lymphocytes, but not T lymphocytes. This finding substantiates up-regulation of the humoral immune system.

Future directions

This review of the state of GMP research clearly shows that several potential clinical/commercial GMP applications exist. All the phenomena cited were observed *in vitro* or in animals. No reference relating to bovine GMP bioactivity in humans was found. Currently, only inference and extrapolation can be made concerning possible benefits of GMP. Reliable GMP applications in humans could benefit human health as well as provide technical support for functional food type claims for some products produced by the dairy industry. Some possibilities are as follows.

Prevention of bacterial infection and toxin binding is one area worthy of pursuit. Kawasaki *et al.* (1995) obtained a patent for use of GMP to neutralize endotoxin. Dosako *et al.* (1992) obtained a patent related to prevention of *E. coli* adhesion and protection against Epstein–Barr virus. A simpler approach may be feeding of whey protein products. If 1 mg of GMP per day was enough to protect a mouse from bacterial toxins then one might imagine that between 0·5 and 1 g of GMP per day might protect a child. Since GMP is 15 % of whey protein, 10–20 g of 35 % WPC would be needed at a cost of only 1·5–3 cents (at $1·43/kg). However, this approach may not be universally applicable. Neeser *et al.* (1988*b*) demonstrated that CFA/I and CFA/II *E. coli* require an N-linked rather than an O-linked saccharide. Since GMP contains O-linked saccharides it is not effective. It may be that intact GMP is ineffective as a protective agent because there are steric or charge-related factors which interfere with the binding of oligosaccharides to the bacterial receptor. Perhaps GMP could provide an inexpensive source for free oligosaccharides or synthetic oligosaccharide derivatives which would afford antibacterial protection.

The findings regarding gastrointestinal contraction are intriguing. Stan *et al.* (1983) proposed that reducing gastric secretions and stomach mixing would reduce proteolysis in young animals thus preserving milk-derived immunoglobulins, lactoferrin, and lysozyme and allowing them to pass through the stomach intact. These proteins could then provide protection in the intestines. If this premise could be extended to humans it would benefit infant feeding and immunocompromised patients.

There are persistent reports of a modest growth-promoting effect by GMP for some bifidus species. Disaccharide II may be involved. Is this a situation where if one feeds more, one will obtain the desired effect?

Perhaps, but Idota *et al.* (1994) present *in vitro* evidence that even that may not be effective as increasing the bovine GMP concentration from 0·01 to 1·0 mg/ml decreased its growth-promoting activity by as much as half. There is much more to be learned about bifidobacterial growth-promotion by GMP and trials in animals and humans are essential.

An extension of the GMP inhibited LPS-induced B lymphocyte proliferation and PHA-induced T lymphocyte proliferation would be down-regulation of the immune system or oral tolerance (Otani *et al.* 1992). This would afford a newborn animal a passive defence mechanism against a broad spectrum of environmental antigens. Could this be extended to allergy?

Conclusion

It is clear that GMP exhibits several useful biological activities. The most promising areas appear to be in protection from toxins, bacteria and viruses and in regulating the immune system. Articles have appeared in food processing magazines (Steijns, 1996; Clare, 1998; LaBell, 1998) extolling the benefits of GMP as a nutraceutical. Initial attempts to incorporate GMP into meringues, biscuits and apple jelly (Marshall, 1991) met with limited success. Snow Brand Milk Products (1999) has received a patent on a stabilizer/viscosifier which contains GMP. The increased interest due to health-promoting aspects of GMP will spark successful food use applications to take advantage of the biological activities.

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