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Purification of glycomacropeptide from non-dialyzable fraction of sweet whey by anion-exchange chromatography

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Abstract

Glycomacropeptide (GMP) was purified from non-dialyzable fraction of sweet whey by anion-exchange chromatography on DEAE-Sephacel at two pHs 6.4 and 3.0. Chromatography at pH 3.0 (but not pH 6.4) gave a GMP fraction of high purity with its yield (1 g from every litre of whey) being approximately 100 times higher than that shown in the previous report. It was concluded that DEAE-Sephacel chromatography at pH 3.0 is a simple useful method to separate GMP from most whey proteins. It may be applicable to a large scale production of GMP.

Introduction

Glycomacropeptide (GMP), a biologically active compound found in sweet whey, has been the subject of growing interest in recent years (Abd El-Salam *et al.* 1996, Dziuba & Minkiewicz 1996). It is a 64-residue glycopeptide, which lacks aromatic amino acids and contains varying amounts of carbohydrates including N-acetylneuraminic acid (sialic acid) (Eigel *et al.* 1984). Glycomacropeptides are thought to be a potential ingredient for dietetic foods and pharmaceuticals, and thus the development of techniques to purify GMP is important.

Glycomacropeptide has been isolated from sweet whey by anion-exchange chromatography (Kawasaki *et al.* 1992, Kawasaki & Dosako 1994, Outinen *et al.* 1995). However, not much is known about the optimal pH for the purification of this glycopeptide. Its pI is also unknown. Kawasaki & Dosako (1994) reported that GMP is not adsorbed on anion exchanger at pH in the range 6–7, while Kawasaki *et al.* (1992) reported that it is adsorbed at pH ≤ 4. These reports contradict each other and thus requires re-examination. This is justified by the following basic concept. A positively charged glycopeptide desorbed on anion-exchanger at pH 6 has its pI > 6. It is still positively charged at pH 3, and thus desorbed on the same ion-exchanger at this pH. Similarly, a negatively charged glycopeptide adsorbed on anion-exchanger at pH 3 is also adsorbed on the same ion-exchanger at pH 6. Thus, desorption at pH 6 and adsorption at pH 3 for the same glycopeptide does not occur.

The objectives of this study were, therefore, (1) to examine the elution pattern of GMP on a diethylaminoethyl (DEAE)-Sephacel anion exchange column at both pH 6.4 and 3.0, and (2) to purify GMP from non-dialyzable fraction of sweet whey. As major whey proteins have pI values between 4.1 and 5.2, anion-exchange chromatography at pH 6.4 or 3.0, may have the potential to yield high purity GMP.

Materials and methods

A sample of sweet whey was prepared from fresh milk by chymosin treatment followed by centrifugation to remove fat and protein precipitate (Nakano & Ozimek 1998). The sweet whey obtained as supernatant was stored at $-25\,^{\circ}$C for approximately 4 months before it was dialyzed in water and freeze-dried to obtain the non-dialyzable fraction of sweet whey. Dialysis was used to remove materials that interfere with both the

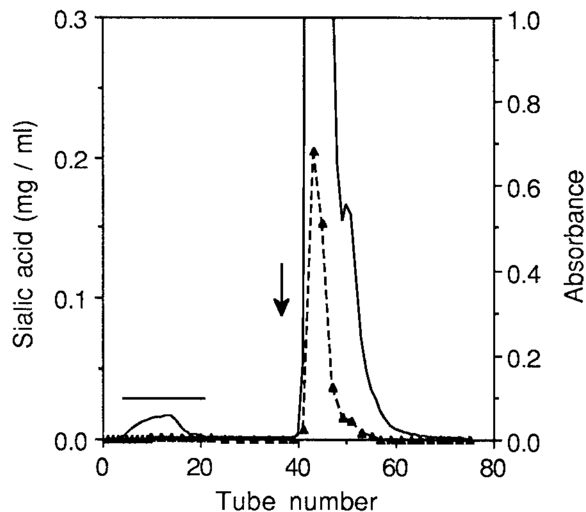

Fig. 1. Chromatography of non-dialyzable fraction of sweet whey on DEAE-Sephacel at pH 6.4. A sample prepared from 78 mg of non-dialyzable fraction of sweet whey was applied to the column. Fractions (1.8 ml) collected at a flow rate of 10 ml h^{-1} were monitored for sialic acid (–▲–) and absorbance at 280 nm (—). An arrow shows the position of application of 1 M NaCl. A bar denotes eluates used for further study. See Materials and methods for other details.

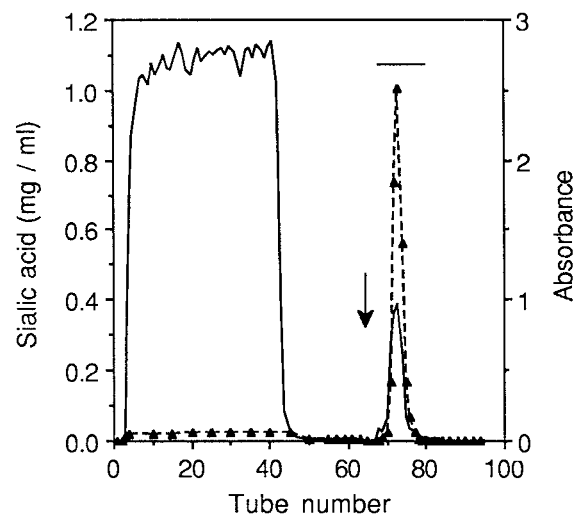

Fig. 2. Chromatography of non-dialyzable fraction of sweet whey on DEAE-Sephacel at pH 3.0. A sample prepared from 300 mg of non-dialyzable fraction of sweet whey was applied to the column. See the legend to Figure 1 for details.

thiobarbituric acid reaction and ultraviolet absorbance determination allowing the sialic acid and protein contents respectively of eluates to be monitored. A reference GMP was prepared from sodium caseinate as described previously (Nakano & Ozimek 1998).

Anion-exchange chromatography on DEAE-Sephacel (Pharmacia Biotech Inc., Canada) was carried out at either pH 6.4 or 3.0. Samples (78 to 300 mg) of non-dialyzable fraction of sweet whey were mixed with water at a ratio of 1 to 200, adjusted to an appropriate pH, and centrifuged at 20000 *g* and 10 °C for 25 min. After centrifugation, the supernatant was filtered through membranes with 0.22 μm pore size. The filtrate was checked for its pH and applied to a 1.5 × 5.2 cm column of DEAE-Sephacel equilibrated with water adjusted to an appropriate pH value. Sodium hydroxide and acetic acid were used to adjust pHs of both the sample and column. Materials adsorbed on the column were eluted by applying 1 M NaCl adjusted at an appropriate pH. Gel chromatography of GMP fraction obtained after DEAE-Sephacel chromatography was carried out as described (Nakano & Ozimek 1998, 1999). The experiment with DEAE-Sephacel was repeated three times, while that with Sephacryl S-200 was repeated twice.

The sialic acid content was determined by the thiobarbituric acid reaction as described previously (Nakano & Ozimek 1998, 1999). Amino acid analysis was performed by using Beckman Model 6300 amino acid analyzer on samples hydrolyzed in 6 M HCl at 110 °C for 24 h.

Results and discussion

A representative DEAE-Sephacel column chromatogram at pH 6.4 is shown in Figure 1. Most sialic acid and protein (both accounting for average 98% of their total amounts recovered) were adsorbed on the column and eluted together, while the remaining small proportion (2%) of each component was not. The dry matter recovered from the unadsorbed fraction averaged 1.0% of the non-dialyzable fraction of sweet whey applied to the column. Its amino acid composition was different from that of GMP (Table 1). Therefore, it is likely that almost all GMPs including sialylated and non-sialylated GMPs are adsorbed on DEAE-Sephacel at pH 6.4. This is consistent with the previous reports demonstrating that GMPs can bind to anion-exchangers at pH 5.0–7.5 (Saito *et al.* 1991, Tanimoto *et al.* 1992, Outinen *et al.* 1995, Nakano & Ozimek 1999), but in disagreement with the findings of Kawasaki & Dosako (1994) who reported that GMPs do not bind to DEAE-Sephadex A-25 at pHs 6–7.

Elution patterns of both sialic acid and protein on DEAE-Sephacel at pH 3.0 differed from those