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Characterization of gliadin–galactomannan incubation mixtures by analytical ultracentrifugation— Part I. Sedimentation velocity

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The aim of this work is to examine the possible interaction and extent thereof of the polysaccharide galactomannan (GAL) with the cereal protein gliadin (GLI) and a peptic–tryptic degraded gliadin (PT–GLI) by analytical ultracentrifugation. The work is part of a series of investigations into the field of coeliac disease (gluten-induced enteropathy) as gliadins are known to be toxic for patients with this disease.

The molecular integrity of the GAL and GLI preparations was first checked by sedimentation velocity and sedimentation equilibrium. Sedimentation velocity showed single boundaries indicating homogeneity and low-speed sedimentation equilibrium gave plausible apparent weight average molar masses of 180,000 g/mol for GAL and 20,000 g/mol for GLI. PT–GLI, GLI and GAL in phosphate buffer (pH 6.5) and the incubated mixtures (stirred for 3 h at 37°C; PT–GLI:GAL = 3.53:1, wt.wt.; GLI:GAL = 0.23 and 0.55:1, wt.wt.) were then investigated by sedimentation velocity at a temperature of 20°C. The plots of $1/s_{20}$ vs. c of GAL, PT–GLI–GAL and GLI–GAL mixtures after incubation show a significantly different shape suggesting the presence of interactions. According to the equation $1/s_{20} = 1/s^{\circ}_{20}(1 + k_s c)$, values for $\{s^{\circ}_{20}, k_s\}$ of {(4.02±0.23) S, (490.9±28.9) ml/g}, {(5.92±0.24) S, (1152±44) ml/g} and {(5.38±0.19) S, (1141±38) ml/g} for GAL and PT–GLI–GAL and GLI–GAL mixtures, respectively, were obtained. The concentration of GAL ranged from 0.75–3.0 mg/ml for GAL alone and from 0.34–1.50 mg/ml in the incubated mixtures. This apparent indication for a weak non-covalent protein–polysaccharide interaction was further supported by UV absorption spectrometry and gel filtration.

INTRODUCTION

There is no doubt that dietary fibres are important physiologically. Dietary fibres influence the digestion of food in general and in particular they alter lipid digestion, cholesterol absorption, reduce the insulin need of diabetics, influence bile acid metabolism and protect against colonic cancer (Sönnichsen & Apostoloff, 1992; Marsh, 1992). Dietary fibres are basically all the polysaccharides and lignin in the diet that are not digested by the endogenous secretions of the human digestive tract (Trowell *et al.*, 1976). Because of the great importance of food proteins in human nutrition the subject of the interaction of such polysaccharides with food proteins is of special relevance. For example, interactions between food proteins and polysaccharides of dietary fibres could protect sensitive persons from harmful effects, e.g. wheat-, soya- and milk-proteins (Stern, 1992; Yamauchi & Suetsuna, 1993; König, 1993).

Interactions between proteins and polysaccharides and the formation of conjugates are well known (Dickinson, 1993; Tolstoguzov, 1992; Harding *et al.*, 1992a; Hill & Mitchell, 1994; Cölfen *et al.*, 1994).

Food proteins from wheat have been of great interest

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in gastroenterology and pediatrics since Dicke (1950) first demonstrated that the clinical manifestations of coeliac disease (CD) (gluten-induced enteropathy) developed after the ingestion of wheat flours and destroys the mucosa of the small intestine. Van de Kamer *et al.* (1953) and Van de Kamer and Weijers (1955) found that the gliadin fraction extracted from wheat flour was toxic to the patients with CD. The etio-patho-mechanism of CD was not sufficiently known until recently (Marsh, 1992; Sönnichsen & Apostoloff, 1992). A critical review of etiology and pathogenesis of coeliac disease has been given by Davidson and Bridges (1987).

Coeliac disease is characterized by a morphological and functional damage of the small intestine mucosa; therefore, investigations of CD should relate as best as possible to the conditions which exist in the small intestinal mucosa. A pH of 6.5 is relevant for the upper small intestine (Paulus & Fricker, 1980; Bauer *et al.*, 1988). A peptic–tryptic digest of whole gliadin approximately corresponds to the state of digestion for this protein in this range of the body also in coeliac disease patients (Mühle & Müller, 1985).

Gliadin itself is one of the main components of wheat proteins (Shewry *et al.*, 1986; Osman, 1992) and on the basis of its ethanol solubility (70% aq. solution) gliadin can be separated from the other wheat proteins (Osborne, 1907). The gliadin fraction is known to consist of more than 40 proteins. According to the electrophoretic mobility differences it can be subdivided into four groups: α-, β-, γ- and ω-gliadins (Shewry *et al.*, 1986).

Locust bean flour derived from the seed endosperm of the carob tree (*Ceratonia siliqua, L.*) contains galactomannan polysaccharides based on a (1–4) linked β-D-mannopyranosyl back-bone partially substituted with single (1–6) linked α-D-galactopyranosyl side-groups (Lopes da Silva & Goncalves, 1990). Locust bean gum has a wide variety of food, pharmaceutical and industrial applications due to its non-toxicity and ability to give very viscous solutions at relatively low concentrations, which are almost unaffected by pH, salts, or heat processing (Dziezak, 1991).

The characterization of gliadins using analytical ultracentrifugation was described over half a century ago (Krejci & Svedberg, 1935; Svedberg & Pedersen, 1940) followed by other studies (Holme & Briggs, 1959; Jones *et al.*, 1965; Beckwith *et al.*, 1966; Booth & Ewart, 2; Sexon & Wu, 1972; Hamauzu *et al.*, 1974). In more recent investigations using this method, molecular weights, sedimentation and diffusion coefficients of gliadin and gliadin fractions have been obtained (Gaugecz *et al.*, 1987). Galactomannan itself has also been investigated with this method (Sharman *et al.*, 1978; Gaisford *et al.*, 1986).

The aim of this work was therefore to examine the possible interaction between a peptic–tryptic-degraded whole gliadin (PT-GLI) or undegraded gliadin (GLI) with galactomannan (GAL) using analytical ultracentrifugation. The undegraded gliadin was also involved in the investigation to reproduce the possibility of incompletely digested gliadin being present in the small intestine. In short, the study should serve as a contribution to a possible protective effect of galactomannan against CD-active gliadin peptides.

EXPERIMENTAL

Materials and methods

Gliadin

Gliadin was prepared and purified according to the large-scale procedure described by Patey and Evans (1973) with slight modifications. The starting material was wheat flour type 550 (750g) obtained from a mixture of the *Triticum aestivum* cultivars MURAS, RAMIRO, ZENTOS and BORENOS harvested in 1991.

The wheat flour was twice extracted with water-saturated *n*-butanol to remove lipids. After centrifugation, the residue was extracted at room temperature with 3 l of 70% ethanol for 2.5 h and centrifuged (2000 x *g*, 20 min) again. The supernatant was concentrated in a rotary evaporator at 40°C. The precipitated gliadin was dissolved in glacial acetic acid (final concentration 0.1 M). The resulting solution was dialysed twice for 24 h against 0.1 M acetic acid and then freeze-dried with 30°C counter heating. A white powder (crude gliadin) was obtained.

Subsequently, cation exchange chromatography was performed to purify the gliadin. Conditions: cation exchanger: CM-Sephadex C50 (Pharmacia, Bromma, Sweden); column: 2.6 x 40 cm; room temperature; sample: 1.5 g crude gliadin dissolved in elution buffer; elution solvent: buffer A: 0.005 M sodium acetate, 1 M dimethylformamide at pH 3.5; buffer B additionally 0.5 M sodium chloride; 750 ml of each buffer was gradually mixed to build a linear gradient (flow rate: 30 ml/h; fraction size: 20 ml; detection: optical absorption at 280 nm).

The fractions were then ultrafiltered (UF) on an Amicon membrane YM 10 to remove traces of salt and washed with 0.05 M acetic acid buffer. Finally the UF-retentate was freeze-dried. The gliadin sample employed for the investigations was analysed by horizontal SDS-PAGE. The electrophoresis was performed on the Pharmacia Multiphor II assembly. Conditions (cf. Pharmacia application note E-65 6/92): gradient gel from 5 to 20% T, buffer kit pH = 8 (Pharmacia No. 18-1031-60), unreduced samples; silver staining according to Blum (1987).

Peptic–tryptic-degraded gliadin

To overcome the problems of enzyme contamination and high salt contents in the gliadin digests, the method

of Frazer *et al.* (1959) was modified: 1 g of gliadin was incubated in 50 ml 0.02 N HCl with at least 50,000 units of immobilized pepsin (attached to agarose beads) under gentle stirring (100 r.p.m.) at 37°C for 3 h.

The pH of the digestive solution was adjusted to 1.9–2.0 and checked every 15 min during the incubation time. After this cycle, particulate matter was removed by filtration (glass filter) and the peptic digest was adjusted to pH 8 with 35% ammonium hydroxide solution. Enzyme units (150) of immobilized trypsin (attached to agarose beads) were added and the digestion was performed for 3 h (37°C, 100 r.p.m). Then the trypsin beads were removed by filtration, the digest was topped up to 100 ml and boiled for 1 min. The resulting dispersion was clarified by centrifugation (10 min, 10,000 x *g*) and the supernatant liquid was freeze-dried. The relative molar mass of the PT–gliadin was estimated by SEC (sample concentration: 12 mg/ml; sample volume: 40 μl, overfill-volume 100 μl; column: Bio-Rad, Bio-Gel TSK 50 XL, 300 x 7.8 mm; flow rate: 0.25 ml/min; pressure: 5–6 kg/cm^2, wavelength: 280 nm, elution buffer: 0.1 m phosphate buffer, pH 6.5). Based on a calibration curve from the retention time, the relative molar mass was determined.

Galactomannan

Galactomannan was isolated from locust bean flour (a kind gift from NESTLE Ltd, Switzerland) according to the procedure of Lopes da Silva and Goncalves (1990), but with some variations: 1 g locust bean flour was dispersed in 100 ml distilled water with vigourous stirring at room temperature for 30 min. Then the dispersion was heated under stirring to 80°C and held at this temperature for 30 min. The resulting slurry was centrifuged at 15,000 x *g* for 10 min. The supernatant was added to a two volume excess of 80% isopropanol and stirred for 30 min to complete the precipitation. The fibrous precipitate was collected by a glass filter and washed twice with isopropanol. The wet galactomannan fibres were then dissolved in water and precipitated again. The resulting galactomannan was dissolved in water, freeze-dried and used for the investigations.

PT-gliadin, gliadin and galactomannan solutions

PT–gliadin, gliadin and galactomannan were dissolved in the same phosphate buffer solution. It was prepared by mixing 25ml aq. solution of Na_2HPO_4 (6.586g/l sol.) and 40ml aq. solution of KH_2PO_4 (5.036g/l sol.) at pH 6.5. No additional electrolyte was employed. The buffer density as measured by an Anton Paar DMA 02C density meter at 20°C (according to Kratky *et al.*, 1973) was 1.00262g/ml and the relative buffer viscosity as determined by an automatic Schott CT 50, AVS. 310 viscometer was 1.0187.

The peptic–tryptic-degraded gliadin (PT–gliadin) was almost completely soluble in the buffer. After stirring for 4 h at room temperature, the turbid solution was filtered through a 0.45μm membrane filter (Sartorius, Minisart® NML) resulting in a clear, colourless solution.

In contrast to the PT–gliadin, the freeze-dried gliadin was difficult to get into solution in the employed buffer with pH 6.5 because the isoelectric point of gliadin is at pH 6.5. After stirring the gliadin sample in the phosphate buffer at room temperature for 6 h, the gliadin solution was separated from the undissolved part. After filtering the solution through a 5μm membrane filter (VESTAR, INC., 650 Cliffside Drive, San Dimas, CA 91773, USA), a clear, colourless solution resulted.

The freeze-dried galactomannan was dissolved by the following procedure. After hydrating the sample in phosphate buffer overnight followed by stirring at room temperature for 4 h, the temperature was raised to 80°C for 2 h. Then, after cooling the solution to room temperature, undissolved components (flocs) were removed by filtering through a 5μm membrane filter as described above giving a clear, colourless, viscous solution.

As a check on the molecular integrity of the gliadin and galactomannan, their molar masses were checked firstly by low-speed equilibrium runs on the Optima XL-A (Beckman Instruments, Palo Alto, California, USA) at 10,000 and 15,000 r.p.m. The monochromator of the XL-A absorption optics was set to 220 and 230 nm. The experiments were performed with solutions of gliadin (0.34–0.68 mg/ml) and galactomannan (1.00–1.50 mg/ml). The equilibrium solute distributions were evaluated using the MSTARA computer evaluation program as described by Harding *et al.* (1992b).

Incubation mixtures of PT–gliadin or gliadin and galactomannan

Mixed solutions of PT–gliadin and galactomannan (3.53:1, wt.wt.) and gliadin and galactomannan (0.55:1 and 0.23:1, wt.wt.) were stirred in an incubator at 37°C for 3 h. The mixtures were then investigated by analytical ultracentrifugation directly after incubation.

The protein content of the solutions of PT–gliadin, gliadin and PT–gliadin/galactomannan and gliadin/galactomannan incubation mixtures was determined by the Kjeldahl procedure (Gerhard, Chemische Apparate GmbH, Bonn, Germany) using a nitrogen to protein conversion factor of 5.7 for wheat gliadin (Schormüller, 1974).

UV absorption spectra

The UV absorption spectra of galactomannan, PT–gliadin, gliadin and the incubation mixtures were determined on both DU 50 (Beckman) and UV-160A (Shimadzu) spectrophotometers.

Gel filtration

The PT–gliadin, galactomannan and incubation mixture (PT–GLI:GAL = 1.67: 1, wt.wt.); incubation condition

as described above but with the following incubation times: 3, 9, 36, 144, 288 h) were all investigated by gel filtration (medium: Biogel P 100; gel: 1.5 x 25.5 cm; elution medium: 0.9% NaCl; elution rate: 0.3 ml/min; detection: PT–gliadin: 280 nm, GAL: phenol/sulphuric acid reaction; sample volume: 0.5 ml).

Sedimentation velocity analysis

In high-speed sedimentation velocity experiments with single-sector cells at 40,000, 48,000 and 50,000 r.p.m. using the Philpot–Svensson–Schlieren (refractive index gradient) optics, the rate of movement of the maximum of the Schlierens peaks was measured to obtain the sedimentation coefficients. The sedimentation coefficient is a function of the size and shape of the dissolved particles, the properties of the solvent and the interaction properties of the system. This technique was also used for investigating the presence of possible impurities in the galactomannan and gliadin preparations in the usual way. Further, to detect the presence of large molecular weight components — particularly in the mixtures — the velocity runs were performed starting at a low speed of 2000 r.p.m. and in some cases with double-sector synthetic boundary cells of the capillary type.

Two different types of analytical ultracentrifuges were used as independent checks: (i) a model 3170B analytical ultracentrifuge (Hungarian Optical Works, MOM, Budapest) employing the 'phase-plate' or Philpot–Svensso–Schlieren optics, (ii) a Beckman Model E (Beckman instruments, Palo Alto, California, USA) also equipped with Philpot–Svensson–Schlierens optics. All measurements were carried out at 20°C. To our knowledge, this is the first time that results obtained with these two types of analytical ultracentrifuges have been compared directly.

Sedimentation velocity runs were carried out on solutions of PT–gliadin (5 mg/ml), gliadin (0.68mg/ml), galactomannan (1.0 – 3.0 mg/ml) and the incubated mixtures of PT–gliadin/galactomannan (PT–GLI:GAL = 3.53:1, wt./wt., GAL concentration 0.375–1.5 mg/ml) and gliadin/galactomannan (GLI:GAL = 0.23:1, wt./wt; GAL concentration 0.67–1.5 mg/ml and 0.55:1, wt./wt.; GAL concentration 0.75 mg/ml). Some runs were also performed with gliadin/galactomannan mixtures (GLI:GAL = 0.23:1, wt./wt; GAL concentration 1.5 mg/ml) directly after mixing and without incubation. These concentrations were the cell loading concentrations.

The optical Schlieren records were photographed and the negatives were evaluated directly (without making positive enlargements) (i) on a magnification equipment (Carl Zeiss Jena, magnification 20-fold) and/or (ii) a photographic enlarger throwing the image onto a graphics digitizing tablet partly using a new computer program for the evaluation of sedimentation velocity experiments with variable time intervals written by Cölfen (1994). A comparison of the plots of $1/s_{20}$ vs. c of galactomannan with those for PT–gliadin or gliadin/galactomannan incubation mixtures are used to provide information about interactions between these components.

RESULTS AND DISCUSSION

The PT–gliadin and the gliadin are fairly heterogeneous and show a broad, but only *one* Schlieren boundary in sedimentation velocity experiments, indicating single sedimenting components (Figs.1a and b). In contrast to this, the galactomannan preparation shows a hypersharp peak (Fig. 1c). In runs (partly with double-sector synthetic boundary cells of the capillary type) starting at a low speed of 2000 r.p.m., the same results were obtained indicating that the samples were free of low and high molecular weight impurities.

The physical integrity of the gliadin and galactomannan was confirmed by sedimentation equilibrium experiments: the evaluation of 'low-speed' sedimentation equilibrium runs using the MSTARA program (Harding *et al.*, 1992b) gave apparent whole cell weight average molar masses $M_{w,\,app.}$ of ~20,000 g/mol for gliadin and about ~180,000 g/mol for galactomannan. The value for gliadin is in good agreement with that of Gaugecz *et al.* (M_w = 23,100 g/mol) which has also been found from 'low-speed' sedimentation equilibrium runs (Gaugecz *et al.*, 1987). This is in contrast to the values of 16,000 and 32,000 g/mol components found from meniscus depletion 'high speed' equilibrium runs (Gaugecz *et al.*, 1987). The sedimentation velocity results of the gliadin sample employed are in agreement with the SDS-PAGE results (see Fig. 2), namely, it was free of low and high molecular weight impurities.

The PT–gliadin/galactomannan (3.53:1, wt.wt.) and gliadin/galactomannan (0.23:1 and 0.55:1, wt.wt.) incubation mixtures also show only one sharp Schlieren peak (see Figs 3a and b). The hypersharp peak in the galactomannan Schlieren patterns might well be caused by a significant concentration dependence of the sedimentation coefficient due to a more or less rodlike solution structure of the polymer. The surprising disappearance of the gliadin's broad Schlieren pattern and the occurrence of only one sharp peak with the incubation mixtures (also with diluted solutions) are the first hint for a gliadin–galactomannan interaction. An explanation could be the formation of galactomannan vesicles which include the gliadin molecules. The assumption of a galactomannan association is supported by the results of rheological measurements (Lopes da Silva & Goncalves, 1990).

Because of the smaller molecular size, the peptic–tryptic-degraded gliadin (relative molar mass 5.930 g/mol from HPLC) should be included to a greater extent

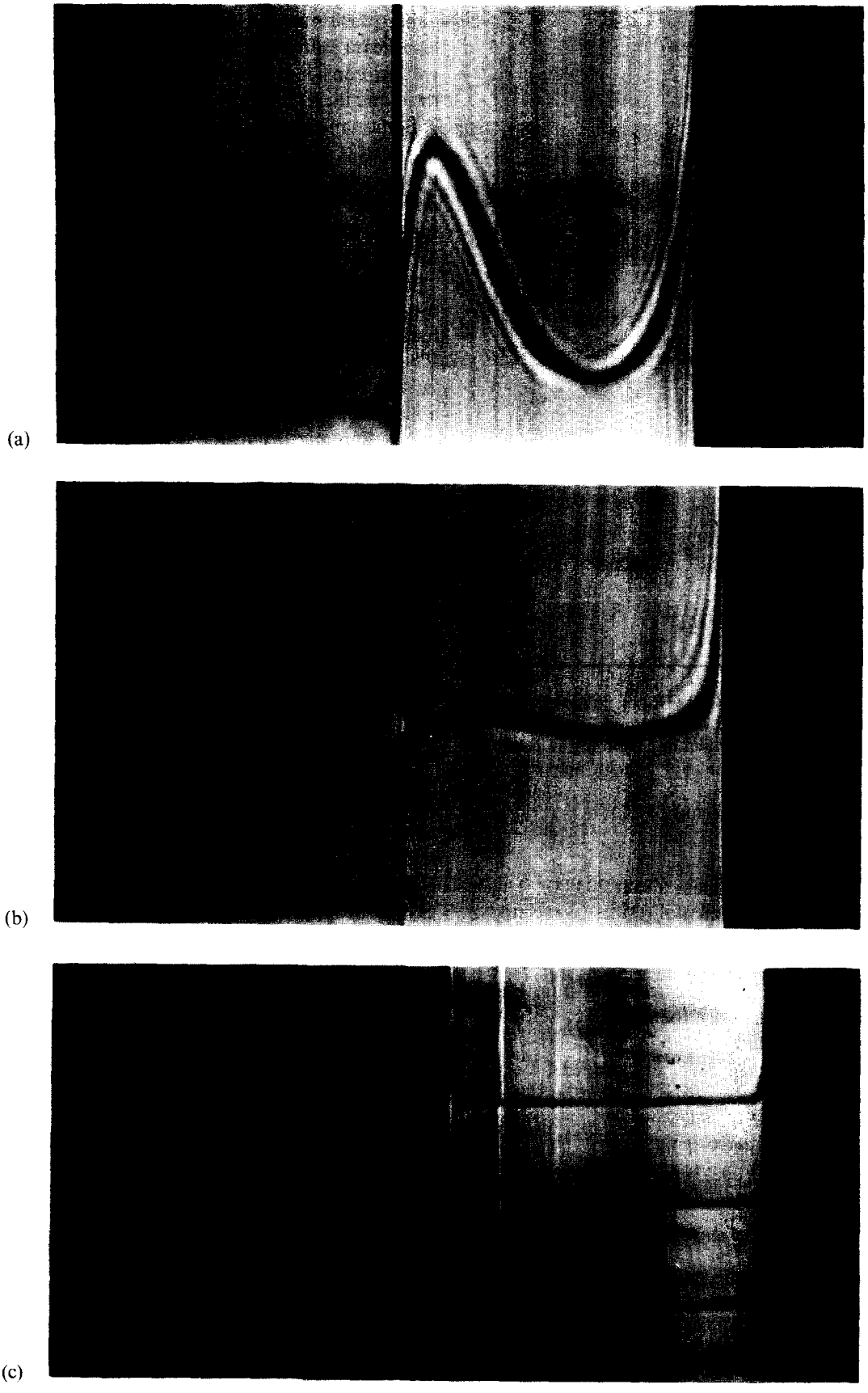

Fig. 1. Sedimentation velocity diagrams of (a) PT–gliadin 2 mg/ml after 79 min at 50,000 rev./min and 5 min at 10,000 rev./min; (b) gliadin 0.68 mg/ml after 105 min at 50,000 rev./min; and (c) galactomannan 3 mg/ml (upper profile), 2.5 mg/ml (middle profile) and 2.0 mg/ml (lower profile) at 48,000 rev./min after 162 min. All samples have been investigated in phosphate buffer at pH 6.5 and 20°C.

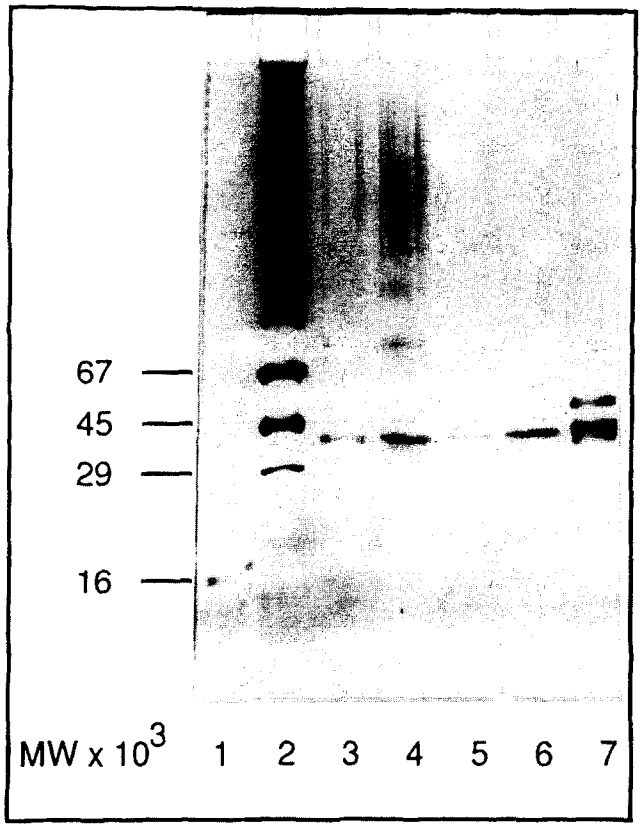

Fig. 2. Horizontal gradient SDS-PAGE of gliadins. The samples investigated are from left to right: 1 and 2 = SERVA marker proteins, 3 and 4 = crude gliadin according to Patey-Evans (3 = 5 μl, 4 = 10 μl), 5 and 6 = gliadin preparation investigated in this paper (5 = 5 μl, 6 = 10 μl), 7 = commercial gliadin from FLUKA.

than the whole gliadin (molar mass about 20,000). This is in agreement with the experimental results (no second peak in the case of PT–GLI–GAL incubation mixture in spite of the essentially higher PT–GLI:GAL ratio of 3.53:1). From Fig. 1a it can be deduced that pure PT-gliadin is able to sediment under the chosen conditions although it is relatively small.

The plots of $1/s_{20}$ vs. c of GAL and the GLI–GAL and PT–GLI–GAL mixtures after incubation show considerably different concentration dependencies (Fig. 4). The regression analysis of $1/s_{20} = 1/s^\circ_{20}(1 + k_s c)$ yields values of $s^\circ_{20} = (4.02 \pm 0.23)$ S, $k_s = (490.9 \pm 28.9)$ ml/g for galactomannan, s°_{20} (5.92±0.24) S, $k_s = (1152 \pm 44)$ ml/g for the PT-gliadin/galactomannan incubation mixture and s°_{20} (5.38±0.19) S, $k_s = (1141 \pm 38)$ ml/g for the gliadin/galactomannan incubation mixture. The errors given are the standard deviations. The higher s°_{20} values at zero concentration of the PT–GLI–GAL and GLI–GAL incubation mixtures provide evidence for a weak protein–polysaccharide interaction, especially if their low molar mass (and the low protein concentration in the case of whole gliadin) is taken into account. The similar k_s values for the mixtures support this view. A rise of the gliadin concentration from GLI:GAL = 0.23:1 to 0.55:1 has no significant influence. Nevertheless, the protein concentration considered in the latter is too low to cause a dramatic increase of the sedimentation coefficient.

Some samples of unincubated gliadin/galactomannan mixtures (GLI:GAL = 0.23:1) investigated directly after mixing also revealed a single sedimenting component and give s_{20} values in agreement with comparable samples from the incubation mixtures. Incubation at 37°C for 3 h results in no further interaction.

These observations are supported by UV absorption measurements (galactomannan has no absorption maximum in the range 200–400 nm). In the same range PT-gliadin and gliadin have an absorption maximum between 272 and 276 nm, and below 250 nm the absorbance increased strongly. The UV spectra of the incubation mixture of PT-gliadin/galactomannan were in agreement with that of the PT-gliadin solution of the same gliadin concentration, indicating that there were no aggregates formed during the incubation procedure which might have influenced the UV absorption.

The results raise the question as to the possible type of interaction. The formation of a covalent binding in analogy to the results of Nakamura *et al.* (1992) for lysozyme/galactomannan is unlikely as the drastic conditions necessary for a Maillard reaction (higher temperature and prolonged reaction times) were not employed in the incubation procedure. For the PT-gliadin/galactomannan and gliadin/galactomannan incubation mixtures and the unincubated mixtures of gliadin/galactomannan, hydrophobic interactions and hydrogen bonding could be expected, however. Furthermore, in the case of the more hydrophilic PT-gliadin the hydrogen binding is more likely.

A non-covalent interaction between PT-gliadin and galactomannan is supported by gel filtration. The result showed no significant difference in the mean elution volumes (ml) between the two single components (GAL: 27.5; PT-GLI 77.5) and the incubation mixture (also two single components) at different incubation times (3 h: GAL: 27.5, PT-GLI 75.0; 9 h: GAL: 25.0, PT-GLI 80.0; 36 h: GAL: 22.5, PT-GLI 75.0; 144 h: GAL: 22.5, PT-GLI 80.0; 288 h: GAL: 22.5, PT-GLI 80.0). In the case of a covalent binding there should be a shift of the peaks: this was not observed. Finally, we see our observations as a springboard for further investigations which will form the basis of further papers in this series. Specifically:

1. More quantitative information of a weak gliadin–galactomannan interaction can be obtained using sedimentation equilibrium data with its various evaluation techniques such as the omega analysis (Milthorpe *et al.*, 1975; Nichol *et al.*, 1976).
2. Additional methods for the investigation of a prolamine–galactomannan interaction should be consid-

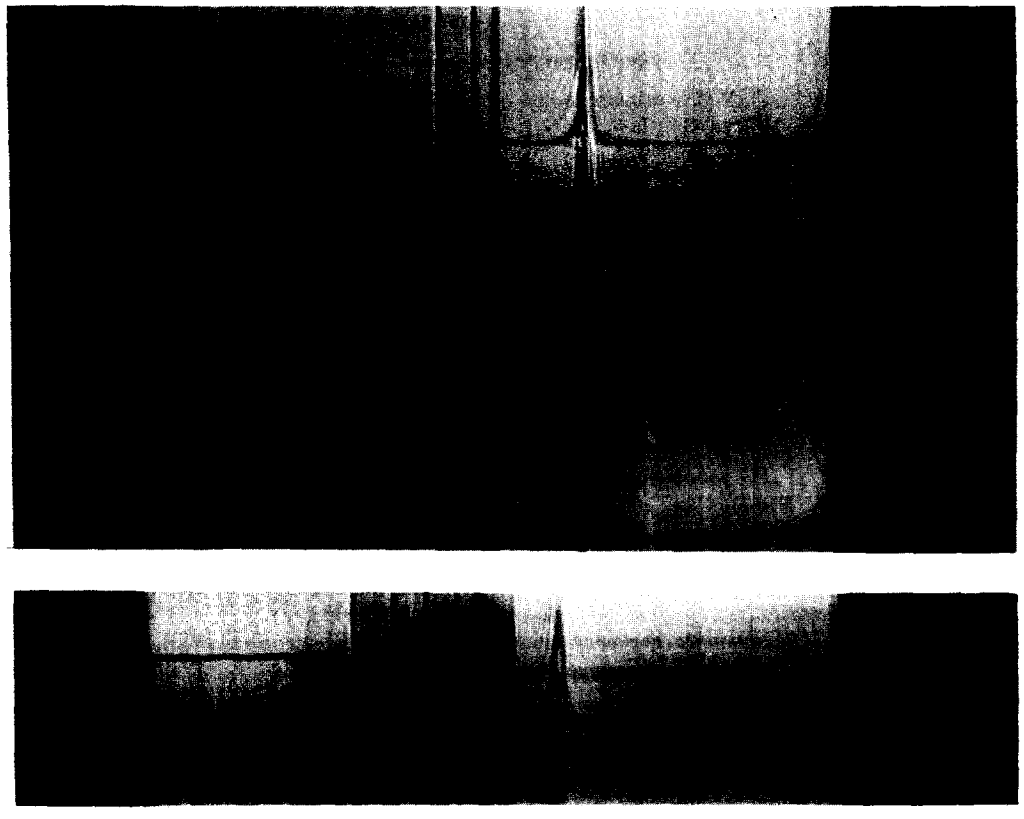

Fig. 3. Sedimentation velocity diagrams of a (a) PT–gliadin/galactomannan incubation mixture (PT–GLI: GAL = 3.53: 1, wt.wt. with GAL = 1.5 mg/ml), incubation time 3h at 37°C, lower profile) and two galactomannan controls (1.5 mg/ml upper and 3.0 mg/ml middle profile) at 48,000 rev./min after 111 min of centrifugation and (b) gliadin/galactomannan incubation mixture (0.23: 1, wt.wt. with GAL = 1.2 mg/ml, incubation time 3h at 37°C) at 50,000 rev./min after 63 min of centrifugation. All samples were in phosphate buffer, pH 6.5 at 20°C.

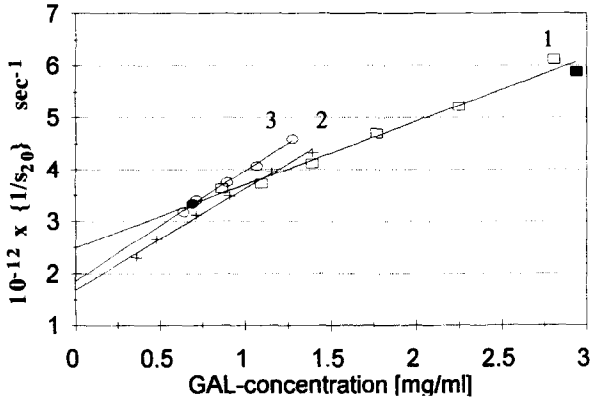

Fig. 4. Plot of the reciprocal sedimentation coefficient $1/s_{20}$ vs. GAL concentration c due to $1/s_{20} = 1/s_o(1 + k_s c)$. The concentrations have been corrected for radial dilution.

1 □ Pure galactomannan Beckman Model E
■ Pure galactomannan MOM Model 3170B
s°_{20} = (4.02±0.23) S; k_s = (490.9±28.9) ml/g

2 + PT–gliadin/galactomannan incubation mixture 3.53:1 (wt.wt.)
s°_{20} = (5.92±0.24) S, k_s = (1152±44) ml/g

3 ○ Gliadin/galactomannan incubation mixture 0.23:1 (wt.wt.)
● Gliadin/galactomannan incubation mixture 0.55:1 (wt.wt.)
s°_{20} = (5.38±0.19) S, k_s = (1141±38) ml/g

ered, e.g. real-time biospecific interaction analysis (BIAcore, Pharmacia) and differential scanning calorimetry.

3. The investigation of the peptic–tryptic-degraded gliadin would seem to be very important. Therefore, further investigations should be focused on the peptic–tryptic-degraded gliadin and single components of α-,β-,γ- and ω-gliadins as starting materials should also be taken into consideration.
4. Further investigations should be performed on the interaction of galactomannan with gliadin or gliadin subunits degraded under the digestion conditions of the stomach (pepsin, pH = 1.3).

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REFERENCES

Bauer, K. H., Lehmann, K., Osterwald, H. P. & Rothgang, G. (1988). *Überzogene Arzneiformen*, Wissenschaftliche Verlagsgesellschaft Stuttgart, pp. 130–135.

Blum, H. (1987). *Electrophoresis*, **8**, 93–99.

Beckwith, A.C., Nielsen, H.C., Wall, J.S. & Huebner, F.R. (1966). *Cereal. Chem.*, **43**, 14–28.

Booth, M.R. & Ewart, J.A.D. (1969). *Biochim. Biophys. Acta*, **181**, 226–233.

Cölfen, H., Harding, S.E., Winzor, D.J. & Vårum, K. (1994). A study of the interaction between Chitosan and lysozyme. In *Abstracts of the 56th Meeting of the Easter school Series in Agricultural and Food Sciences "Biopolymer Mixtures"*, University of Nottingham, Sutton Bonington Campus, 19–21st Sept., 33.

Cölfen, H. (1994), unpublished.

Davidson, A.G.F. & Bridges, M.A. (1987). *Clinical Chimica Acta*, **163**, 1–40.

Dicke, W.K. (1950). *Coeliakie*, M.D. Thesis, Utrecht.

Dickinson, E. (1993). *Protein-polysaccharide interactions in food colloids*. In *Food Colloids and Polymers: Stability and Mechanical Properties*, eds. E. Dickinson & P. Walstra. Cambridge, Royal Society of Chemistry, pp. 77–93.

Dziezak, J.D. (1991). *Food Technology*, **3**, 116.

Frazer, A.C., Fletcher, R.F., Ross, C.A.C., Shaw, B., Sammons, H.G. & Schneider, R. (1959). *Lancet*, **2**, 252–255.

Gaugecz, J.S., Békés, F. & Lásztity, R. (1987). Analytical ultracentrifugal investigation of gliadin fractions. In *Gluten Proteins* (Proc. 3rd International Workshop, Budapest, Hungary, May 9–12, 1987). eds. R. Lásztity & F. Békés. World, Scientific Singapore, New Jersey & Hong Kong, pp. 578–587.

Gaisford, S.E., Harding, S.E., Mitchell, J.R. & Bradley, T.D. (1986). *Carbohydrate Polym.*, **6**, 423.

Harding, S.E., Jumel, K., Kelly, R., Godo, E., Horton, J.C. & Mitchell, J.R. (1992a). The structure and nature of protein–polysaccharide complexes. In *Food Proteins — Structure and Functionality* (4th Symposium on Food Proteins "Structure-Functionality Relationships", Reinhardsbrunn, Germany, October 5–8, 1992), Schwenke, K.D. & Mothes, R., Verlag Chemie, Weinheim, 1993, p. 216.

Harding, S.E., Horton, J.C. & Morgan, P.J. (1992b). *Analytical Ultracentrifugation in Biochemistry and Polymer Science*, eds S.E. Harding, A.J. Rowe & J.C. Horton, The Royal Society of Chemistry, pp. 275–294.

Hamauzu, Z., Toyomasu, T. & Yonezawa, D. (1974). *Agr. Biol. Chem.*, **38**, 2445–2450.

Hill, S. E., Mitchell, J. R. (1994). Chemical interactions in mixed biopolymer systems. Abstracts of the 56th Meeting of the Easter School Series in Agricultural and Food Sciences "Biopolymer Mixtures", University of Nottingham, Sutton Bonington Campus, 19–21st Sept.

Holme, J. & Briggs, D.R. (1959). *Cereal. Chem.*, **36**, 321–340.

Jones, R.W., Babcock, G.E. & Dimler, R.J. (1965). *Cereal. Chem.*, **42**, 210–214.

König, E. (1993). *Die Milcheiweißallergie — Ursachen, Diagnose, Behandlung*. In *Milchwissenschaft Giessen*, ed. E. Renner, 15.

Kratky, O., Leopold, H. & Strabinger, H. (1973). *Meth. Enzymol.*, **27**, 98.

Krejci, L.E. & Svedberg, T. (1935). *J. Am. Chem. Soc.*, **57**, 946–951.

Lopes da Silva, J.A.M. & Goncalves, P. (1990). *Food Hydrocolloids*, **4**, 277–287.

Marsh, M.N. (1992). *Gastroenterology*, **102**, 330–354.

Milthorpe, B.K., Jeffrey, P.D. & Nichol, L.W. (1975). *Biophys. Chem.*, **3**, 169.

Mühle, W. & Müller, F. (1985). *Z. Klin. Med.*, **40**, 1413–1419.

Nakamura, S., Kato, A. & Kobayashi, K. (1992). *J. Agric. Food Chem.*, **40**, 735–739.

Nichol, L.W., Jeffrey, P.D. & Milthorpe, B.K. (1976). *Biophys. Chem.*, **4**, 259–267.

Osborne, Th.B. (1907). *The Proteins of the Wheat Kernel*, Carnegie Inst. Publ., **84**.

Osman, A.A. (1992). *Dissertation*, Universität Leipzig, Germany, (1973).

Patey, A.L. & Evans, D.J. (1973). *J.Sci. Fd. Agric.*, **24**, 1229–1233.

Paulus, K. & Fricker, A. (1980). *Lebensm. Technol.*, **31**, 128.

Schormüller, J. (1974). *Lehrbuch der Lebensmittelchemie*, Springer-Verlag, Berlin, p. 37.

Sexon, K.R. & Wu, Y.V. (1972). *Biochim. Biophys. Acta*, **263**, 651–657.

Sharman, W.R., Richards, E.L. & Malcolm, G.N. (1978). *Biopolymers*, **17**, 2817–2833.

Shewry, P.R., Tatham, A.S., Forde, J., Kreis, M. & Miflin, B.J. (1986). *J. Cereal Sci.*, **4**, 97–106.

Sönnichsen, N. & Apostoloff, E. (1992). *Autoimmunkrankheiten*, Gustav Fischer Verlag Jena, Stuttgart, p. 384

Stern, M. (ed.) (1992). Proceedings of the 7th meeting on Prolamin Analysis and Toxicity, Schloß Weitenburg, Germany, 4–6th Nov., Universitäts-Kinderklinik Tübingen, Germany.

Svedberg, T. & Pedersen, K.O. (1940). *Die Ultrazentrifuge*. In *Handbuch der Kolloidwissenschaft in Einzeldarstellungen, Band VII*. ed. W. Ostwald, Theodor Steinkopff, Dresden, p. 349.

Tolstoguzov, V.B. (1992). Functional properties of food proteins. Role of interactions in protein systems. In *Food Proteins — Structure and Functionality* (4th Symposium on Food Proteins "Structure–Functionality Relationships", Reinhardsbrunn, Germany, 5–8 October, 1992), eds K.D. Schwenke & R. Mothes, Verlag Chemie, Weinheim u.a. 1993, p. 203

Trowell, H., Southgate, D.A.T., Wolever, T.M.S., Leeds, A.R., Gassull, Ma. & Jenkins, D.J.A. (1976). *Lancet*, **1**, 967.

Van de Kamer, J.H., Weijers, H.A. & Dicke, W.K. (1953). *Acta Paediatr.*, **42**, 223–231.

Van de Kamer, J.H. & Weijers, H.A. (1955). *Acta Paediatr.*, **44**, 465–469.

Yamauchi, F. & Suetsuna, K. (1993). *J. Nutr. Biochem.*, **4**, 450.