STUDIES ON GASTRIC MUCOSAL IgA: SEPARATION OF IMMUNOGLOBULIN RICH FRACTION FROM GASTRIC MUCOPROTEINS

by

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Received October 5, 1977

Summary.

Human gastric mucosal scrapings were subjected to fractionation on an isopycnic CsCl gradient. Immunoglobulin A was found between the 5th and 10th ml from the top of the tube. (Total volume 12ml). After two-fold fractionation the combined IgA containing fraction accounted for 4%-7% of the total carbohydrate content of the original gastric mucosal scrapings. Gas liquid chromatography of sugars showed the fraction to be enriched in Mannose and N-Acetyl glucosamine. The total carbohydrate content of the material was 5.5%-7% by weight. Immunodiffusion against specific anti Secretory component serum failed to demonstrate the presence of the secretory component in this fraction. It is concluded that gastric mucosal IgA, which appears to differ from a typical sIgA in lacking the characteristic secretory component activity, can be separated from the carbohydrate-rich gastric mucoproteins by CsCl fractionation. This indicates the absence of covalent bonding between IgA and the mucoproteins of gastric mucus.

Introduction.

IgA is the predominant immunoglobulin in most secretions, including those of the gastrointestinal tract. (1-5). The role which this immunoglobulin plays in the defence of the mucosa and its structural and functional relationship to the carbohydrate-rich mucoproteins secreted by the tissues are unknown. Investigations of gastric mucus are hindered by the difficulty of solubilising the viscous gel which lines the mucosa, and by the inadequacy of gel filtration techniques in separating gastric mucoproteins from non-covalently bound proteins (6,7). In our work we have used the method of isopycnic fractionation on CsCl gradient (8) to investigate the distribution of IgA

ISSN 0006-291X

of human gastric mucus on the gradient, and to compare it with the distribution of mucoproteins.

Materials and Methods.

Mucosal scrapings of fresh gastric mucosa, removed from the antrum during operations for gastric or duodenal ulcers, were used. In each case only areas which appeared normal to the naked eye and were at least 5 cm away from pathological lesions were scraped. Prior to scraping the tissue was washed with physiological saline to remove any loosely adhering luminal material, and thus only mucus which adhered firmly to the mucosa and was presumably associated with the particular part of the stomach was investigated. The washing and scraping was performed within 10 min. of resection. The viscous scrapings were suspended in 0.1M Tris HCl buffer pH8.3 containing 5mM EDTA. Approximately 10ml of buffer were used per ml of the gel. Solid CsCl was added to the suspension (0.425g CsCl per ml of the suspension). The mixture was stirred and allowed to stand at room temperature for 15 minutes before loading into tubes. It was then centrifuged in a Beckman L 65 Ultracentrifuge for 72 hours at 5°C in a fixed angle rotor 40 at 40000rpm (106 500g av.) At the end of this period the contents of the tubes were removed in 1 ml aliquots from the top of the tube. The separated 1 ml fractions were analysed for total hexose by the phenol/H_2SO_4 method of Dubois et al (9), and for the presence of IgA by immunodiffusion against specific anti IgA (α-chain) serum. (Behring, Hoechst Pharmaceuticals). The fractions were pooled to give : L1 - the top layer free of IgA, L2 - the middle layer which was IgA positive, and L3 - the bottom layer which again showed absence of IgA but did contain a viscous gel sediment. These three fractions were dialysed and lyophilised, the solid material was weighed and the protein content was determined by the method of Lowry (10).

The IgA containing fraction L2 was further purified second fractionation of the lyophilised material on CsCl gradient. It was the purified IgA-positive fraction L2 which was used for carbohydrate analysis and for immunodiffusion against anti Secretory component serum.

Carbohydrate analysis of the three fractions L1, L2 and L3 was performed by GLC of Trimethylsilyl derivatives of methoxy sugars after methanolysis in dry methanolic HCl (IN HCl in dry methanol) overnight at 75°C (11). GLC was carried out in a Perkin-Elmer Gas Chromatograph, model F11, on a column of 2.5% Silicone gum rubber, E301 on Chromosorb GAW-DMCS. The samples were run for 20 minutes at 200°C and then programmed to 250°C at 3°/min. Arabinitol and Mannitol were used as internal standards.

Results and Discussion.

Fig.1. shows the distribution of IgA on the original CsCl gradient, and Fig.2 the relative distribution of total hexose in the separated 1 ml fractions. Fraction

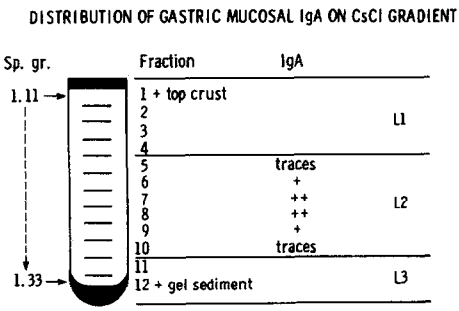

Figure 1

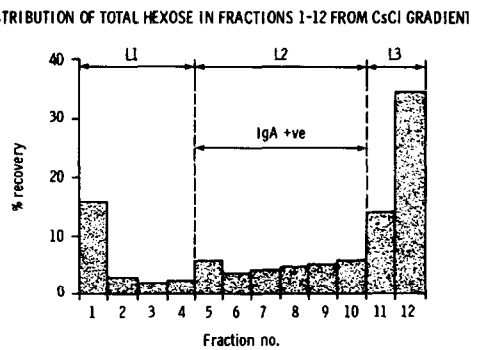

Figure 2

L3 includes the gel sediment at the bottom of the tube. The recoveries of total hexose, protein and solid material in the original combined fractions L1, L2 and L3 are shown in Table 1. The results indicate that the IgA positive fraction L2, is well separated from the bulk of the carbohydrate-rich mucoproteins found in L3. Repeated fractionation of the material in L2 further removed carbohydrate containing material from the fraction, and the resultant, purified IgA-positive fraction L2 accounted for between 4% and 7% of the total hexose of the original gastric mucosal scrapings. It is concluded that gastric mucosal IgA, which may itself be associated with a small amount of intramolecular carbohydrate, is not covalently bound to the characteristic viscous mucoproteins of gastric mucus, and that it can be separated from these macromolecules by fractionation in CsCl gradient. This does not imply that structural association between these two types of compounds must be ruled out. Recently Snary and his co-workers (6,7) indicated irreversible conformational transitions of gastric mucoproteins on exposure to CsCl. The effects of such conformational changes on the forces involved in inter molecular associations of mucus components cannot be elucidated at the present time.

TABLE 1. Distribution of Total Hexose, protein and lyophilised solid between fractions L1, L2 and L3.

	Total Hexose	Total Protein	Lyophilised Solid
L1	22.5%	36.2%	25%
L2, IgA +ve fraction	28.9%	52.8%	43%
L3	48.5%	11.0%	32%

Results are expressed as % recovery

TABLE 2. Carbohydrate composition and content of fractions L1, purified L2 and L3.

	L1		L2		L3	
	MR	%	MR	%	MR	%
Galactose	1	24.6	1	15.6	1	24.3
Fucose	1.01	22.6	1.23	17.5	1.11	24.6
Mannose	0.18	4.4	1.10	17.2	0.12	3.0
Glucose	0.18	4.4	0.36	5.6	0.09	2.2
N-Acetyl Glucosamine	0.80	24.1	1.63	31.2	0.67	20.0
N-Acetyl Galactosamine	0.66	19.9	0.67	12.9	0.87	25.9
Total carbohydrate content % by weight	7.5		6.4		37.5	

Carbohydrate composition is expressed as (a) molecular ratios MR of the different sugars, relative to galactose, and (b) as % by weight of total carbohydrate.
The carbohydrate content is expressed as % by weight of the total solid in the fraction.

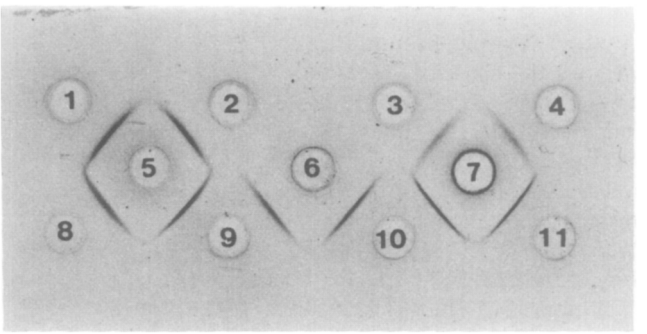

Fig. 3. Immunodiffusion of human colostral IgA (well 5), gastric IgA fraction L2 (well 6) and saliva (well 7) against specific anti Secretory component serum (wells 1-4) and specific anti IgA (α-chain) serum (wells 8-11)

The carbohydrate composition and content of the three fractions L1, purified L2 and L3, as determined by GLC are shown in Table 2. Total sugar content of the IgA positive fraction L2 is low between 5.5% and 7% of the weight of the material, and its sugar composition differs from that of L1 and L3, by containing a significantly higher proportion of Mannose and N-Acetyl Glucosamine. Immunodiffusion of the material in fraction L2 against specific anti secretory component sera (Behring anti free SC and Nordic anti SP) shows the gastric mucosal IgA found in this fraction to be devoid of the characteristic secretory component activity associated with sIgA found in other secretions, e.g. colostrum and saliva (Fig. 3). This finding is also true for intact gastric mucosal scrapings suspended in non-dissociating buffers and is not a result of the exposure of the material to CsCl.

Acknowledgement

We wish to thank Mr Simon Haskoll for technical assistance.
We are indebted to the Hayward Trust for the grant which made this work possible.

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