THE USE OF ION EXCHANGE RESINS IN THE ISOLATION OF BLOOD GROUP A-SPECIFIC SUBSTANCE FROM HOG GASTRIC MUCIN*

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Ion exchange resins (1) have been used in the investigation of A substance hydrolysates (2), but apparently no attempt has been made to determine whether they can be profitably used in the isolation or purification of undegraded A substance[1] from sources such as hog gastric mucin. Studies along these lines are reported in this communication.

EXPERIMENTAL

Ion Exchange Resins—De-Acidite (The Permutit Company, New York), washed on a 40 mesh screen with distilled water, was treated several times with aqueous 4 per cent sodium carbonate and repeatedly washed with distilled water, by suspension and decantation, until the supernatant liquid was colorless and had a pH of 8.0 or less. The resin collected on a suction filter contained 70 to 80 per cent water. 1 gm. (dry weight) of the resin contributed less than 0.05 milliequivalent of base to 75 ml. of distilled water when the suspension was allowed to stand, with frequent shaking, for 2 days at 25°. Under the same conditions 1 gm. (dry weight) of the resin removed 95 per cent of the hydrochloric acid from 75 ml. of a 0.066 M solution and 85 per cent from 75 ml. of a 0.093 M solution.

Amberlite IR-4 (The Resinous Products and Chemical Company, Philadelphia) prepared as described above, contained approximately 35 per cent water, and 1 gm. (dry weight) of the resin removed more than 95 per cent of the hydrochloric acid present in 25 ml. of a 0.166 M solution.

Amberlite IR-100 (The Resinous Products and Chemical Company), treated with 1 per cent hydrochloric acid and washed free of chloride, gave a product containing approximately 35 per cent water.

A Substance Preparations—A 2 per cent suspension of hog gastric mucin granules (Wilson), adjusted to pH 4.4 to 4.5 with glacial acetic acid, was

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[1] Defined as a substance effective in inhibiting isoagglutination of human blood group A cells by group B serum and also effective in inhibiting lysis of sheep erythrocytes by human blood group A cell immune rabbit sera.

centrifuged twice in the open bowl of a Sharples centrifuge at 20,000 R.P.M. The centrifugate was used either directly or after ethanol fractionation (3, 4).

Procedure

Sufficient exchange resin was added, unless otherwise indicated, to a 1 to 2 per cent aqueous solution of the A substance preparation to provide 4 to 5 gm. (wet weight) of freshly washed resin for each gm. of dissolved solid. The suspension was stirred for 2 to 3 hours at 5°, filtered, and the operation

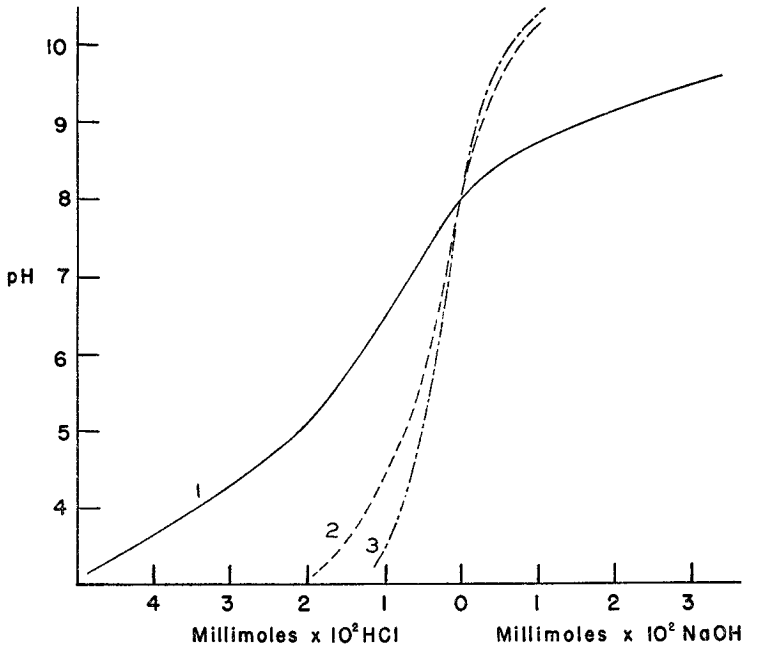

FIG. 1. Titration curves of A substance preparations. Curve 1, Fraction 136; Curve 2, Fraction 128; Curve 3, Fraction 141.

repeated. The resulting solution was either lyophilized or subjected to further treatment with a different exchange resin. Equivalent N-acetylglucosamine contents and inhibition of hemolysis titers were determined as described previously (4, 5). Titration curves were determined by adding sufficient hydrochloric acid to 50 mg. of solid in 5 ml. of water to decrease the pH to 3.0 or less and then titrating with 0.040 M sodium hydroxide. Since an inflection point was observed at pH 8, the data are plotted in Fig. 1 with reference to the number of millimoles of acid or base required to change the pH of the above solution from pH 8 to some other pH.

DISCUSSION

Within limits the equivalent N-acetylglucosamine content (5) of an A substance preparation can be taken as an index of the A substance activity of the preparation, evaluated in terms of inhibition of isoagglutination or inhibition of hemolysis (4, 5). Preliminary experiments based upon the determination of the equivalent N-acetylglucosamine content of an A substance solution before and after treatment with a solid adsorbent previously equilibrated with water indicated that of a number of adsorbents tested the anion exchange resins De-Acidite and IR-4 and the cation exchange resin IR-100 were sufficiently promising to warrant further investigation.[2]

The results obtained by treating an aqueous solution of hog gastric mucin with the above ion exchange resins, either singly or in combination, are given in Table I. It is noteworthy that successive application of De-Acidite and IR-100 gave a preparation which was as active in the hemolysis test[3] as those obtained from mucin by ethanol fractionation (3, 4). The separation of a resin-treated mucin solution into a clear supernatant and a less active viscous precipitate is reminiscent of the behavior observed when A substance preparations obtained from mucin by ethanol fractionation are electrodialyzed (4).[4] Although resin treatment will give products[3] as active as those obtained by ethanol fractionation, there is no evidence that further treatment of the product with any of the above resins will lead to more active preparations such as those obtained by a combination of ethanol fractionation and electrodialysis.[4]

A substance preparations of varying purity, obtained from hog gastric mucin by ethanol fractionation or electrodialysis, were subjected to resin treatment and, in contrast to the experience with mucin, only in one instance was an increase in activity observed (Table II). In the isolation of A substance from hog gastric mucin, it has been noted that an increase in A substance activity is usually accompanied by an increase in the equivalent N-acetylglucosamine content of the preparation (4).[4] However, with A substance preparations containing more than 10 per cent equivalent N-acetylglucosamine, the equivalent N-acetylglucosamine content may not necessarily increase with A substance activity upon further purification and in some instances may actually decrease.[4] This behavior is not unexpected, since it has been pointed out that blood group-specific substances other than the A substance contain alkali-labile bonds involving N-acetylglucosamine

[2] It is possible that with a different test or under different conditions other adsorbents may have been selected.

[3] Comparable titers were also obtained with the inhibition of the isoagglutination test which would indicate that resin treatment does not cause the degradation of A substance.

[4] Holzman, G., and Niemann, C., unpublished experiments.

TABLE I

Inhibition of Hemolysis Titer and Equivalent N-Acetylglucosamine Content of Hog Gastric Mucin Suspension after Successive Treatments with Several Exchange Resins

Description of fraction*	Fraction No. of product	Yield	Inhibition of hemolysis titer†	Equivalent N-acetyl-glucos-amine content‡
Aqueous suspension of hog gastric mucin granules centrifuged twice at pH 4.4; centrifugate	135	72% from mucin	0.19 ± 0.02	7.6
Fraction 135 treated twice with De-Acidite	136	83% from No. 135	0.16 ± 0.01	8.9
Fraction 136 treated twice with IR-100; clear supernatant and	137 (ppt.)	50% from No. 136	0.11 ± 0.01	10.8
viscous ppt. obtained upon standing 24 hrs.; separated	138 (supernatant)	23% from No. 136	0.105 ± 0.005	11.6
Fraction 137 treated twice with IR-4	139	100% from No. 137		11.2
Fraction 138 treated twice with IR-4	140	100% from No. 138		11.7
Fraction 136 treated twice with IR-100 and twice with IR-4	141	67% from No. 136	0.12 ± 0.01	11.2
Fraction 135 treated alternately 4 times with De-Acidite and	152 (supernatant)	32% from mucin	0.14 ± 0.02	10.5
IR-100; allowed to stand 4 days; turbid supernatant and viscous ppt. obtained; separated	154 (ppt.)	20% from mucin	0.19 ± 0.02	9.2
Fraction 152 treated with De-Acidite and IR-100	153	88% from No. 152	0.12 ± 0.01	11.2

* 4 to 5 gm. (wet weight) of the exchange resin per gm. of material treated were used for each treatment, except in the preparation of Fractions 167 and 168, in which 8 gm. of De-Acidite per gm. were used for each treatment, and in the preparation of Fractions 152, 154, and 153, in which 0.5 gm. portions of De-Acidite and IR-100 per gm. were used for each treatment.

† Micrograms of test substance present in system in which sheep erythrocytes are 50 per cent hemolyzed by an anti-human A cell immune rabbit serum. The titers reported are the average of two or three determinations and are reported together with the average deviation of the several determinations.

‡ Expressed as equivalent per cent of N-acetylglucosamine in test substance (5). The analytical data reported are the mean results of duplicate or triplicate analyses. The absolute deviation was in no case larger than 0.3 per cent (equivalent N-acetylglucosamine).

(5, 6), and it is known that A- and O-specific substances can be separated, at least in part, by relatively simple fractionation procedures (7).[4]

TABLE II

Inhibition of Hemolysis Titer and Equivalent N-Acetylglucosamine Content of Some Blood Group A-Specific Substance Preparations after Treatment with Exchange Resins

Description of fraction*	Fraction No. of product	Yield	Inhibition of hemolysis titer†	Equivalent N-acetylglucosamine content‡
Aqueous suspension of hog gastric mucin granules centrifuged twice at pH 4.4; centrifugate (Fraction 135) fractionated with ethanol; material soluble in 40% (by volume) ethanol, insoluble in 65% (by volume) ethanol	143	22% from mucin	0.11 ± 0.01	10.6
Fraction 143 treated twice with De-Acidite	144	77% from No. 143	0.105 ± 0.015	12.0
Fraction 144 treated twice with IR-100	145	100% from No. 144	0.105 ± 0.010	12.3
Fraction 145 treated twice with IR-4	147	100% from No. 145	0.11 ± 0.01	12.1
Aqueous suspension of hog gastric mucin granules; centrifugate fractionated with ethanol; material soluble in 30% (by volume) ethanol, insoluble in 65% (by volume) ethanol; upon reprecipitation, insoluble in 45% (by volume) ethanol; dialyzed, then electrodialyzed	110		0.20 ± 0.02	11.8
Fraction 110 treated twice with De-Acidite	167	74% from No. 110	0.21 ± 0.02	13.4
Fraction 110 treated twice with IR-4	165	91% from No. 110	0.20 ± 0.02	11.8
Same as Fraction 110, except upon reprecipitation, material soluble in 45% (by volume) ethanol, insoluble in 65% (by volume) ethanol; dialyzed, then electrodialyzed	126		0.088 ± 0.005	10.4
Fraction 126 treated twice with De-Acidite	168	60% from No. 126	0.070 ± 0.005	11.9
Fraction 126 treated twice with IR-4	166	88% from No. 126	0.086 ± 0.005	10.5

See the corresponding foot-notes to Table I.

The effectiveness of De-Acidite and IR-100 in reducing the " buffering capacity" of mucin solutions and partially purified A substance preparations is clearly illustrated in Fig. 1. The anion exchange resin IR-4 was found to be markedly inferior to De-Acidite in this respect. Analysis of Fractions

135, 136, and 137 for total nitrogen, amino nitrogen, and amino acid nitrogen (Table III) revealed that De-Acidite was instrumental in removing acidic nitrogenous non-blood group-specific substances containing little or no amino nitrogen, whereas the substances removed by IR-100 contained substantial amounts of amino and amino acid nitrogen. At least part of the materials removed by De-Acidite are non-dialyzable.[5] It is interesting to note that in a centrifuged mucin solution approximately 20 per cent of the solids are not precipitated by 66 per cent ethanol, whereas approximately 30 per cent are removable by successive treatment with De-Acidite and IR-100.

It has been observed that many A substance preparations are contaminated by non-blood group-specific substances[6] exhibiting marked specific absorption in the 260 to 270 mμ region (8). An A substance preparation (Fraction 110) containing a substantial amount of the "260 mμ component" ($E^{1\%}_{1\text{ cm.}}$ at 260 mμ = 13.2) was treated with De-Acidite and the resulting preparation (Fraction 167) was found to have a value of $E^{1\%}_{1\text{ cm.}}$ at 260 mμ of 3.6. Treatment with IR-100 or IR-4 caused little or no decrease in extinction. The fact that De-Acidite was effective and IR-4 was relatively ineffective in removing the "260 mμ component" would indicate that the removal of this component by De-Acidite is not a simple anion exchange. An explanation of the mode of action of De-Acidite leading to the loss of the "260 mμ component," a gain in the equivalent N-acetylglucosamine content, and no significant change in A activity must await the accumulation of additional data.

TABLE III

Nitrogen Content of A Substance Preparations

Fraction No.	Total N	Amino N	Amino acid N
	per cent	*per cent*	*per cent*
135	8.4	1.9	1.0
136	7.3	2.0	1.2
137	6.4	0.16	0.09

SUMMARY

The treatment of hog gastric mucin with the two ion exchange resins De-Acidite and IR-100 has given apparently undegraded A substance preparations which are as active as those obtained from the same source by

[5] Bennett, E. L., unpublished data.

[6] While the "260 mμ component" has not been obtained free of A substance, all evidence (8) points to its non-blood group specific character.

ethanol fractionation. This enrichment in A substance is due to the partial removal by these two resins of some of the non-blood group-specific components normally present in hog gastric mucin.

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