Coomasie blue stained gel from an unpurified human interferon preparation, MEM containing 2% fetal bovine serum from cell cultures, and fresh media containing 2% fetal bovine serum revealed that the major impurities in this human fibroblast interferon preparation have mol. wts. of 60,000 to 100,000 daltons (Fig. 2). A major portion of these impurities have an electrophoretic mobility identical to that for bovine serum albumin.

These data raise the possibility that the multiple mol. wts. previously reported for human fibroblast interferon were due to aggregation of the interferon with other proteins contaminating the preparation, although oligomer formation cannot be completely excluded. Furthermore, the major contaminant occurring in these preparations appears to be bovine albumin added with the fetal bovine serum used in preparing tissue culture interferon. Bovine serum albumin is known to bind a number of hydrophobic molecules (19). Interferon was recently partially purified by chromatography or a bovine serum albumin affinity column (20). Retention of human fibroblast interferon on affinity chromatography columns of CH-Sepharose (ω-carboxypentyl-agarose) suggests that human fibroblast interferon may contain a hydrophobic region (21). These results dramatized the need for purification before ascribing additional physico-chemical and biological properties to interferon.

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was lyophilized or directly applied onto a Sephadex G 150 superfine column (90 x 1.5 cm) which had been equilibrated with 0.1 M ammonium acetate, pH 6.7. Amino acid compositions were determined by hydrolysis of the samples in 6N HCl with norleucine as an internal standard for 20 hours at 110°C, in a chamber sealed under nitrogen, and applied to a Durrum D500 analyzer. Cysteic acid was determined after performic acid oxidation (10). Sialic acid was determined by the method of Warren (11). Carbohydrates were analyzed as the trimethyl silyl derivatives of methylglycosides by gas liquid chromatography (Hewlett-Packard 5710 B gas chromatograph with integrator 3373B) as described elsewhere (12). Glycopeptide samples were analyzed by acrylamide gel electrophoresis in the presence of SDS using standard techniques (4).

RESULTS. Three peaks are obtained in the included volume when the soluble glycopeptide mixture extracted from human red blood cell membranes is subjected to gel filtration in the presence of Ammonyx-LO (Fig. 1). All three

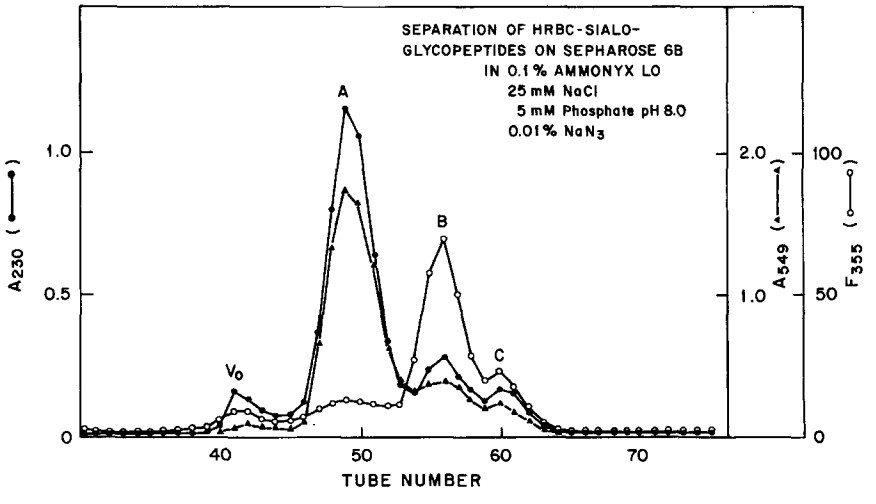

Fig. 1. Gel filtration of the soluble sialoglycopeptides of human erythrocyte membranes. The effluent was monitored for protein (●—●), sialic acid (▲—▲) and fluorescence (○—○). Vo is the void volume.

peaks have similar sialic acid/protein ratios, however, only fractions B and C contain detectable amounts of tryptophan. Peaks B and C represent 15% and 10% of the dry weight of the recovered material. Peak A, which represents approximately 75% of the recovered material, contains one major electrophoretic species when analyzed by SDS gel electrophoresis (Fig. 2A). This band corresponds to the PAS-1 component described previously. A second minor band appears in the PAS-2 position, and the intensity of this can be increased if the sample is pre-heated in SDS before electrophoresis.

The material from peaks B and C produce multiple bands on SDS gels (Fig. 1 B,C). Peak B is composed largely of a single band which corresponds to PAS-2, but if the same material is treated with chloroform-methanol before electrophoresis, significant amounts of higher molecular weight bands appear

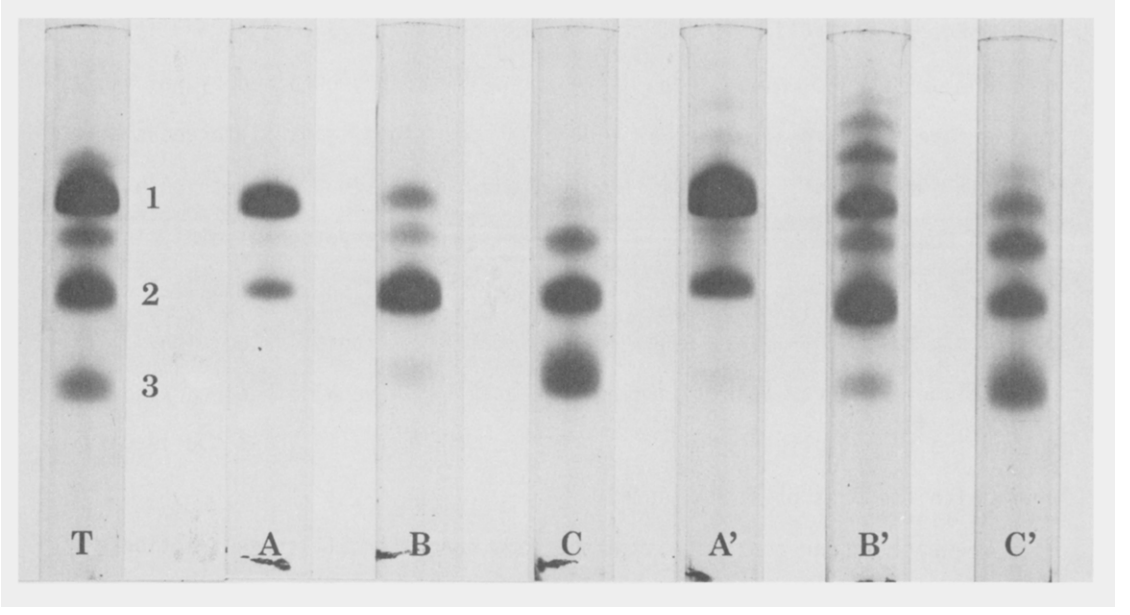

Fig. 2. SDS gel electrophoresis of the fractions prepared by gel filtration in Ammonyx. Gels A, B, and C were taken from pooled samples of peaks A, B, and C (Fig. 1) and electrophoresed directly after dialysis and freeze drying. Gels A', B', and C' were samples of the same fractions subjected to extraction with chloroform-methanol (2:1) before electrophoresis. Gel T is the pattern obtained of the entire sialoglycopeptide mixture before fractionation. All gels were stained with PAS. Companion gels stained with Coomassie blue gave identical patterns.

(Fig. 1 B') which cannot be converted back to the lower molecular weight forms by pre-heating the samples.

Peak C is composed predominantly of bands which correspond to PAS-2 and PAS-3 (Fig. 1-C), but higher molecular weight forms also can be generated by pretreatment with chloroform-methanol (Fig. 1-C'). Neither B nor C contain significant amounts of PAS-1 unless they are pretreated with the organic solvents. Peak A contains only trace amounts of PAS-3 regardless of whether the material is exposed to chloroform-methanol or not.

A number of minor PAS-positive bands are also evident on these gels, some of which have been noted by others, e.g. the band between PAS-1 and PAS-2 has been called PAS-1a (13) or 4 (14,15). Since it appears that some of these bands are interconvertible forms while others may represent aggregates created during processing of the samples for analysis, it seems simplest to describe our results in terms of the original nomenclature suggested by Fairbanks, et al. (4).

The amino acid compositions of the three fractions obtained by gel filtration are superficially similar (Table 1), but significant differences were found in the content of proline, glycine, alanine, valine, and methionine between peaks A and C in addition to the presence of tryptophan solely in fractions B and C. Some preparations of peaks B and C showed identical compositions, but in others it appeared that peak B was contaminated with material derived from peak A. Amino acid analyses have also been carried out on individual glycopeptides isolated from SDS gels (16), and the results agree with the data presented here.

The total sugar content was in the same range for all three fractions, (approximately 50-60%) and the relative amounts of individual sugars were similar. Differences seen in Table 1 can be attributed to experimental error, but detailed analyses of individual oligosaccharide units are needed before any conclusions can be drawn.

Gel Filtration profiles were obtained of tryptic digests of the glyco-

TABLE 1

Amino Acid and Carbohydrate Composition of Human Erythrocyte Membrane Sialoglycopeptides. [1]

	mol%				
	Peak A	Peak B	Peak C	Major Glycopeptide found[2]	expected[3]
aspartic acid	6.9	7.2	7.5	9	8
threonine	10.9	11.1	10.6	14.3	15
serine	11.8	10.6	9.4	15.4	18
glutamic acid	11.7	9.8	11.4	15.3	15
proline	7.4	6.2	5.2	9.7	10
glycine	4.7	7.1	7.3	6.1	6
alanine	5.6	9	9	7.3	6
1/2 cystine [4])	-	-	-	-	-
valine	8.5	5.6	5.5	11.1	11
methionine	1.4	3.3	3.1	1.8	2
isoleucine	7.5	6.3	5.9	9.8	10
leucine	6.1	7	7.5	8	8
tyrosine	2.9	3	3.2	3.8	4
phenylalanine	1.9	2.5	2.6	2.5	2
histidine	4	3.1	3.3	5.2	5
lysine	4	3.2	3.4	5.2	5
tryptophan [5])	-	.6	.5	-	-
arginine	4.7	4.6	4.7	6.1	6
		g/100g			
fucose	1	.5	.6		
mannose	2.9	2.4	1.6		
galactose	10.7	11.8	11.3		
glucose	.6	1	1.3		
galNAc	10.8	12.6	10.2		
glcNAc	6.7	7.0	8.2		
sialic acid	20.7	23.2	19.4		

1) Average values from 3 different samples, uncorrected for losses during hydrolysis of Thr,Ser,Tyr,Met. A dash denotes values below 0.1.
2) Peak A material calculated as residues/peptide.
3) Data obtained from amino acid sequence (cf. 7).
4) Determined as cysteic acid after performic acid oxidation.
5) Determined by fluorometry; no values given from amino acid analysis. Tryptophan was also determined on the amino acid analyzer after hydrolysis in methane sulfonic acid, but the high sugar content makes it difficult to obtain reliable absolute values (9).

peptides in peaks A, B, and B plus C (Fig. 3). The elution pattern from peak A is strikingly different from that obtained from peak B or digests of peaks B plus C together.

DISCUSSION. These results are consistent with the idea that the major sialoglycopeptides of the human red blood cell membrane represent at least two distinct molecular species. The predominant form is a glycopeptide composed of 131 amino acids and 16 oligosaccharide chains. Recently the complete amino acid sequence of this molecule has been determined (7). This glycopeptide is present in an aggregated form in detergents and represents the major PAS-staining band (PAS-1) seen when red cell membranes are analyzes by SDS gel electrophoresis. Since this molecule represents the bulk (∿ 75%) of the sialoglycopeptides previously designated glycophorin (3) we now suggest that this component be designated glycophorin A.

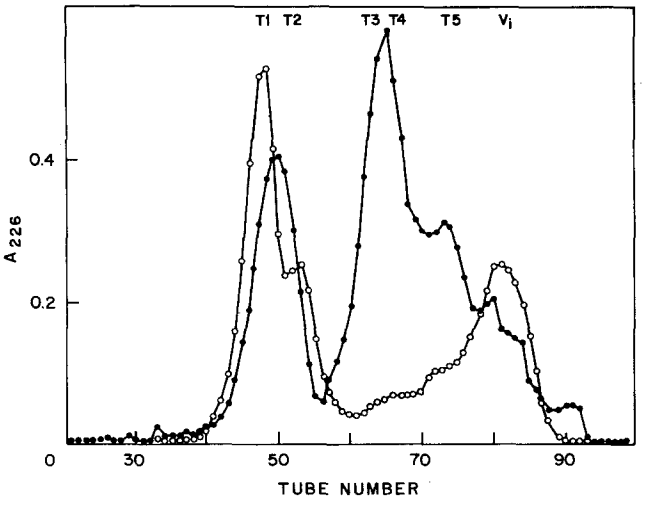

Fig. 3. Gel filtration of the soluble peptides produced by tryptic digestion of samples from peak A, Fig. 1 (●—●) and peak B, Fig. 1 (○—○). The peptides labeled T1 - T5 represent the major tryptic peptides which were isolated and characterized in the course of determining the amino acid sequence of the predominant glycopeptide species (7).

The material separated from glycophorin A by gel filtration in Ammonyx seems to share certain common features, e.g. similar amino acid and sugar composition, capacity to form high molecular weight aggregates, and it co-purifies with the major species, yet it has certain distinct properties. The content of glycine, alanine, and methionine are significantly greater than in the A peptide. The higher content of methionine in this second species probably accounts for the anomalous results obtained earlier during a study of the cyanogen bromide fragments of the total glycoprotein mixture (2). Cyanogen bromide cleavage of purified glycophorin A now gives results which are consistent with this interpretation. Tryptophan has been detected only in the minor sialoglycopeptide, but no cystine has been detected in either chain. These results differ from the findings of others (17).

Comparative tryptic digests were done on both glycopeptides to rule out the possibility that the differences in amino acid composition between the two were due to the presence of small amounts of contaminating peptides. The peptides obtained from peak A eluted in the same positions as those prepared for the sequence studies of the major component. In that study several minor peptides were also obtained which were not part of the major chain, and they probably represent peptides of the B-C fractions (7).

A great source of confusion during this study was the finding that ostensibly different glycopeptides generated bands of identical mobilities on SDS gels. We were initially misled by the fact that the glycopeptide with the fastest mobility (PAS-3) could be eluted from gels and, upon re-electrophoresis, was capable of forming all the high molecular weight bands seen in Fig. 2 C'. According to our present understanding, the bulk of the glycopeptides which elute in peak A (or run at the PAS-1 position) represent a dimer of glycophorin A which can dissociate upon heating (5,6,13) or after selective alkylation (18) into a monomeric form which migrates in the PAS-2 position. However, other sialoglycopeptides clearly not identical to glycophorin A can also migrate to both positions on SDS gels, particularly if they are pretreated with organic solvents.

These results settle some of the earlier ambiguities concerning the composition and subunit size of the glycophorin molecule, and they also indicate that another major sialoglycopeptide is present in the human RBC membrane. It remains to be determined whether this second component is a single polypeptide chain, and what relation, if any, it bears to glycophorin A. These results also suggest that the analyses of sialoglycopeptides by SDS gel electrophoresis must be approached with caution since it is evident that such glycopeptides cannot be identified solely on the basis of their electrophoretic mobilities.

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