THE SECRETION OF A SIALIC ACID-FREE IMMUNOGLOBULIN

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1. Introduction

A number of studies have shown that proteins destined to be secreted are synthesized largely or exclusively on membrane-bound ribosomes [1–4]. A small proportion of the carbohydrate moiety of secreted glycoproteins may be incorporated before the polypeptide chains are discharged from the polysomes [5, 6]. Thereafter, there is a slow translocation of completed chains through the rough and smooth membranes of the endoplasmic reticulum [7–9] while monosaccharide residues are added sequentially to the glycoprotein molecule.

The addition of sialic acid, which constitutes the terminal monosaccharide in many glycoproteins, has been shown to take place largely in the smooth endoplasmic reticulum [9, 10], and it has been suggested that this sugar is added late in the synthetic process. Thus, in rabbit lymph node tissue, there is a lag of 20 min between the appearance of intracellular and secreted [^{3}H]glucosamine-labelled immunoglobulin, but there is no such delay between the appearance of intracellular and extracellular [^{3}H]sialic acid-labelled antibody [11]. This observation is consistent with the difference in electrophoretic mobility between intracellular and secreted MOPC 21 myeloma protein reported by Notani et al. [18]. It seemed possible, therefore, that the completion of the carbohydrate moiety might constitute the signal for secretion [12].

This paper reports the results of experiments designed to investigate the sialic acid content of the immunoglobulin synthesized by two cloned lines of the IgG-secreting mouse myeloma MOPC 21.

2. Materials and methods

The P3K (MOPC 21) mouse myeloma line was a gift from Dr. K. Horibata [13]. Cells were grown under conditions previously described [14, 15]. D-1-[^{14}C]glucosamine HCl (specific radioactivity 52 mCi/mmole), D-6-[^{3}H]glucosamine HCl (specific radioactivity 10 Ci/mmole) and [^{14}C]lysine (specific radioactivity 336 mCi/mmole) were purchased from the Radiochemical Centre, Amersham, Bucks, U.K. Ampholines were bought from LKB Produkter, AB, Bromma, Sweden. Neuraminidase (type VI, from *Clostridium perfringens*, EC 3.3.1.18) was obtained from the Sigma Chemical Co., London, S.W.6, U.K. All other reagents were of analytical grade.

Isolation of clones from the parent P3K culture, incorporation of labelled compounds and isoelectric focusing of labelled intracellular and secreted IgG were accomplished as described by Cotton et al. [15]. In some experiments, incubation media containing labelled, secreted IgG or cell lysates obtained by treatment with Na deoxycholate [15] were incubated for a further 4 hr with 10 μg/ml of neuraminidase. Radioactive bands located by radioautography were cut from the isoelectric focusing gels and extracted in the presence of 1 mg of unlabelled MOPC 21 protein by homogenisation in 2.0 ml of 0.6 M lithium acetate, 10 mM Tris-HCl, pH 7.2 containing 0.4% SDS. The resulting suspensions were shaken overnight, centrifuged to remove the polyacrylamide and the supernatant clarified by passage through a Millipore filter. Protein was recovered by the addition of one-tenth volume of 100% trichloroacetic acid. Sialic acid was removed from the trichloro-

acetic acid-insoluble material by hydrolysis in 5% trichloroacetic acid for 1 hr at 80° [11]. The aqueous phases were extracted three times with ether to remove the trichloroacetic acid, and applied to 2.0 × 0.5 cm columns of Dowex-1 (Cl^-) ion exchange resin. The columns were eluted with 0.2 N HCl. A portion of the eluate was retained for radioactivity measurement by scintillation counting; the remainder was characterised by chromatography on paper in butanol: propanol: 0.1 N HCl (1:2:1, v/v) [16]. The precipitates remaining after treatment with trichloroacetic acid at 80° were further hydrolyzed *in vacuo* in 200 µl of 6.0 N HCl for 5 hr at 105°. HCl was removed under reduced pressure; the residues were redissolved in the aqueous eluates from the corresponding Dowex-1 (Cl^-) columns, and the solutions applied to 2.0 × 0.5 cm columns of Dowex-5 (H^+) ion exchange resin. These columns were eluted with 2.0 N HCl, and the aqueous and acid eluates retained for radioactivity measurement by scintillation counting.

3. Results and discussion

Isoelectric focusing of secreted [^{14}C]lysine-labelled P3K myeloma protein typically yields the band pattern shown in fig. 1C. By contrast, isoelectric focusing of labelled intracellular material obtained after 15 min of incubation in the presence of the same labelled amino acid yields a single major band (band o, fig. 1A). This band corresponds in position to extracellular band o, and yields authentic heavy and light chains on reduction with β-mercaptoethanol and analysis on SDS polyacrylamide gels (not shown). Similar isoelectric focusing patterns have been previously reported by Awdeh et al. [17] and by Cotton et al. [15]. Differences between the electrophoretic mobility of intracellular and secreted MOPC 21 IgG have also been noted by Notani et al. [18].

Treatment of the labelled material synthesized by wild type P3K cells or by the mutant clone IF 1 [15] with neuraminidase gave the results shown in fig. 1B, D, F. No change is observed in the position of the intracellular or extracellular wild type bands; however, the bands obtained by isoelectric focusing of IgG secreted by the IF 1 clone are displaced towards the cathode, presumably as a result of the loss of one or more sialic acid residues. Chemical analysis of

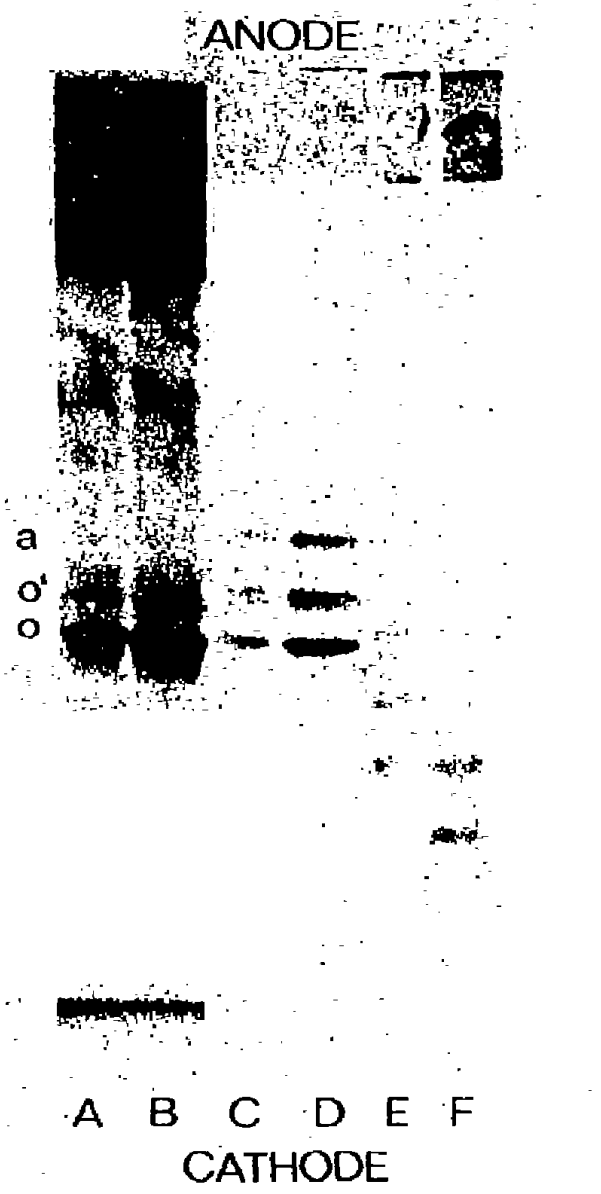

Fig. 1. Effect of neuraminidase on intracellular and secreted immunoglobulin. Intracellular and secreted [^{14}C]lysine-labelled immunoglobulin was prepared from incubated wild type (P3K) or mutant (IF 1) cells and isolated by isoelectric focusing as previously described [15]. In some experiments, the intracellular or secreted products were incubated with neuraminidase prior to isoelectric focusing as decribed in the text. Bands were located by radioautography. A) Intracellular (P3K); B) intracellular (P3K) incubated with neuraminidase; C) secreted (P3K); D) secreted (P3K) incubated with neuraminidase; E) secreted (IF 1); F) secreted (IF 1) incubated with neuraminidase.

[^{3}H]glucosamine- and [^{14}C]glucosamine-labelled material eluted from similar isoelectric focusing gels con-

Table 1
The sialic acid content of IgG secreted by P3K (MOPC 21) cells.

Clone designation (cf. [15])	Band designation (fig. 1 and [17])	Radioactivity retained by Dowex-1 (Cl^-) columns (cpm)	Radioactivity retained by Dowex-50 (H^+) columns (cpm)	Radioactivity unretained by Dowex-50 (H^+) columns (cpm)	Radioactivity retained by Dowex-1 (Cl^-) columns identified as sialic acid (%)
P3K	o	120	3,400	3,840	–
	o'	80	2,500	2,870	–
	a	60	2,400	2,240	–
IF 1	o	3,900	3,200	5,000	68
	o'	1,920	1,400	2,720	65
	a	2,060	1,300	2,200	66

Approx. 3×10^5 wild type (P3K) or mutant (IF 1) myeloma cells [15] were incubated in 1.0 ml of Dulbecco-modified Eagles medium [20] containing 50 μCi D-[1-^{14}C]glucosamine and 500 μCi D-[6-^{3}H]glucosamine. The cells were removed by centrifugation and the labelled, secreted immunoglobulin isolated by isoelectric focusing [15]. Radioactive bands located by radioautography were eluted and analyzed for their sialic acid content as described in the text.

firmed that the IF 1 bands, but not the wild type bands, contained sialic acid (table 1). The absence of sialic acid from the wild type myeloma protein was further confirmed by our inability to obtain the colour reaction for sialic acid [19] from 20 mg of unlabelled MOPC 21 protein purified from the sera of tumour-bearing mice (see also [5]).

Since sialic acid is absent from both intracellular and freshly secreted wild type IgG, it is clear that the secretion of MOPC 21 protein is not normally mediated by the addition of this terminal monosaccharide. Sialic acid addition cannot therefore account for the difference in electrophoretic mobility between intracellular and secreted MOPC 21 IgG [18]. Moreover, the absence of sialic acid from the myeloma protein purified from the sera of tumour-bearing animals precludes the possibility that addition of this sugar might occur in the serum.

The nature of the differences between the three bands observed on isoelectric focusing of myeloma proteins remains obscure. Awdeh et al. [17] inferred from the behaviour on DEAE columns of labelled IgG secreted by myeloma 5663 that the charge differences between bands o and a could not be ascribed to the acquisition of a carboxyl group. This conclusion is consistent with our findings, since the product of the wild type P3K cells is devoid of sialic acid and, in the case of IF 1, the relative content of sialic acid is similar in each band (table 1).

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