Glycoconjugate Journal (1995) **12**: 211–218

Glycosylation of alpha-1-proteinase inhibitor and haptoglobin in ovarian cancer: evidence for two different mechanisms

G. A. TURNER*, M. T. GOODARZI and S. THOMPSON
Department of Clinical Biochemistry, The Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK
Received 30 December 1994

The change in glycosylation of the two acute-phase proteins, alpha-1-proteinase inhibitor (API) and haptoglobin (Hp), in progressive ovarian cancer is different. This has been shown by monosaccharide analysis and lectin-binding studies of proteins purified from serum. In the glycan chains of API, there is decreased branching (more biantennary chains), less branches ending in alpha 2-3 sialic acid, more branches ending in alpha 2-6 sialic acid and more fucose, probably linked alpha 1-6 to the core region. On the other hand, Hp shows increased branching (more triantennary chains), more branches ending in alpha 2-3 sialic acid, less branches ending in alpha 2-6 sialic acid, and more fucose, probably in the alpha 1-3 linkage at the end of the chains. This is surprising because API and Hp are thought to be glycosylated by a common pathway in the liver. We have also shown that the fucose-specific lectin, *lotus tetragonolobus*, extracts abnormal forms of both Hp and API in ovarian cancer, but the expression of this Hp is related to tumour burden and the expression of this API is related to lack of response to therapy. It is suggested that this difference in the behaviour of API and Hp in ovarian cancer may be associated with the different changes in their glycosylation. Of the many mechanisms that could explain these findings, a likely one is that a pathological process is removing API with triantennary chains from the circulation. In addition to their normal roles (API-enzyme inhibitor and Hp-transport protein) these proteins are reported to have many other effects in biological systems, such as immunosuppression. As correct glycosylation of API and Hp is required for their normal stability/activity, changes in glycosylation could affect their functions in ovarian cancer and these modifications could alter the course of the disease.

Keywords: Glycosylation, alpha-1-proteinase-inhibitor, haptoglobin, ovarian cancer

Introduction

Changes in the glycosylation of some serum proteins in certain diseases are well established [1, 2] and are already being used for monitoring disease activity, for prognostic purposes and in differential diagnosis. However, their importance in reflecting particular pathological processes, or in altering the functional activity of a molecule in disease is still very unclear. In a number of previous studies, we investigated the glycosylation of two serum glycoproteins, alpha-1-proteinase inhibitor (API) [3–5] and haptoglobin (Hp) [6–9], in ovarian cancer. The results indicated that the change in glycosylation for each glycoprotein was related to a different aspect of the disease, and that the type of change occurring for each molecule was different. From these results it was possible to speculate that the function of API or Hp could be affected by the cancer-related changes, and that this could have a direct impact on the progress of the disease. It was also important to ask why the observed glycosylation changes were different for API and Hp, because carbohydrate is attached to both of these proteins by the same pathway in the liver. This further suggests that these proteins are involved in different mechanisms in the disease process. The aim of this paper is to review the evidence (including recent unpublished material on the lectin-binding properties of purified Hp) describing the differences in the glycosylation of API and Hp in ovarian cancer, address in depth all the questions raised by these findings, and attempt to arrive at possible explanations for this situation. Before addressing these aims, the properties and structure of API and Hp will be described. More detailed descriptions can be found elsewhere [10–14].

API and Hp are two serum proteins that are synthesized by hepatocytes in the liver. The role of API is to inhibit the

*To whom correspondence should be addressed.

enzyme activity of serine proteases such as elastase and cathepsin G. This protects the body from damage, and the deficiency state is associated with emphysema in adults and liver disease in neonates. The major role of Hp, on the other hand, is to combine with any free haemoglobin (Hb) that is released by intravascular red cell destruction. The Hp/Hb complex is then rapidly broken down by the reticulo-endothelial system and this mechanism conserves iron stores and protects the renal tubules from damage by the Hb. Hp has also been reported to be involved in other processes, particularly in relation to the regulation of inflammation and infection. The normal concentrations of API and Hp in the blood are 2–3.5 g l^{-1} and 0.5–2 g l^{-1} respectively.

In many abnormal situations in the body (inflammation, infection, malignancy and trauma), the concentration of API and Hp in the blood increase rapidly and substantially (two–four fold) in the so called acute-phase reaction (APR). This reaction is thought to be a physiological response to limit the injury caused by the pathological process and return the body to homeostasis. In ovarian cancer for example, the concentration of API and Hp in the blood can be as high as 8 g l^{-1} [15]. It is produced by the release of cytokines (IL1, IL6 and TNFα) from leukocytes and other cells involved in the pathology, and these substances stimulate the *in vitro* synthesis of API and Hp in isolated hepatocytes and cultured hepatoma cell lines [14].

Despite the similarity in the overall patho/physiological behaviour of API and Hp, they have very different structures. API is a single polypeptide chain of 394 residues (M_r = 54 kDa), which carries three N-linked 'complex' oligosaccharide units at positions 46, 83, and 247 (12% by weight). In normal API, the biantennary units reportedly outnumber the triantennary by 3:1 [16], although there seems to be controversy over the exact proportions [16–18]. There is also disagreement over the presence of fucose on normal API, some studies have reported very low amounts [18, 19], whereas other studies could not confirm the presence of fucose [16, 17, 20]. More precise details are unknown.

In contrast, Hp is composed of two alpha chains of 9.1 kDa and two beta chains of 40 kDa and these chains are linked by disulphide bridges. The carbohydrate content of Hp is 16–20% and is found exclusively as N-linked 'complex' oligosaccharide chains on the beta chain at positions 23, 46, 50 and 80. Biantennary and triantennary chains have been detected; both terminating in *N*-acetylneuraminic acid (Neu5Ac) [21]. The ratio of alpha 2-6 to alpha 2-3 is about 4:1; the latter being located on the triantennary chains [21]. Fucose is present in a core alpha 1-6 position or in an external alpha 1-3 position linked to *N*-acetylglucosamine (GlcNAc) [21].

Studies of API and Hp extracted from cancer sera with lotus tetragonolobus

Sera from ovarian cancer patients were extracted with the fucose-specific lectin, *lotus tetragonolobus*, and the protein composition of the extract was examined by electrophoresis in polyacrylamide gels under denaturing conditions followed by silver staining. All the techniques have been previously described [3, 4, 6, 7]. Figure 1 shows representative electrophoretic patterns obtained after the separation of the lectin extracts from eight ovarian cancer patients. It can be seen that there is an association between the intensity of a diffusely-staining band at 40–45 kDa and increasing tumour burden. This band was shown to be the β-chain of Hp using Western blotting with an anti-Hp antibody [6].

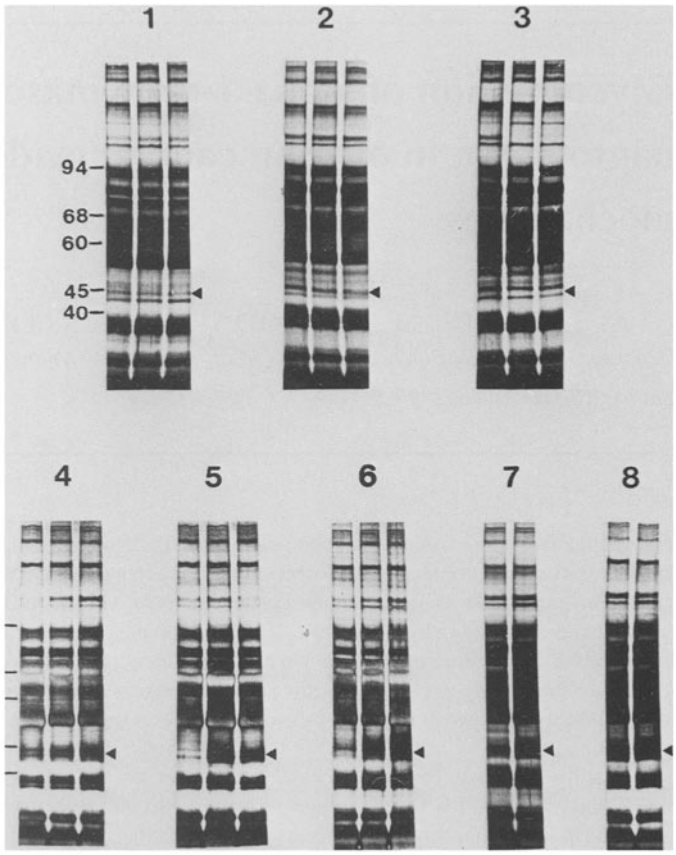

Figure 1. Silver-stained electrophoretic separations of lotus-extracted sera from eight women with ovarian cancer and different tumour burdens. Groups 1-3 are from women who had a low tumour burden; groups 4-6 are from women who had an increasing tumour burden; and groups 7 and 8 are from women who had a high tumour burden. Hp is shown as a diffuse band at 40–45 kDa and its position is indicated by an arrow-head on the right-hand side of the gel. The sharp bands at 40–45 kDa in groups 1-3 were shown by Western blotting not to be Hp. All women were undergoing cytotoxic chemotherapy. The time interval between the first and last samples for each patient was 148, 203, 278, 266, 348, 226, 154 and 34 days respectively. In Figs 1 and 2, the position of the molecular weight markers (kDa) are indicated on the left-hand side of the gel. Taken with permission from reference [7].

A summary of the amount of lotus-extractable Hp in 77 specimens collected from 21 cancer patients is given in Table 1. The data are presented as a semi-quantative estimation of the amount of Hp extracted. There is a highly significant asso-

Table 1. Lotus-extractable Hp in serum from ovarian cancer patients.

Patient group (tumour burden)	Intensity of Hp band (no. of specimens)		
	Not present or weak band	*Moderate band*	*Strong band*
Low	22	5	0
Moderate	12	9	1
High	4	8	16

The association between Hp assessment and tumour burden is highly significant ($p < 0.0001$).
The χ^2 test was carried out by pooling data for the 'moderate band' and 'strong band' groups. Taken with permission from reference [7].

ciation between the amount of Hp extracted and increasing tumour burden.

Another band at approximately 56 kDa was detected in electrophoretic patterns of lectin extracts of sera if lower amounts of extract were loaded and the protein separation was understained. There was a large increase in this band in those specimens that were collected at tumour recurrence (Fig. 2). Surprisingly, this component was expressed only weakly at the start of therapy, despite large amounts of tumour being present. This band was identified by blotting as API [3]. Hp is only weakly detected in Fig. 2 because of the experimental conditions, but it can still be seen both before and after remission.

Using an arbitrary grading system, a blind assessment was made of the API levels in lectin extracts from 29 women with ovarian cancer (Table 2). Two specimens were analysed for each patient (one at the start of therapy and one at a later date). The patients are shown as two groups; those who did not respond to therapy (non-responders) and those who had complete clinical remission (responders). At the start of therapy, there was no difference in the median grading of both groups, but after a period of therapy the extracted API levels were very different. In the non-responders, 75% of the women had significantly higher levels of extracted API in the second specimen than in the first ($0.05 > p > 0.02$; paired Wilcoxon). In the responders, the amount of extracted API was either very low in both specimens or was lower in the second specimen; but the difference in expression was not statistically significant ($p > 0.05$).

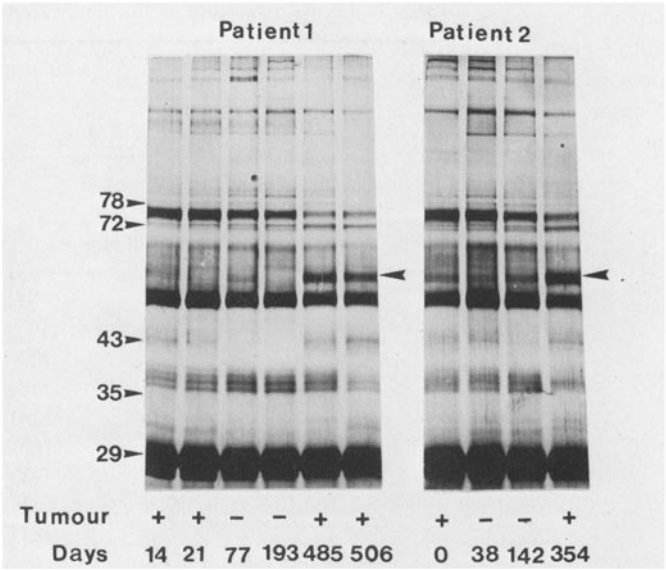

Figure 2. The electrophoretic separation of lotus-extracted sera from two women with ovarian cancer who provided specimens throughout their cytotoxic chemotherapy. The presence of clinically-detectable tumour and the time after the start of therapy are indicated below the separation. API is indicated by an arrow-head on the right-hand side of the gel and its identity was confirmed by Western blotting. Taken with permission from reference [3].

Monosaccharide composition of API and Hp purified from cancer sera

API and Hp were purified from sera of healthy women and women with progressive ovarian cancer by an affinity chromatography method which used either rabbit anti-human API antibody (Sigma) or sheep anti-human Hp antibody (The Binding Site). The monosaccharide composition of the purified material was determined using a Dionex carbohydrate analyser. The procedures used have been described in detail elsewhere [5, 9]. Results from the monosaccharide analysis of purified Hp from 'healthy' and 'cancer' sera are shown in Table 3. These indicate that there is a large increase in the fucose content of the Hp in 'cancer' sera ($p < 0.0001$). There was a smaller, but very significant ($p < 0.0005$), increase in the GlcNAc content of 'cancer' Hp and a small increase in galactose (Gal) which was of borderline significance ($p < 0.05$). There was no difference in the Neu5Ac content of 'healthy' and 'cancer' Hp.

The changes in the monosaccharide content of purified API from the 'healthy' and 'cancer' sera were different from those obtained for Hp (Table 3). The increase in fucose content for API in the 'cancer' sera was less, and the GlcNAc and Gal contents were both significantly decreased (see Table 3 for p values). Like Hp, there was no difference in the Neu5Ac content of the two groups.

Probing the carbohydrate structure of purified API and Hp using lectins

As this method has only just recently been reported [5, 22, 23], it will be described in more detail. Purified API or Hp was dissolved in 25 mmol l^{-1} Tris-HCl, pH 7.5, containing 100 mmol l^{-1} NaCl (TBS) and 100 µl was incubated for 2 h at 37°C in each well of a multiwell plate. For each protein and each lectin used, preliminary experiments were carried out to

Table 2. Lotus-extractable API in serum from responding and non-responding ovarian cancer patients.

	Intensity of API band (grade)							
	Non-responders				*Responders*			
	Specimen				*Specimen*			
	1	*2*	*Change*		*1*	*2*	*Change*	
	4	2	D	(81)	1	0	D	(7)
	2	4	I	(147)	2	2	N/C	(38)
	2	3	I	(53)	0	0	N/C	(119)
	0	3	I	(114)	1	1	N/C	(17)
	4	4	N/C	(24)	2	0	D	(64)
	2	3	I	(168)	2	1	D	(134)
	3	4	I	(41)	1	0	D	(218)
	0	1	I	(31)	0	0	N/C	(63)
	1	2	I	(21)	0	0	N/C	(14)
	1	4	I	(49)	1	1	N/C	(18)
	2	2	N/C	(21)	3	0	D	(21)
	1	2	I	(35)	4	1	D	(14)
	0	0	N/C	(35)	0	0	N/C	(35)
	0	2	I	(62)	2	3	I	(35)
	1	1	N/C	(28)				
Median	1	3		(45)	1	0		(37)

'Responders' specimen 1 vs 2, $p > 0.05$. 'Non-responders' specimen 1 vs 2, $0.05 > p > 0.002$ (Wilcoxon paired analysis). D, I and N/C decrease, increase and no change respectively. Values in parenthesis are the number of days between specimens 1 and 2. Specimen 1 was obtained within 14 days of starting therapy. Taken with permission from reference [3]

Table 3. Monosaccharide content of purified Hp and API from healthy women and women with ovarian cancer.

Group	*Fuc*	*GlcNAc*	*Gal*	*Neu5Ac*
	(mol per 3 mannoses)			
Hp				
Healthy women (7)	0.16 ± 0.08	3.7 ± 0.2	2.3 ± 0.3	2.2 ± 0.4
Ovarian cancer (7)	0.54 ± 0.14	4.3 ± 0.3	2.7 ± 0.3	2.1 ± 0.5
p value	< 0.0001	< 0.0005	< 0.05	> 0.05
API				
Healthy women (8)	0.26 ± 0.05	4.7 ± 0.5	2.6 ± 0.3	2.4 ± 0.3
Ovarian cancer (12)	0.43 ± 0.14	3.8 ± 0.5	2.3 ± 0.2	2.4 ± 0.3
p value	< 0.005	< 0.001	< 0.05	> 0.05

In Tables 3 and 4, all values are mean ± SD, the number of individuals is given in parenthesis, and the Student's t-test was used for statistical analysis.

determine optimum protein and lectin concentrations. The optimum concentrations of API used for Sambucus nigra agglutinin (SNA), Concanavalin (Con A) and Maackia amurensis agglutinin (MAA) were 0.15 μg ml^{-1}, 0.15 μg ml^{-1} and 0.5 μg ml^{-1} respectively. The optimum concentrations of Hp used with the same lectins were 0.05 μg ml^{-1}, 0.05 μg ml^{-1} and 0.125 μg ml^{-1} respectively. Unbound protein was removed by three washes in TBS containing 0.1% (by vol) Tween 20 (TTBS) and each well was incubated in the same buffer for 1 h at 37°C and at 4°C overnight. After washing twice with 1 mmol l^{-1} Tris-HCl, pH 7.5, containing 0.1% (by vol) Tween and mmol l^{-1} $CaCl_2$, $MgCl_2$ and $MnCl_2$, 200 μl of digoxi-

Table 4. Lectin reactivity of purified Hp and API from healthy women and women with ovarian cancer.

Group	*Lectin*		
	Con A	*SNA*	*MAA*
		(Absorbance 492nm)	
Hp			
Healthy women (8)	1.40 ± 0.35	1.70 ± 0.22	0.18 ± 0.07
Ovarian cancer (11)	0.86 ± 0.15	1.69 ± 0.29	0.45 ± 0.10
p value	< 0.0005	> 0.05	< 0.0001
API			
Healthy women (8)	1.29 ± 0.28	1.31 ± 0.19	0.47 ± 0.08
Ovarian cancer(12)	1.87 ± 0.14	1.95 ± 0.09	0.23 ± 0.07
p value	< 0.0001	< 0.0001	< 0.0001

Each value used to calculate the mean was the average from triplicate determinations in three separate experiments.

genin (DIG) labelled lectin was added and incubated at 37°C for 1 h. For API, the optimum lectin concentrations for SNA, Con A and MAA were 1 μg ml^{-1}, 1 μg ml^{-1} and 1.5 μg ml^{-1} respectively. For Hp, these were 0.5 μg ml^{-1}, 1 μg ml^{-1} and 1 μg ml^{-1} respectively. The plate was washed four times with TTBS and each well incubated with 150 μl of anti-DIG antibody labelled with peroxidase (0.05 U ml^{-1}) for 1 h. After four more washes the colour was developed with phenylenediamine and H_2O_2 and read at 492 nm on a plate reader. The background absorbances (without API or Hp but with the blocking step, lectin and antibody conjugate) for Con A, SNA and MAA were (mean ± SD, observations) 0.28 ± 0.02 (9); 0.16 ± 0.03 (9); 0.14 ± 0.02 (9) respectively. All measurements were adjusted for background absorbance. The lectins and the labelled anti-DIG antibody were supplied by Boehringer Mannheim.

Hp purified from ovarian cancer patients with progressive disease showed a large and significant reduction ($p < 0.0005$) in the reactivity with the lectin Con A; no change in the reactivity with SNA and a three-fold increase ($p < 0.0001$) in the reactivity with MAA (Table 4). In contrast, API purified from the 'cancer' sera (Table 4) bound significantly more Con A and SNA ($p < 0.0001$) and two-fold less MAA ($p < 0.0001$).

Discussion

Despite both API and Hp being extracted by lotus lectin in ovarian cancer, other data from our studies clearly indicate that the changes in the glycosylation of these two proteins in this disease are considerably different. Both the GlcNAc measurements and the Con A binding studies suggest that in cancer, the triantennary chains on API are being replaced by biantennary chains, whereas in Hp the opposite situation is occurring. Furthermore, the results for the Neu5Ac binding lectins, MAA and SNA suggest that in cancer there is a shift from chains terminating in alpha 2-3 to alpha 2-6 Neu5Ac for API and vice versa for Hp. As alpha 2-3 Neu5Ac is normally more frequently found on triantennary structures, this conclusion is consistent with the proposed changes in branching.

It also seems very likely that the distribution of fucose on API and Hp is different in cancer. This molecule is found in three positions on carbohydrate side chains [1]; at the end of the chain attached by an alpha (1-2) bond to Gal or an alpha (1-3) bond to GlcNAc; and in the main core attached by an alpha (1-6) bond to GlcNAc. If high amounts of alpha (2-6) Neu5Ac are present, fucose cannot be added in the 1-2 or the 1-3 positions [24]. This does not occur if Neu5Ac is present in the 2-3 position. In summary, therefore, API in ovarian cancer contains many biantennary chains with a lot of Neu5Ac in the alpha (2-6) position and possibly fucose in the alpha (1-6) position; whereas Hp contains many tri or tetraantennary chains with a lot of Neu5Ac in the alpha (2-3) position and possibly fucose in the alpha (1-3) position. If the latter Neu5Ac and fucose are present on the same lactosamine unit this is called the sialyl Lewis x (sLex) antigen and the possible relevance of this to immunosuppression will be discussed later.

The different changes in the glycosylation of API and Hp in ovarian cancer are difficult to reconcile because these two molecules are synthesized in the liver by the same pathway [10, 14]. *In vitro*, API and Hp are secreted by primary cultures of hepatocytes [27, 30] and human hepatoma cell lines (HepG2 and Hep-3B) [25, 26, 28, 29] and their production can be stimulated by treatment with glucocorticoids or cytokines such as IL-6. These processes are thought to mimic the APR *in vivo*. These treatments also affect glycosylation *in vitro*. Two types of change have been identified [31]. For human hepatocytes and Hep-3B cells, there is a decrease in the branching of acute-phase proteins (APP), the so called Type I change. This resembles that observed in the sera of patients with acute

inflammatory states. For Hep-G2 cells, there is an increase in the branching of APP, the so called Type II change. This change is observed in the sera of patients with chronic inflammatory states.

Unfortunately, only one study [27] has reported on the glycosylation of API and Hp in the same *in vitro* investigation, and this has been done with rat hepatocytes. It was found that dexamethasone treatment decreased the branching of Hp but not API. Although it is difficult to extrapolate directly from rat cells *in vitro* to man, it does suggest that either Hp is being glycosylated by a different pathway to API within the hepatocyte or that these proteins are synthesized in different liver cells.

It is interesting to note that the glycosylation change in API resembles the Type I change, and that the glycosylation change in Hp resembles the Type II change. It is possible that the API changes are caused by an acute event, and that the Hp changes are due to some underlying chronic response. There is a precedence for this situation in the inter-current infections that are observed in the chronic inflammatory diseases such as rheumatoid arthritis and systemic lupus erythromatosus [1]. Our lotus extraction results would tend to agree with this hypothesis, because the lectin only extracted API in large amounts when there was a recurrence of the disease; whereas Hp was extracted throughout disease development.

An obvious explanation of our results is that API and Hp are produced by different sources in ovarian cancer. It is known that the tissue in which a molecule is glycosylated is very important in affecting the carbohydrate structure of the glycan chain [32]. Other sources of these proteins have been described, and these may be more important in cancer. A polymeric form of Hp has been isolated from the ascitic fluid of ovarian cancer patients that has potent immunosuppressive properties [33]. Another Hp related protein (HPR), has been detected in many different types of cancers [34–36]. Hp has also been detected in lymphocytes, monocytes and granulocytes [10]. The synthesis of API in macrophages and neutrophils is well established [13], and in the former case it appears to play, a definite but limited, role in restricting the destructive potential of elastase. However, all these non-hepatic sources of API and Hp are produced in very low concentrations and have only local functional significance. The changes we detected on the purified proteins were occurring on the majority of molecules that were present in the blood. It would seem very unlikely, therefore, that the production of API and Hp by extra-hepatic tissues can account for the observed differences in glycosylation.

Another explanation of the discrepancy between API and Hp glycosylation is that post-secretory mechanisms are operating which modify the glycosylation of one of these proteins more than the other. Although there is evidence for the release of glycosyl transferases into the blood in disease (see [31] for references), which could modify the glycosylation of circulating API or Hp, this could not explain the alterations which occur in branching, as this is determined very early in the synthesis of a glycan [37]. Even the modification of terminal carbohydrates such as Neu5Ac and fucose, which is theoretically possible, is unlikely, due to a lack of the appropriate nucleotide sugar substrates in the blood. Glycosidases are also released into the blood in disease (see [31] for references) and these could reduce the sialylation and fucosylation of a molecule, but they could not change the branching of a circulating glycoprotein. In any case, it is difficult to envisage how any blood-mediated change could effect one glycoprotein more than another.

Complex N-glycan chains on glycoproteins such as API and Hp have a common core structure of three mannose and two GlcNAc residues, which terminates in two-four branches composed of GlcNAc, galactose, fucose, and Neu5Ac [1]. The composition of the latter is very variable and within any single population there exists many molecules with different types of extension (glycoforms) [27]. Thus, any change in the carbohydrate composition of a glycoprotein in disease indicates a shift in the weighted average of the population. Therefore, observed differences in the carbohydrate composition of API and Hp may be due to the differential removal of one particular glycoform from the blood. This is difficult to test without using animal systems. There is no evidence for a striking difference in turnover of API or Hp in health or disease [10, 12, 14, 38, 39]. In healthy individuals, the half-lives of API and Hp are very similar (3.5–4.5 days). These appear to change when disease is present, but the changes are small and the results not well documented. A study of the APR in rats [40] has shown that increased branching slightly reduced the half-life of alpha-1-acid glycoprotein in the blood, and decreased branching slightly increased it. It is clear, however, that when API or Hp are bound to elastase [11] or Hb [12] respectively, these complexes are rapidly removed from the blood; also, removal of Neu5Ac, or deglycosylation [10, 11] results in their rapid clearance even in the uncomplexed form.

Our findings could be explained if a pathological process was specifically removing API, but not Hp, with increased branching from the blood. This possibility seems likely for many reasons. Firstly, API is known to form complexes with other proteins and itself in the blood [41]. A previous study of benign and malignant ovarian effusions has detected extra API-containing components in malignant specimens [42]. It was suggested that there were protease-API complexes, caused by the tumour excreting proteases into the peritoneal effusion. Our previous studies showed that API extracted by lotus lectin in ovarian cancer was composed of aggregates [3]. This could be caused by prolonged storage at –20°C or repeated cycles of freezing and thawing of fresh cancer sera. This effect was not observed in sera from healthy individuals. If API with increased branching formed these complexes, then they would be rapidly removed from the circulation [11]. Secondly, because changes in the glycosylation of Hp are related to tumour burden, whereas those with API are associated with unresponsiveness to therapy, this suggests that the API changes are caused by a different pathological mechanism. Thirdly, there is evidence for the sequestration of API

by tumour tissue. A component of the same molecular weight as API has been detected in very close association with many different types of tumour, [15] and tumour sections from stomach cancer that stained strongly for API were associated with poor prognosis [43].

It is not known what effects these particular changes in the glycosylation have on the biological activity of API and Hp. One study of API synthesized in the presence of glycosylation inhibitors [44] has shown that incomplete glycosylation has no effect on protease inhibitory activity, but increased the sensitivity of API to heat. Furthermore, genetically-engineered API that is devoid to carbohydrate has normal inhibitory activity, but an increased tendency to aggregate [45]. This suggests that carbohydrate has an important role in stabilizing the API structure. In the deficiency phenotype (ZZ), the formation of API aggregates in the liver is also associated with changes in the glycosylation of the molecule [46]; particularly, a decrease in Neu5Ac content [47]. In a human hepatoma cell line, an incompletely sialylated form of API is synthesized, which accumulates in intracellular inclusions [48].

On the other hand, the carbohydrate portion of Hp appears to be essential for the active form of the molecule, but has no direct role in the binding to Hb [49, 50]. Removal of about 40% of the total weight of the carbohydrate moiety totally inhibited the ability of Hp to form a complex with Hb [49]. In another study of tryptic fragments of Hp [50], only those fragments binding to Con A exhibited peroxidase activity with Hb equivalent to the unfractionated material.

The glycosylation changes in API and Hp could be affecting processes not normally thought to involve these molecules. Clinical studies have shown that there is a direct association between elevated levels of API and Hp in cancer patients and immunosuppression [51]. *In vitro*, it has been shown that addition of API at physiological concentrations can inhibit NK cell activity [52, 53], modulate neutrophil chemotaxis [54], superoxide production [55], and adhesion to endothelium [56]; whereas *in vitro* studies with Hp have shown that it inhibits lymphocyte activation by PHA [57, 58], and modulates prostaglandin synthesis [59, 60] and superoxide production [61]. Although some of these additional effects of API and Hp are well documented, their importance in disease and the role of glycosylation in these processes, is unclear. Finally, our results suggested that Hp in cancer could have increased expression of the sLex antigen; this grouping has been shown to be very important in the binding of leukocytes to endothelial cells in inflammation [62]. Increased sLex has been detected on AGP in inflammation [31] and it has been suggested that this change could inhibit the leukocyte/endothelium interaction and dampen the acute-phase response. A similar mechanism may be operating with cancer Hp.

Concluding remarks

We are only just starting to describe in detail the changes in the glycosylation of acute-phase proteins in disease, let alone the effect of these changes on the function of these molecules. This review of the glycosylation of API and Hp in ovarian cancer not only illustrates the complexity of the current situation, but also demonstrates the possibilities for improving our understanding of the pathological processes involved, and so developing better disease markers. We and others have also shown that the glycosylation of API and Hp vary in different diseases (see [1, 2, 31] for references). In the near future, there will be major advances in this field due to the development of many new procedures to investigate glycoprotein structure, and increasing knowledge, through molecular and cell biology, of the mechanisms involved in the synthesis of glycans.

Acknowledgements

We wish to acknowledge the technical assistance of E. Dargan, and E. Wong; the staff of various hospitals in the Newcastle area for supplying the specimens and clinical details; and the financial support of the North of England Cancer Research Campaign, The GO fund and the Islamic Republic of Iran.

References

1. Turner GA (1995) In: *EQAS '94 Proceedings* (in press).
2. Turner GA (1992) *Clin Chim Acta* **208**:149–71.
3. Thompson S, Guthrie D, Turner GA (1988) *Br J Cancer* **58**:589–93.
4. Thompson S, Wong E, Cantwell BMJ, Turner GA (1990) *Clin Chim Acta* **193:**13–26.
5 Goodarzi MT, Turner GA (1995) *Clin Chim Acta* (in press).
6. Thomson S, Turner GA (1987) *Br J Cancer* **56**:605–10.
7. Thomson S, Cantwell BMJ, Cornell C, Turner GA (1991) *Br J Cancer* **64**:386–90.
8. Thompson S, Dargan E, Turner GA (1992) *Cancer Lett* **66**:43–48.
9. Dargan E, Thompson S, Cantwell BMJ, Wilson RG, Turner GA (1994) *Glycosyl & Dis* **1**:37–43.
10. Gordon AH, Koj A (1985) *Research Monographs in Cell and Tissue Physiology,* vol 10. Netherlands: Elsevier Science Publishers BV.
11. Carrell RW (1986) *Clin Invest* **77**:1427–31.
12. Dobryszycka W (1993) In: Mackiewicz A, Kushner I, Baumann H, eds. Acute phase proteins: molecular biology, biochemistry, and clinical applications. Boca Raton: CRC Press, 1993; 185–206.
13. Perlmutter DH (1993) In: *Acute Phase Proteins: Molecular Biology, Biochemistry, and Clinical Applications,* (Mackiewicz A, Kushner I, Baumann H, eds) pp. 150–67, Boca Raton: CRC Press.
14. Kushner I, Mackiewicz A (1993) In: *Acute Phase Proteins: Molecular Biology, Biochemistry, and Clinical Applications,* (Mackiewicz A, Kushner I , Baumann H, eds) pp. 3–19, Boca Raton: CRC Press.
15. Turner GA, Skillen AW, Baumah P, Guthrie D, Welsh J, Harrison J, Kowalski A (1985) *J Clin Path* **38**:588–92.
16. Hodges LC, Laine R, Chan SK (1979) *J Biol Chem* **254**:8208–12.

17. Mega T, Lujane E, Yoshida A (1980) *J Biol Chem* **255**:4057–61.
18. Saitoh A, Aoyagi Y, Asakura H (1993) *Arch Biochem Biophys* **303**:281–87.
19. Chan SK, Luby J, Wu YC (1973) *FEBS Lett* **35**:79–83.
20. Bayard B, Kerkaert JP, Laine A, Hayem A (1982) *Eur J Biochem* **124**:371–76.
21. Nilsson B, Lowe M, Osada J, Ashwell G, Zopf D (1981) In: *Glycoconjugates Proc 6th Int Sym Glyco Tokyo: Japan Scientific Societies Press.* (Yamakawa T, Osawa T, Handa F, eds) pp. 275–76.
22. Katnik I, Jadach J, Krotkiewski H, Gerber J (1994) *Glycosyl & Dis* **2**:97–104.
23. Goodarzi T, Rafiq M, Turner GA (1995) *Biochem Soc Trans* **23**:168S.
24. De Vries T, van Den Eijnden DH (1992) *Histochem J* **24**:761–70.
25. Gauldie J, Richards C, Harnish D, Lansdorp P, Baumann H (1987) *Proc Natl Acad Sci USA* **84**:7251–55.
26. Baumann H, Richards C, Gauldie J (1987) *J Immunol* **139**:4122–28.
27. Pos O, van Dijk W, Ladiges N, Linthorst C, Sela M, van Tiel D, Boers W (1988) *Eur J Cell Biol* **46**:121–28.
28. Mackiewicz A, Kushner I (1989) *Scand J Immunol* **29**:265–71.
29. Mackiewicz A, Kushner I (1990) *Inflammation* **14**:485–97.
30. Mackiewicz A, Pos O, van der Stelt M, Yap SH, Kapcinska M, Kaciak M, Dewey MJ, Berger FG, Baumann H, Kushner I, van Dijk W (1992) In: *Affinity Electrophoresis: Principles and Application* (Breborowicz J, Mackiewicz A, eds) pp. 135–53. Boca Raton: CRC Press.
31. van Dijk W, Turner GA, Mackiewicz A (1994) *Glycosyl & Disease* **1**:5–14.
32. Rademacher TW, Parekh RB, Dwek RA (1988) *Ann Rev Biochem* **57**:785–838.
33. Oh S-K, Very DL, Ettinger R, Walker J, Giampaolo C, Bernado J (1989) *Hybridoma* **8**:449.
34. Shurbaji MS, Pasternack GR, Kuhajda FP (1991) *Am J Clin Path* **96**:238–42.
35. Shurbaji MS, Thurmond TS, Pasternack GR, Kuhajda FP (1991) *Lab Invest* **64**:A52.
36. Redston MS, Kern SE, Vogelstein B, Hamilton SR (1992) *Lab Invest* **66**:A47.
37. Schachter H (1991) *Glycobiology* **1**:453–61.
38. Koj A (1974) *Structure and Function of Plasma Proteins* vol 1 (Allison AC, ed.) pp. 73–120. London: Plenum Press.
39. Cooper EH, Stone J (1979) Acute-phase reactant proteins in cancer. *Adv Cancer Res* **30**:1–44.
40. Parivar K, Tolentino L, Taylor G, Oie S (1992) *J Pharm Pharmacology* **44**:447–50.
41. Laurell C-B, Jeppsson J-O (1975) In: *The Plasma Proteins vol 1 2nd edition* (Putnam FW, ed) pp. 229–264. New York: Academic Press.
42. Bleasby AJ, Knowles JC, Cooper EH (1986) *Int J React* **VIII**: 393–400.
43. Tahara E, Itoh H, Taniyama K, Yokoyaki H, Hata J (1984) *Human Path* **15**:957–64.
44. Guzdek A, Potempa J, Dubin A, Travis J (1990) *FEBS Lett* **72**:125–27.
45. Powell LM, Pain R (1992) *J Mol Biol* **2224**:241–52.
46. Putnam FW (1984) In: *The Plasma Proteins. Structure, Function and Genetic Control,* vol IV (Putnum FW, ed) pp. 45–166. London: Academic Press.
47. Cox DW (1975) *Am J Hum Gen* **27**:165–77.
48. Carlson J, Ariksson S, Alm R, Kjellstrom T (1984) *Hepatology* **4**:235–41.
49. Kaartinen V, Mononen I (1988) *Biochim Biophys Acta* **953**:345–52.
50. Katnik I (1984) *Biochim Biophsy Acta* **790**:8–14.
51. Baskies AM, Chretien PB, Weiss JF, Makich RW, Beveridge RA, Catalona WJ, Spiegel HE (1980) *Cancer* **45**:3050–60.
52. Okumura Y, Kudo J, Ikuta T, Kurokawa S, Ishibashi H, Okubo H (1985) *Inflammation* **9**:211–19.
53. Lejeune P-J, Mallet B, Farnarier C, Kaplanski S (1989) *Biochim Biophs Acta* **990**:122–27.
54. Aoshiba K, Natashi A, Ishihara Y, Kagawa J, Takizawa T (1993) *J Clin Lab Med* **122**:333–40.
55. Shasby M (1989) *Am Rev Resp Dis* **1**:451–53.
56. Smith CW, Hollers JC, Patrick RA, Hassett C (1979) *J Clin Invest* **63**:221–29.
57. Samak R, Edelstein R, Israel L (1982) *Cancer Immunol Immunother* **38**:38–43.
58. Baseler MW, Burrel R (1983) *Inflammation* **7**:387–400.
59. Beisembaeva RU, Mursagalieva AT, Dzhumalieva, Shaikenova TE, Mevkh AT (1990) *FEBS Lett* **269**:125–27.
60. Frohlander N, Ljunggren O, Lerner UH (1991) *Biochem Biophys Res Comm* **178**:343–51.
61. Oh S-K, Pavlotsky N, Tauber AI (1990) *J Leukocye Biol* **47**:142–48.
62. Brandley BK, Swiedler SJ, Robbins PW (1990) *Cell* **63**:861–63.