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Biosynthesis of complement C1 inhibitor by Hep G2 cells

Reactivity of different glycosylated forms of the inhibitor with C1s

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The biosynthesis of C1 Inh (C1 inhibitor) was studied in a human hepatoma cell line (Hep G2) by metabolic labelling, immunoprecipitation with anti-(C1 Inh) serum, analysis on SDS/polyacrylamide gel slabs and fluorography. Two forms of C1 Inh are secreted by Hep G2: a minor form of M_r 90000 and a major form of $M_r \sim 100000$. The latter form is also found in small amounts intracellularly in co-existence with an 80000-M_r form. Accumulation of the 80000-M_r C1 Inh is favoured when the cells are labelled at 23 °C instead of 37 °C or when they are treated with monensin. In the presence of tunicamycin, a compound that blocks the formation of *N*-asparagine-linked oligosaccharide chains, a decrease in M_r of both secreted and intracellular major forms is observed, indicating that secreted and intracellular C1 Inh contain *N*-linked oligosaccharide units. The 100000 M_r secreted C1 Inh is sensitive to endoglycosidase F but resistant to endoglycosidase H, and it incorporates [^{3}H]galactose, [^{3}H]glucosamine and [^{3}H]galactosamine, indicating the presence of both *N*-linked oligosaccharides of the complex type and *O*-linked oligosaccharides. The intracellular C1 Inh contains *N*-linked oligosaccharide units of the high-mannose type as demonstrated by endoglycosidase H-sensitivity. The functional activity of C1 Inh during its biosynthesis was tested by studying its reactivity towards C1s. Both secreted and intracellular C1 Inh form covalent-like complexes with purified plasma C1s. The underglycosylated C1 Inh secreted in presence of tunicamycin is still reactive with purified C1s. These results clearly show that sugars are not essential for this inhibitory activity of C1 Inh.

INTRODUCTION

C1 inhibitor (C1 Inh) is a single-chain glycoprotein which contains an unusually high level (about 35%) of carbohydrate (Pensky & Schwick, 1969; Haupt *et al.*, 1970). It is the only known plasma inhibitor of the C1 proteinases C1r and C1s (Sim *et al.*, 1979), but it also inactivates several other enzymes of plasma, such as kallikrein (Gigli *et al.*, 1970), factor XIa and XIIa (Forbes *et al.*, 1970) and plasmin (Harpel & Cooper, 1975). C1 Inh was originally identified as an inhibitor of the activated form of the first component of complement (Levy & Lepow, 1957; Ratnoff & Lepow, 1957), but recent studies have shown that C1 Inh also regulates the activation of C1 (Ziccardi, 1982).

Detailed studies of C1 subcomponents and C1 biosynthesis have been carried out in our laboratory (Bensa *et al.*, 1983; Colomb *et al.*, 1984; Reboul *et al.*, 1985, 1986). In this context we were interested in the biosynthesis of C1 Inh, since this protein is likely to control the state of activation or the activity of subcomponents C1r and C1s of C1 during their biosynthesis. Besides, as this inhibitor shows a broad spectrum of inhibition, it may be involved in a versatile manner in intracellular or extracellular regulation, as has been recently shown for the homologous α_1-proteinase inhibitor (Perlmutter *et al.*, 1985). It thus appears of primary importance to analyse the reactivity of C1 Inh as a function of its state of glycosylation during its biosynthesis. Although human monocytes and monocyte-like cells (U 937) have been shown to produce C1 Inh (Reboul *et al.*, 1985; Yeung Laiwah *et al.*, 1985; Randazzo *et al.*, 1985), liver is considered to be the major site of C1 Inh synthesis (Johnson *et al.*, 1971). As liver contains hepatocytes, epithelial cells and Küpffer cells, it appeared more specific to choose Hep G2 cells for these studies. This human hepatoma cell line is also known for its high protein-synthesizing capacity (Morris *et al.*, 1982).

MATERIALS AND METHODS

Materials

RPMI 1640 medium, FCS, penicillin, streptomycin and fungizone were purchased from Flow Laboratories. Selectamine was from Flobio, Courbevoie, France; IgG Sorb was purchased from the Enzyme Center, Malden, MA, U.S.A. Goat anti-(human C1 Inh) antiserum was from Atlantic Antibodies, Westbrook, ME, U.S.A. Tunicamycin was from Calbiochem and monensin from Sigma. L-[^{35}S]Methionine (specific radioactivity ~ 1000 Ci/mmol), D-[6-^{3}H]glucosamine hydrochloride (40 Ci/mmol) and [^{14}C]methylated mole-

Abbreviations used: FCS, heat-inactivated foetal-calf serum; phosphate-buffered saline, 20 mM-phosphate/145 mM-NaCl, pH 7.4; SEA, staphylococcal enterotoxin A; iPr_2P-F, di-isopropyl phosphofluoridate; k.i.u., kallikrein-inhibitory unit; The nomenclature of the complement components and subcomponents is that recommended by the World Health Organisation (1968), the activation of a component being indicated by an overbar.

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cular-mass markers were obtained from Amersham International. D-[1-^{3}H]galactose (15–20 mCi/mmol) was from Oris, Gif-sur-Yvette, France. D-[1-^{3}H]Galactosamine hydrochloride (~ 12.4 Ci/mmol), En^{3}Hance, endoglycosidase F and endoglycosidase H were purchased from New England Nuclear Corp.

Methods

The purification of C$\bar{1}$ Inh from plasma was performed as described previously (Reboul *et al.*, 1977), with slight modifications: fresh plasma was used and aprotinin (10 k.i.u./ml final concn.) was added to all samples, except at the last stage of purification. C$\bar{1}$s was purified from recalcified plasma as described by Arlaud *et al.* (1979).

Purified C$\bar{1}$ Inh and C$\bar{1}$s were labelled with ^{125}I by the iodogen method of Fraker & Speck (1978).

Cell culture and biosynthetic labelling. Hep G2 was kindly given by Dr. T. Meo (Institut Pasteur, Paris, France). The cells were grown almost to confluence in 25 cm^2 plastic flasks (Falcon) at 37 °C in a CO_2/air (1:19) atmosphere, using RPMI 1640 medium supplemented with 20 mM-Hepes, 1 mM-pyruvate and 10% (v/v) FCS. Penicillin (100 units/ml), streptomycin (100 μg/ml) and fungizone (2.5 μg/ml) were added to the medium. Before labelling, the cells were incubated for 48 h in the same medium containing 10% (v/v) of lymphocyte conditioned medium which had been prepared as described by Bensa *et al.* (1983) from SEA-stimulated lymphocytes. After washing twice with Hanks balanced salt solution, the cells were incubated for 17 h with 4 ml of selectamine lacking methionine supplemented with 10% (v/v) FCS, 10% (v/v) conditioned medium and L-[^{35}S]methionine (250 μCi/ml). Labelling with [^{3}H]monosaccharides was performed in a similar manner, except that the cells were labelled in RPMI containing 125 μCi of either D-[1-^{3}H]galactose, D-[6-^{3}H]glucosamine hydrochloride or D-[1-^{3}H]galactosamine hydrochloride/ml, and incubated at 37 °C for 18 h. After incubation the medium was removed, centrifuged for 10 min at 1500 *g*, dialysed against PBS and stored at −20 °C. The cell monolayer was washed twice with phosphate-buffered saline, pH 7.4, and lysed by the addition of 4 ml of 0.05 M-Tris/HCl (pH 7.4)/0.1 M-KCl/0.01 M-EDTA/0.5% sodium deoxycholate, followed by two cycles of freezing and thawing.

In a few experiments, lysates were prepared by sonication to avoid the presence of detergents. After the washing with phosphate-buffered saline, 4 ml of phosphate-buffered saline/EDTA (5 mM) was added and the cells were left for 10 min at room temperature, then 30 min at 4 °C. The cells were scraped off with a rubber policeman and the suspension was placed in an ice bath and sonicated twice for 45 s with a interval of 1 min between bursts. The lysate was centrifuged at 110000 *g* for 30 min at 4 °C in a Kontron TST 54 rotor, then dialysed against phosphate-buffered saline and stored at −20 °C.

Immunoprecipitation. Medium or cell lysates corresponding to about 10^7 total c.p.m. were diluted with equal volumes of detergent buffer (1% Triton X-100/0.5% sodium deoxycholate/1% SDS in phosphate-buffered saline, pH 7.4) containing human serum albumin (5 mg/ml). The mixture was incubated at 4 °C overnight with 10 μl of antiserum to C$\bar{1}$ Inh. Then 50 μl of IgG Sorb, washed once with 1 ml of detergent buffer containing human albumin (5 ml/ml), were added and the incubation was continued for 30 min at 4 °C. The immunoadsorbent was collected by centrifugation and washed once with 1 ml of detergent buffer containing albumin and five to nine times with 1 ml of detergent buffer. After washing, the adsorbed immune complexes were dissociated by boiling for 5 min in 2% (w/v) SDS/5% (v/v) 2-mercaptoethanol/20% (v/v) glycerol/0.1 M-Tris/HCl, pH 6.8. Electrophoretic separation of the labelled components was performed by the method of Laemmli (1970) in 7.5% (w/v) polyacrylamide/0.1% SDS slab gels. After electrophoresis the gels were fixed by soaking them for 1 h in 25% trichloroacetic acid, washed with water, treated with En^{3}Hance according to the instructions of the manufacturer, and dried. The dried slab gels were autoradiographed with Kodak X-Omat AR film at −70 °C.

Standards for M_r determination were myosin (M_r 200000), phosphorylase *b* (M_r 92500), bovine serum albumin (M_r 69000) and ovalbumin (M_r 46000).

Endoglycosidase treatment. After immunoprecipitation, C$\bar{1}$ Inh was recovered from the immunoadsorbent by boiling for 5 min in SDS/2-mercaptoethanol/glycerol/Tris/HCl, pH 6.8. After centrifugation, 50 μl aliquots of the supernatant were diluted with 100 μl of 50 mM-EDTA/0.5% Nonidet P40/0.1 M-sodium phosphate buffer, pH 6.1, for endoglycosidase F treatment, and with 50 μl of 0.1 M-sodium citrate buffer, pH 5.5, for endoglycosidase H treatment.

To inhibit potential proteinase activity during the treatment, iPr_2P-F (5 mM) was added to the samples. Endoglycosidase F was added to 10 units/ml and endoglycosidase H to 5 μl/ml (final concns.). Enzymic digestion was performed for 17 h at 37 °C and then terminated by adding 10 μl of 10% SDS/10% glycerol/0.1 M-Tris/HCl, pH 6.8, and 4 μl of 2-mercaptoethanol, and heating 5 min at 100 °C.

Reactivity of C$\bar{1}$ Inh with C$\bar{1}$s. Washed C$\bar{1}$ Inh immunoprecipitates were rinsed once with 200 μl of 5 mM-triethanolamine/145 mM-NaCl buffer, pH 7.4, and the whole Ig Sorb-bound C$\bar{1}$ Inh–anti(C$\bar{1}$ Inh) complex was resuspended in 200 μl of the buffer containing C$\bar{1}$s (25 μl/ml). Then they were left to incubate at 37 °C for 45 min. After centrifugation, the immunoprecipitates were prepared for electrophoresis as described above. In some experiments, unlabelled cell lysates prepared by sonication in the absence of detergent were incubated with ^{125}I-C$\bar{1}$s at 37 °C for 1 h before immunoprecipitation. After incubation, the immunoprecipitation was carried out as described above.

RESULTS

Synthesis of C$\bar{1}$ Inh by Hep G2 cells

Cultures of Hep G2 cells were incubated in medium containing [^{35}S]methionine for 17 h at 37 °C. C$\bar{1}$ Inh was immunoprecipitated either from culture medium or cell lysates. Analysis by SDS/polyacrylamide gel electrophoresis is shown in Fig. 1. Two forms of C$\bar{1}$ Inh were immunoprecipitated from culture medium (lane 1): a major form whose apparent M_r is slightly higher than that

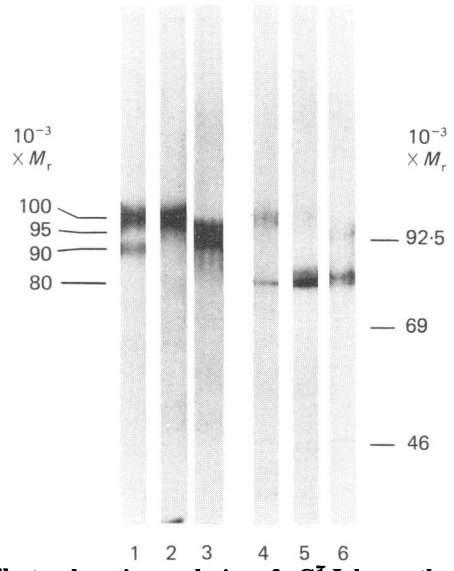

Fig. 1. Electrophoretic analysis of C1 Inh synthesized and secreted by Hep G2 cells

Electrophoretic separation of samples and fluorography were as described in Materials and methods section. Lanes 1 and 2, immunoprecipitates from culture medium of Hep G2 cells radiolabelled with [^{35}S]methionine at 37 and 23 °C respectively; lane 3, C1 Inh purified from plasma and labelled with ^{125}I; lanes 4, 5 and 6, immunoprecipitates from lysates of Hep G2 cells labelled at 37 °C (lane 4), at 23 °C (lane 5) and at 37 °C in the presence of monensin (lane 6).

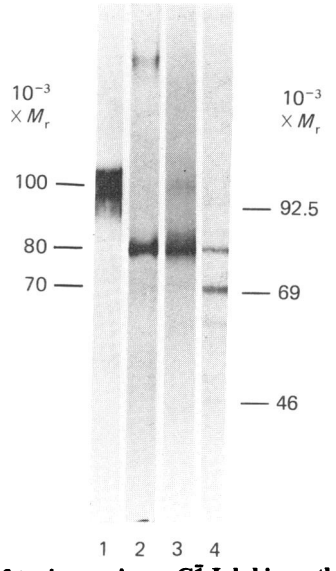

Fig. 2. Effect of tunicamycin on C1 Inh biosynthesis

Hep G2 cells were incubated for 17 h at 37 °C in the absence or presence of tunicamycin (5 μg/ml) and subsequently labelled for 4 h at 37 °C with [^{35}S]methionine in the absence or presence of tunicamycin. Lanes 1 and 2, C1 Inh immunoprecipitated from the supernatant of control Hep G2 cells (lane 1) and tunicamycin-treated Hep G2 cells (lane 2); lanes 3 and 4, C1 Inh immunoprecipitated from the lysates of control (lane 3) and tunicamycin-treated (lane 4) Hep G2 cells. Immunoprecipitates from supernatants and lysates were treated and analysed as described in the Materials and methods section.

of C1 Inh purified from plasma (M_r ~ 95000, lane 3) and a minor form with an apparent M_r of 90000.

In the case of cell lysates, two forms are immunoprecipitated in trace amounts (Fig. 1, lane 4). One has an electrophoretic mobility identical with that of the 100000-M_r C1 Inh observed in the culture medium. The other has an apparent M_r of 80000. An accumulation of this latter form was observed when the biosynthetic labelling was performed at 23 °C instead of 37 °C (Fig. 1, lane 5). When Hep G2 cells were treated with monensin, two forms, of M_r 82000 and 94000, were present in the intracellular medium besides the 80000-M_r form (Fig. 1, lane 6).

Effect of tunicamycin

To study the glycosylation of synthesized C1 Inh, we used tunicamycin, an antibiotic that blocks *N*-glycosylation of proteins (Takatsuki *et al.*, 1971; Struck & Lennarz, 1980). Hep G2 cells were preincubated with tunicamycin (5 μg/ml), then labelled with [^{35}S]methionine in the presence of the drug. The results are illustrated in Fig. 2. C1 Inh secreted from tunicamycin-treated cells (lane 2) has an apparent molecular size less than that from control culture (lane 1). An increase in mobility is also noted in intracellular C1 Inh (lane 4). These results indicate that both secreted and intracellular C1 Inh are glycoproteins with asparagine-linked oligosaccharides.

Biosynthetic incorporation of different tritiated monosaccharides

Hep G2 cells were labelled with either [^{3}H]galactose, [^{3}H]glucosamine or [^{3}H]galactosamine for 17 h at 37 °C. As shown in Fig. 3, all three sugars labelled the major secreted form of M_r 100000, suggesting the presence of *O*-linked oligosaccharides and *N*-linked oligosaccharides of the complex type. Under these conditions, labelled intracellular C1 Inh was not detected.

Treatment of C1 Inh with endoglycosidases

We tested the susceptibility of C1 Inh immunoprecipitated either from the Hep G2-cell lysates or from the medium to two types of endoglycosidases which hydrolyse the linkage between glycan and asparagine of the protein backbone. Endoglycosidase F cleaves oligosaccharide chains of both high mannose and complex type (Elder & Alexander, 1982), and endoglycosidase H cleaves only high-mannose oligosaccharides (Tarentino & Maley, 1974). When C1 Inh immunoprecipitated from the medium was treated with endoglycosidase F, the 100000-M_r form disappeared and several lower-M_r products were detected (Fig. 4, lanes 1 and 2). Among them, the lightest form (M_r ~ 80000) had nearly the same electrophoretic mobility as the intracellular inhibitor and the C1 Inh secreted after tunicamycin treatment. When C1 Inh was treated with endoglycosidase H, no change in electrophoretic mobility was observed (Fig. 4, lanes 3 and 4), indicating that the oligosaccharides of this form are of the complex type. C1 Inh found in the cells was susceptible to both endoglycosidase F and H. Two products, of respective M_r 68000 and 71000, appeared in the electrophoretic pattern after endoglycosidase F treatment (Fig. 4, lane 6), whereas only one, of M_r ~ 67000, is

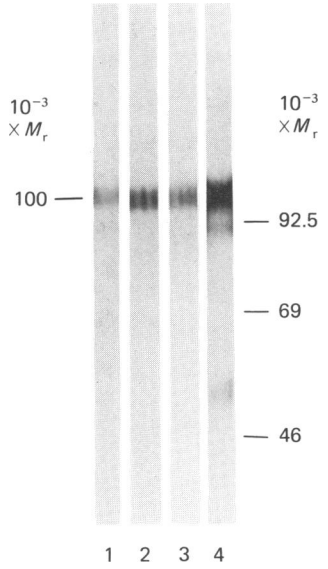

Fig. 3. Incorporation of tritiated monosaccharides in C1 Inh

C1 Inh was immunoprecipitated from culture medium of Hep G2 cells incubated for 17 h with [^{3}H]galactose (lane 1), [^{3}H]glucosamine (lane 2), [^{3}H]galactosamine (lane 3) and analysed as described in the Materials and methods section. [^{35}S]Methionine-labelled C1 Inh (lane 4) is shown as a reference.

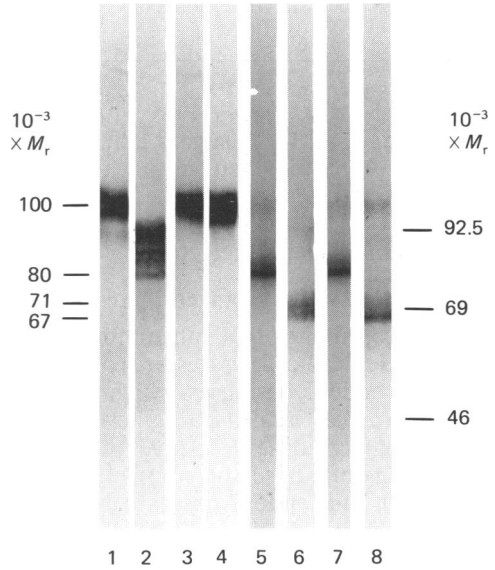

Fig. 4. Endoglycosidase treatment of C1 Inh labelled with [^{35}S]methionine

C1 Inh immunoprecipitates were treated with endoglycosidases F and H before separation by SDS/polyacrylamide-gel electrophoresis and fluorography as described in the Materials and methods section. Lanes 1 and 2, reference secreted C1 Inh and secreted C1 Inh treated with endoglycosidase F respectively; lanes 2 and 3, reference secreted C1 Inh and secreted C1 Inh treated with endoglycosidase H respectively; lanes 5 and 6, control intracellular C1 Inh and intracellular C1 Inh treated with endoglycosidase F respectively; lanes 7 and 8, control intracellular C1 Inh and intracellular C1 Inh treated with endoglycosidase H respectively.

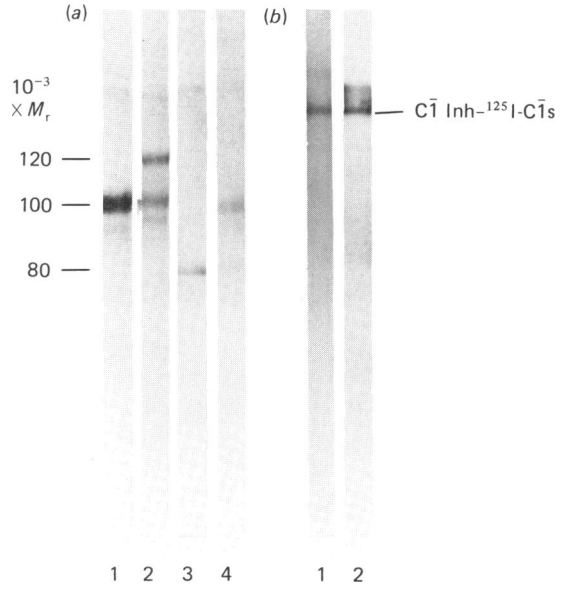

Fig. 5. Reactivity of C1 Inh with C1s

(*a*) Labelled C1 Inh. C1 Inh was immunoprecipitated from culture medium after biosynthetic labelling with [^{35}S]methionine as described in the Materials and methods section. After washing, the immunoprecipitates were resuspended in 200 μl of buffer in the absence or presence of unlabelled C1s (25 μg/ml) and were incubated at 37 °C for 45 min. The immunoprecipitates were then boiled for 5 min and were prepared for electrophoresis under reducing conditions. Secreted C1 Inh was incubated with buffer (lane 1) or with C1s (lane 2); C1 Inh secreted after tunicamycin treatment was incubated with buffer (lane 3) or C1s (lane 4). (*b*) Labelled C1s. Cell lysates of unlabelled Hep G2 cells were incubated with ^{125}I-C1s before immunoprecipitation as described in the Materials and methods section. After incubation, C1 Inh–^{125}I-C1s complexes were immunoprecipitated with anti-C1 Inh and subjected to SDS electrophoresis under non-reducing conditions. Lane 1, C1 Inh–^{125}I-C1s complex immunoprecipitated from Hep G2-cell lysate; lane 2, C1 Inh–^{125}I-C1s complex immunoprecipitated from the lysate of Hep G2 cells treated with monensin.

observed after endoglycosidase H treatment (Fig. 4, lane 8).

Treatment of C1 Inh with C1s

To determine whether synthesized C1 Inh was functional, we tested its reactivity towards C1s. The results are illustrated in Figs. 5(*a*) and 5(*b*).

When C1 Inh immunoprecipitated from the culture medium was incubated for 45 min with unlabelled C1s, a band of $M_r \sim 120000$ was visible in the electrophoretic pattern (Fig. 5*a*, lane 2). The presence of this band, which corresponds to a complex between C1 Inh and the B-chain of activated C1s (the samples are reduced), indicated that secreted C1 Inh is reactive towards activated C1s.

The underglycosylated C1 Inh secreted by Hep G2 cells treated with tunicamycin also formed a complex with C1s (Fig. 5*a*, lane 4), indicating that sugars are not essential to the development of activity.

Complexing of intracellular C1 Inh with C1s was examined by treatment of the unlabelled cell lysate prepared by sonication in absence of detergent with

^{125}I-C1s. After incubation, immunoprecipitation was carried out as described in the Materials and methods section. Fig. 5(*b*) shows that C1 Inh present in cell lysates reacts with ^{125}I-C1s to form a complex which can be immunoprecipitated with anti-(C1 Inh) serum. When Hep G2 cells were treated with monensin, several bands were visible on the gel (Fig. 5*b*, lane 2). They represent complexes between ^{125}I-C1s and the different forms of C1 Inh synthesized in the cells in the presence of monensin.

DISCUSSION

Previous studies have suggested that the liver is the primary site of C1 Inh synthesis (Johnson *et al.*, 1971); in the present study we have demonstrated that a human hepatoma cell, Hep G2, is also able to secrete C1 Inh. Recent work in this laboratory (Reboul *et al.*, 1986) has shown that Hep G2 cells secrete C1 subcomponents C1r and C1s in stoichiometric amounts, but at a 3-fold lower level than it secretes C1 Inh. Since C1r and C1s are secreted in their non-activated form (Reboul *et al.*, 1986), C1 Inh might be involved in the prevention of C1r activation.

Hep G2 cells secrete two forms of C1 Inh: a major form that exhibits a slightly higher M_r ($\sim$ 100000) than the C1 Inh purified from plasma ($M_r \sim 95000$) and a minor form ($M_r \sim 90000$). The latter form, which is not found when Hep G2 cells are labelled at 23 °C instead of at 37 °C, may correspond either to an incompletely glycosylated protein or to a proteolysed form of the inhibitor.

An intracellular C1 Inh of M_r 80000 is immunoprecipitated in co-existence with a 100000-M_r form. These two forms of C1 Inh are detected in trace amounts when the biosynthetic labelling is performed at 37 °C. However, an accumulation of the 80000-M_r C1 Inh is observed when the labelling is carried out at 23 °C. Similar results were obtained when the Hep G2 cells were treated with monensin, a cationic ionophore known to affect protein secretion, through interferences with the intracellular translocation of proteins and the late glycosylation (Tartakoff, 1983). However, besides the 80000-M_r form, two heavier M_r forms, corresponding probably to different steps of glycosylation, were also found. Pretreatment of Hep G2 cells with tunicamycin, an inhibitor of *N*-glycosylation, results in a decrease in M_r of both secreted and intracellular C1 Inh. These results indicate that both C1 Inh species contain *N*-linked oligosaccharides and that complete glycosylation is not required for the secretion of C1 Inh.

From the above data it thus appears probable that the secreted 100000-M_r form represents the fully glycosylated protein with *N*-linked carbohydrate chains of the complex type (resistance to endoglycosidase H, cleavage by endoglycosidase F, incorporation of [^{3}H]galactose and [^{3}H]glucosamine). Incorporation of [^{3}H]galactosamine is observed in secreted C1 Inh; as this sugar is often found in *O*-linked oligosaccharides (Kornfeld & Kornfeld, 1980), it is likely that secreted C1 Inh contains this type of oligosaccharide. These results agree with the data of Harrison (1983), who observed an unusual composition of carbohydrate in C1 Inh and suggested that the molecule contains a number of *O*-glycosidic oligosaccharide units in co-existence with *N*-linked oligosaccharides.

The intracellular inhibitor ($M_r \sim 80000$) appears also as a glycoprotein of the high-mannose type, as demonstrated by endoglycosidase H-sensitivity. The difference in size between intracellular and extracellular C1 Inh suggests that stepwise glycosylation(s) occur before secretion; this is also exemplified by the M_r difference observed between the above-described forms of C1 Inh and a 60000-M_r form observed as a cell-free-translation product of C1 Inh mRNA (Tosi *et al.*, 1986). Studies of the reactivity of C1 Inh towards C1s clearly show that both intracellular and secreted C1 Inh form covalent-like complexes with C1s. Thus C1 Inh-mediated inhibitions are likely to exist at the intracellular as well as at the extracellular level. The observation that the underglycosylated C1 Inh (intracellular or secreted in the presence of tunicamycin) form complexes with C1s confirms previous results (Minta, 1981) showing that sugars are not essential for the inhibition of C1s to take place. From these experimental results, it is not possible to determine whether the different forms of biosynthesized C1 Inh undergo proteolytic cleavage as has been reported for purified plasma C1 Inh reacting with C1s (Weiss & Engel, 1983; Nilsson *et al.*, 1983; Agostini *et al.*, 1985).

Structural studies of C1 Inh have suggested that C1 Inh is a highly elongated molecule with a rod-like moiety and a globular domain (Odermatt *et al.*, 1981) and that its reactive site is in the *C*-terminal part of the molecule (Salvesen *et al.*, 1985). Recent data from our laboratory, obtained from small-angle neutron scattering with soluble C1 Inh, also confirm the above finding, with the additional observation of a 90° bend in the middle of the rod-like part of the molecule. In addition, recent findings on the sequence of the *C*-terminal half of C1 Inh indicate that *N*-glycosylation sites are absent in the 150 *C*-terminal residues and that *O*-glycosylation sites, although present, are less abundant than they are upstream towards the *N*-terminal of this known sequence (Tosi *et al.*, 1985). The arginine residue of the reactive site, located at 34 residues from the *C*-terminus of C1 Inh, is thus unlikely to reside in a highly glycosylated area. Accordingly, the extent of glycosylation of C1 Inh would have little effect on its activity. The significance of the exceptionally high glycosylation of C1 Inh is still unclear; further studies are necessary to elucidate the role of carbohydrate either in the catabolism of the protein or in the control of various intracellular or extracellular proteinases.

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