Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 679–683, February 1988
Biochemistry

Complete amino acid sequence of human plasma Zn-α_2-glycoprotein and its homology to histocompatibility antigens

(plasma protein structure/glycan structure/HLA class I antigens/secretory major histocompatibility complex-related protein/immunoglobulin gene superfamily)

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Contributed by Frank W. Putnam, September 22, 1987

ABSTRACT **In the present study the complete amino acid sequence of human plasma Zn-α_2-glycoprotein was determined. This protein whose biological function is unknown consists of a single polypeptide chain of 276 amino acid residues including 8 tryptophan residues and has a pyroglutamyl residue at the amino terminus. The location of the two disulfide bonds in the polypeptide chain was also established. The three glycans, whose structure was elucidated with the aid of 500 MHz ^{1}H NMR spectroscopy, were sialylated *N*-biantennas. The molecular weight calculated from the polypeptide and carbohydrate structure is 38,478, which is close to the reported value of ≈41,000 based on physicochemical measurements. The predicted secondary structure appeared to be comprised of 23% α-helix, 27% β-sheet, and 22% β-turns. The three *N*-glycans were found to be located in β-turn regions. An unexpected finding was made by computer analysis of the sequence data; this revealed that Zn-α_2-glycoprotein is closely related to antigens of the major histocompatibility complex in amino acid sequence and in domain structure. There was an unusually high degree of sequence homology with the α chains of class I histocompatibility antigens. Moreover, this plasma protein was shown to be a member of the immunoglobulin gene superfamily. Zn-α_2-glycoprotein appears to be a truncated secretory major histocompatibility complex-related molecule, and it may have a role in the expression of the immune response.**

Zn-α_2-glycoprotein (Zn$\alpha_2$gp) (M_r ≈38,500) is one of several human plasma proteins of unknown function that have been highly purified and characterized in terms of chemical and physical chemical properties (1, 2). This protein was first isolated from fraction VI of the Cohn ethanol procedure, and its name derives from the fact that it was precipitated by addition of zinc ions (1). The purified protein appeared homogeneous on electrophoresis and on amino-terminal amino acid analysis, which revealed a blocked end group. However, polymorphism of Zn$\alpha_2$gp was observed electrophoretically both for the native and the asialo form of the protein (3). The carbohydrate content was reported to be 18% and to consist of *N*-acetylneuraminic acid, galactose, mannose, fucose, and *N*-acetylglucosamine (1). The lack of *N*-acetylgalactosamine indicated the absence of *O*-glycans. Although the possible biological function of this protein is not known, it has been speculated that Zn$\alpha_2$gp has a lectin-like determinant in common with nephritogenic urinary glycoproteins (2, 4).

In this paper we describe the determination of the complete amino acid sequence of Zn$\alpha_2$gp, including the location of the two disulfide bridges and the structure of the three carbohydrate units, and we discuss the possible secondary structure of this protein. An unexpected finding made by computer analysis of the sequence data is that Zn$\alpha_2$gp is closely related to antigens of the major histocompatibility complex (MHC) in amino acid sequence and in domain structure. This suggests that Zn$\alpha_2$gp may have a role in the expression of the immune response.

MATERIALS AND METHODS

Materials. The details of the preparation of the immunochemically homogeneous Zn$\alpha_2$gp procedure will be published elsewhere. In brief, pooled normal human plasma was fractionated with the Cohn ethanol procedure (1); the resulting supernatant of Cohn fraction V was concentrated by ultrafiltration followed by ammonium sulfate fractionation, zone electrophoresis, and gel filtration. The remaining traces of serum proteins were successfully removed using solid-phase immunoadsorption employing antisera against human serum and human α_1-acid glycoprotein. A yield of 400 mg of homogeneous Zn$\alpha_2$gp was obtained from 100 liters of plasma. The preparation used was number 050584 from Behringwerke.

Methods. Prior to cleavage, Zn$\alpha_2$gp was enzymatically desialized using *Vibrio cholera* neuraminidase (Calbiochem) followed by reduction and alkylation with iodoacetic acid. Specific chemical cleavage was done initially with CNBr at 4°C in 70% (vol/vol) formic acid, followed by a second cleavage in 70% (vol/vol) formic acid at room temperature to split an Asp-Pro bond, and then by incubation in dilute HCl (0.012 M, 23°C) to break an Asp-Thr bond. Enzymatic digestion was carried out with the following enzymes: trypsin, lysyl-endopeptidase, *Staphylococcus aureus* V8 protease, chymotrypsin, proline-specific endopeptidase, carboxypeptidase Y, and pyroglutamate aminopeptidase. The purification of the resulting peptides was carried out by reversed-phase HPLC (Varian model 5000 liquid chromatograph with an Altex-Ultrasphere-ODS or Toyo-Soda TSK-

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Abbreviations: Zn$\alpha_2$gp, Zn-α_2-glycoprotein; MHC, major histocompatibility complex.

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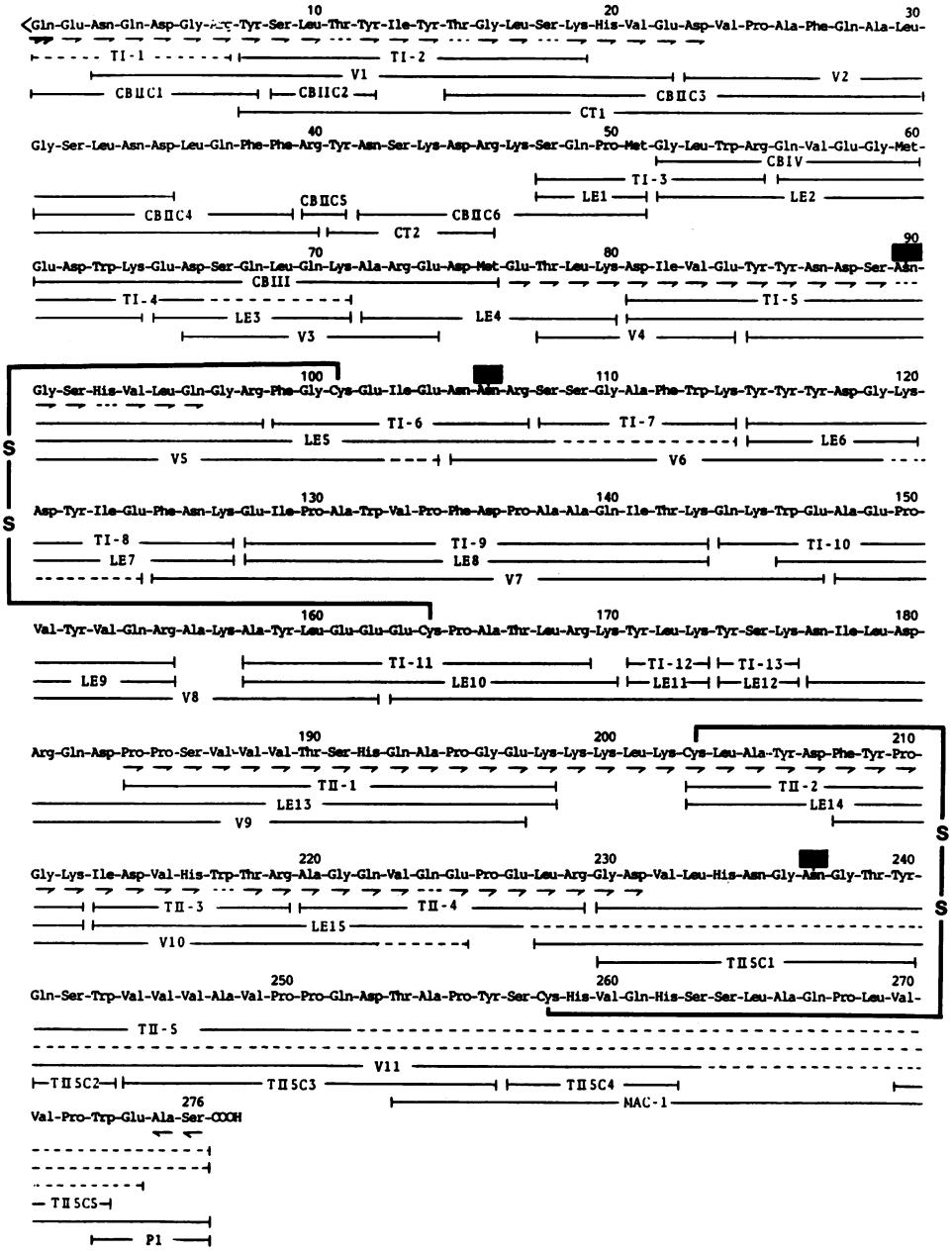

FIG. 1. The complete amino acid sequence of human plasma Zn$\alpha_2$gp. CB, cyanogen bromide cleavage fragments; TI, tryptic peptides of acid-cleavage fragment I; TII, tryptic peptides of acid-cleavage fragment II; LE, lysyl-endopeptidase peptides; V, *S. aureus* V8 protease peptides; CT, tryptic peptides of citraconylated CBII; MAC, mild-acid-cleaved peptides; P1, proline-specific endopeptidase peptide of MAC-1; ⊸, carboxypeptidase Y; and –, pyroglutamate aminopeptidase digestions of Zn$\alpha_2$gp. Solid lines indicate those regions of the peptides that were sequenced, whereas the dotted lines indicate residues or regions of peptides that were not sequenced. Disulfide bonds are indicated by the bold lines with —S-S—. The solid squares above Asn-90, Asn-106, and Asn-237 indicate *N*-glycans. In this figure the amino acid sequences of only the major peptides are included, but it should be noted that additional sequences were established to confirm the position of each amino acid residue.

120 C_{18} column and a Bio-Rad Hi-Pore C_4 column). Automated sequence determination was performed with a Beckman 890C sequencer, while for the manual sequence determination the dimethylaminoazobenzene isothiocyanate (DABITC) procedure was used (5).

For the location of the disulfide bonds, asialo Zn$\alpha_2$gp was digested at pH 1.8 with *Aspergillus saitoi* acid protease (Sigma) and then with thermolysin. The cystine-containing peptides isolated by HPLC were identified with 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (6) and oxidized, and the resulting cysteic acid peptides were subjected to sequencing.

Preparation of Glycopeptides. Native Zn$\alpha_2$gp was first digested with trypsin and then exhaustively digested with Pronase. After preliminary fractionation on Bio-Gel P-4, the resulting glycopeptides were purified to homogeneity by HPLC. They were characterized by their amino acid sequences and by determining the structure of the carbohydrate moiety employing 500 MHz ^{1}H-NMR spectroscopy by methods described (7) except that a Varian VXR 500 MHz spectrometer was used with 32K data blocks. Assignments were based on various reports (8–10).

Computer Analysis of Sequence Data. The Protein Sequence Database of the Protein Identification Resource and the programs SEARCH, FASTP, ALIGN, PRPLOT, CHOFAS, and DOTMATRIX were provided by the National Biomedical Research Foundation.§§

§§Protein Identification Resource (1987) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), release 11.0.

RESULTS AND DISCUSSION

The Complete Amino Acid Sequence. The strategy for the amino acid sequence analysis of carbohydrate-rich proteins has been presented in our papers on the amino acid sequence of α_2HS-glycoprotein¶¶ (11). Small and large peptides and glycopeptides were readily purified by HPLC. Using this approach, an unambiguous amino acid sequence was established for Zn$\alpha_2$gp (Fig. 1). The detailed data of this study are available on request from our laboratory.

CNBr cleavage was carried out first and afforded four fragments (CBI, CBII, CBIII, and CBIV; Fig. 1); these could readily be separated by gel filtration on Sephadex G-75. One fragment (CBII) consisting of 51 residues was devoid of a free amino-terminal amino acid and thus represented the amino terminus of the protein. The largest fragment (CBI), which begins at glutamic acid-77 and consists of 200 residues, was free of homoserine indicating its carboxyl-terminal position. The sequence of the two shorter CNBr peptides (CBIII and CBIV) located between the amino- and carboxyl-terminal fragments was deduced from the amino acid sequences of peptides derived from the tryptic and lysyl endopeptidase digests of Zn$\alpha_2$gp.

To elucidate its amino acid sequence (Fig. 1), the large CNBr fragment (CBI) was treated with 70% (vol/vol) formic acid at room temperature effecting cleavage of the Asp-Pro bond (between residues 183 and 184), which is located almost in the middle of this fragment. Interestingly, the other Asp-Pro bond (between residues 136 and 137) was not cleaved. The resulting carboxyl-terminal half of this fragment could be further split by mild acid hydrolysis between residues 253 and 254, which simplified the elucidation of the carboxyl-terminal 23 residues. An additional advantage was that a proline-specific enzyme cleaved the Pro-Trp bond between positions 272 and 273 yielding the terminal tetrapeptide.

Polypeptide Structure. Zn$\alpha_2$gp consists of a single polypeptide chain containing 276 residues (Asp_{18}, Asn_{11}, Thr_9, Ser_{18}, Glu_{21}, Gln_{20}, Pro_{17}, Gly_{16}, Ala_{18}, $\frac{1}{2}Cys_4$, Val_{21}, Met_3, Ile_8, Leu_{19}, Tyr_{18}, Phe_8, Lys_{20}, His_7, Arg_{12}, and Trp_8) and has a molecular weight of 31,889 in the unglycosylated form. Pyroglutamic acid was found to be the amino terminus of this protein. The unusually high content of tryptophan and tyrosine accounts for the high extinction coefficient of 18.0 (1). Three of the four asparagine residues that are present in the signal sequence of Asn-Xaa-Ser/Thr carry a glucosamine (Asn-90, Asn-106, and Asn-237) (see below).

Location of the Disulfide Bonds. The amino acid sequence of Zn$\alpha_2$gp contains four half-cystine residues. Determination of the positions of the disulfide bonds demonstrated the presence of one bond between residues 101 and 164 and a second one between residues 203 and 258. The loops formed by the disulfide bonds are similar in length, 64 and 56 residues, respectively. Yet, analysis of the amino acid sequence of Zn$\alpha_2$gp by the computer program RELATE gave no statistically significant evidence for internal duplication.

Structure of Glycans. The oligosaccharides at the three glycosylation sites were identical (Fig. 2) except that the glycosylation site Asn-237 was not completely sialylated (90%) on the mannose α2,6-arm. The three glycans possessed the typical N-linked biantennary structure, being substituted with *N*-acetylneuraminic acid in α2,6-linkage and lacking fucose. They were identical, except for the peptide moiety, to the glycans described for serotransferrin, α-fetoprotein, and many other glycoproteins (11–13). Thus, the molecular weight of the carbohydrate moiety of Zn$\alpha_2$gp is 6644 and that of the native glycoprotein is 38,478, a value that is ≈6% lower than that determined earlier by physicochemical techniques (1, 2).

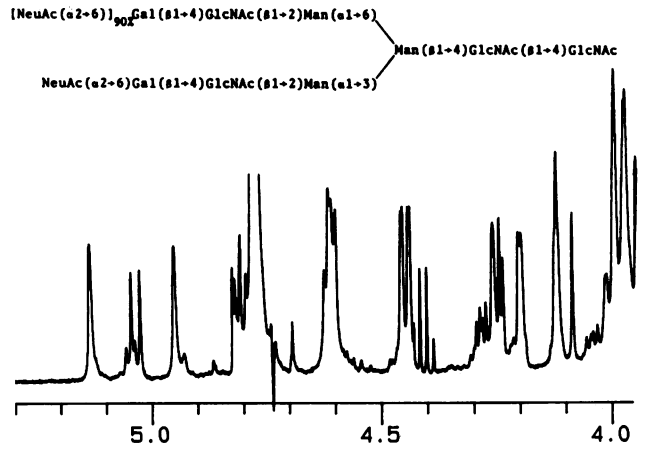

FIG. 2. ^{1}H-NMR spectrum (500-MHz) of the glycans of Zn$\alpha_2$gp. The glycan at Asn-237 was only partially sialylated on the mannose α1,6-branch as indicated in this figure. Only the essential section of the spectrum (4.0–5.5 ppm) is reproduced here. The peak due to the *N*-acetyl protons would be at 2.0 ppm.

Secondary Structure. The secondary structure of Zn$\alpha_2$gp was estimated by the CHOFAS computer program based on the method of Chou and Fasman (14), which predicted the presence of ≈23% α-helix, 27% β-sheet conformation, and 22% β-turns. The three *N*-glycans linked to residues 90, 106, and 237 are located in β-turn regions and are thus probably at the surface of the protein. The hydropathic profile (data not shown) indicated that three carbohydrate chains are located in the hydrophilic regions. This is further supported by the observation that the sialic acid residues of the protein can readily be cleaved enzymatically. Another notable feature of the secondary structure is that 8 of the 16 β-sheet conformations (residues 9–16, 94–98, 131–135, 151–154, 187–191, 232–234, 241–248, and 269–272) essentially coincide in their positions in the amino acid sequence with the major hydropathic profile. Of particular interest is the type of conformations found within the two disulfide loops: two β-turns, three α-helices, four β-sheets, and one glycan in the first loop and four β-turns, one α-helix, three β-sheet conformations, and one glycan in the second loop.

Sequence Homology to Histocompatibility Antigens. Zn$\alpha_2$gp is highly homologous to the α chains of HLA (human), H-2 (mouse), and RLA (rabbit) class I histocompatibility antigens (15–21) in amino acid sequence and in domain structure and is also homologous but somewhat less so to class II antigens (Fig. 3). The 18 highest scores obtained from a computer search of the Protein Identification Resource data base§§ were for homology to the α chain of class I antigens, which consist of a highly polymorphic α chain noncovalently bound to a nonpolymorphic light chain (β_2-microglobulin) (20, 21). The identities ranged from as high as 36–39% for overlaps of 272–279 amino acids with HLA class I α chains such as the pHLA12.4 clone and the serologically defined antigens Cw3, B40, B27, and B7; the initial similarity scores for these ranged from 188 to 141 compared to a mean score of 22 for all 4028 sequences in the data base. Three HLA class II antigens of the DR (formerly DC) subclass followed class I antigens with a score of 81 and had 25–27% identity for 165 amino acid overlaps. A similarity score of 80 corresponds to a z value of 10, which is statistically significant (22). Fig. 3 shows the amino acid sequence homology for representative examples of human class I and II antigens.

The sequence similarity of Zn$\alpha_2$gp to histocompatibility antigens reflects a shared framework structure that is not specific for the class, subclass, or species. The five highest

¶¶α_2HS-glycoprotein is an abbreviation for α_2-glycoprotein discovered by J. F. Heremans and K. Schmid.

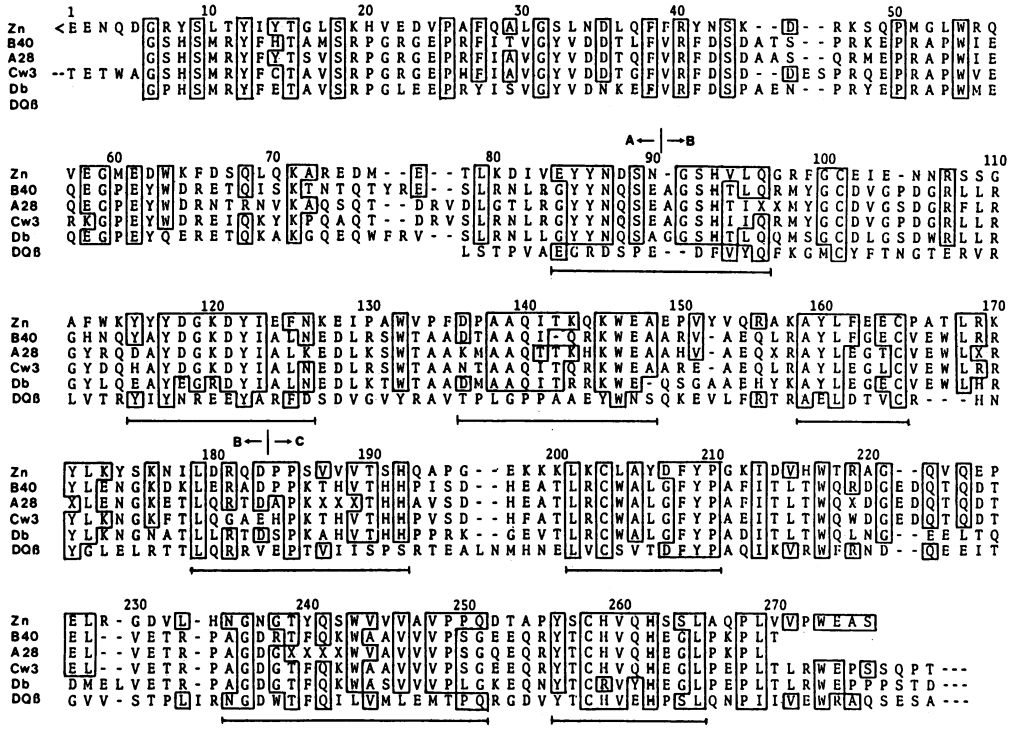

FIG. 3. Comparison of amino acid sequences of Zn$\alpha_2$gp and MHC antigens. Zn, Zn$\alpha_2$gp; B40, human histocompatibility antigen (HLA) class I B40 α chain (15); A28, HLA class I A28 α chain (16); Cw3, HLA class I Cw3 α chain (17); Db, murine histocompatibility antigen (H-2) class I Db α chain (18); DQβ, HLA class II DQ β chain (19). The amino acid residues identical with Zn$\alpha_2$gp are in boxes. The homologous cores are indicated by solid bars under the sequences. A, B, and C indicate the domains A, B, and C. The single-letter amino acid code is used.

scores included three human HLA class I antigens of various subclasses, a mouse H-2-related, but secreted, antigen (clone pH16), and a rabbit RLA class I antigen. These scores represent identities of 36–38% with Zn$\alpha_2$gp. Although highly significant, these percentages are much less than those for interspecies or intraspecies sequence comparison for antigens of the same class. For example, human pHLA12.4 and B7, cited above, have 87% identity in the region compared to Zn$\alpha_2$gp, whereas rabbit RLA PR9 and human pHLA12.4 have 80% identity (23). Thus, despite its striking structural relationship to MHC molecules, Zn$\alpha_2$gp is not identifiable with any subclass of HLA class I or class II molecules yet reported.

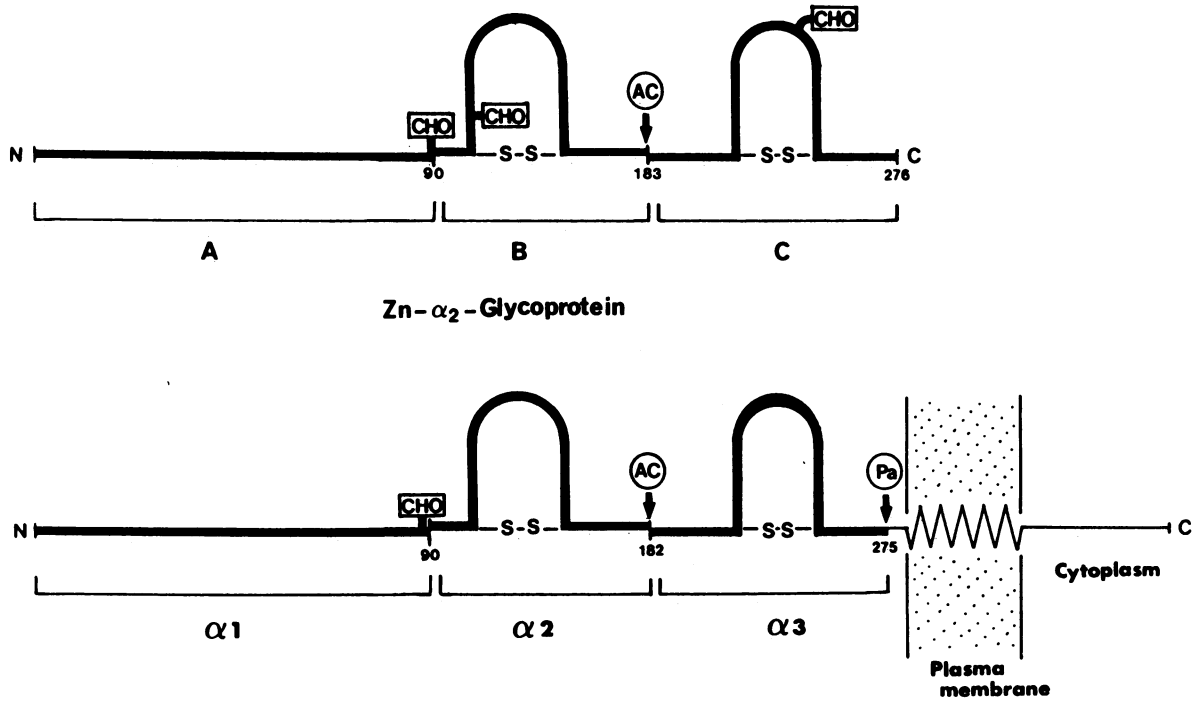

FIG. 4. Schematic diagram of the domain structures of Zn$\alpha_2$gp and HMC class I α chain. AC, acid-cleavage position; Pa, papain-cleavage position; CHO, carbohydrate. The highly homologous regions between Zn$\alpha_2$gp and MHC class I α chain are indicated by bold lines on the MHC class I α chain.

Similarity in the Domain Structure of Zn$\alpha_2$gp and MHC Antigens of Classes I and II. The domain structure of Zn$\alpha_2$gp is very similar to the extracellular domain structure of MHC class I α chains (Fig. 4). The α chain has three extracellular domains (α1, α2, and α3); each domain contains ≈90 residues and is precisely encoded by an exon. The domain structure of Zn$\alpha_2$gp is determined by the β-turn and the carbohydrate at Asn-90, by the β-turn and the acid-cleavage site at Asp-183, and by sequence homology to the MHC class I α chain. The A domain of Zn$\alpha_2$gp and the α1 domain of the MHC α chain both lack a disulfide bond and end with a glucosamine glycan at a similar position. Both the second and third domains of Zn$\alpha_2$gp and the MHC class I α chain have disulfide bonds in homologous positions. Acid cleavage of the Asp-Pro bond of the polypeptide chain occurs at the same place between the second and third domains for both proteins. Zn$\alpha_2$gp lacks the transmembrane and cytoplasmic domains characteristic of insoluble class I α chains, but the latter are removed by Pronase to yield a solubilized α chain of the same size as Zn$\alpha_2$gp.

Zn$\alpha_2$gp has four potential *N*-glycosylation sites, of which three are glycosylated, Asn-90, Asn-106, and Asn-237. The latter two have no counterpart in class I antigens. The first glycan occurs at the second asparagine in the sequence Asn-Asp-Ser-Asn-Gly-Ser (residues 87–92), which has two potential glycosylation sites. The first of these (Asn-87) is homologous to the highly conserved glycosylation site at Asn-86 in HLA, H-2, and RLA antigens; however, Asn-87 is not glycosylated in Zn$\alpha_2$gp, probably because it is followed by aspartic acid that adversely affects the tripeptide acceptor sequence. Thus, the glycan at Asn-90 in Zn$\alpha_2$gp is the homologue of that at Asn-86 in class I antigens.

Class II molecules are heterodimers composed of an α chain and a β chain; both chains have two extracellular domains each of which contains ≈90 amino acids. In the β chain, such as the human DQβ illustrated in Fig. 3, both domains have a 60-residue disulfide-bonded loop similar to that in Zn$\alpha_2$gp, class I antigens, and immunoglobulins. Thus, class II polypeptides exhibit structural homology to Zn$\alpha_2$gp for only two domains rather than three as for class I.

Zn$\alpha_2$gp is a Member of the Immunoglobulin Gene Superfamily. Zn$\alpha_2$gp is a member of the immunoglobulin gene superfamily on the basis of both its amino acid sequence and its domain structure. The 46 highest similarity scores for amino acid sequence homology to Zn$\alpha_2$gp were for class I MHC antigens (the 19 highest), followed by class II MHC antigens. The latter were interspersed among IgG, IgE, and IgM constant regions, the T-cell-receptor γ-chain constant region, and immunoglobulin heavy-chain variable regions. All these gave z values ranging from 6.0 (probably significant) to 10.0 (significant). Furthermore, Zn$\alpha_2$gp has the repeated domain structure characteristic of the immunoglobulin gene superfamily, namely a series of disulfide-bonded loops enclosing ≈60 amino acid residues (Fig. 4). This suggests that the Zn$\alpha_2$gp gene descended from the ancestral gene of the immunoglobulin gene superfamily that encoded a domain of 90–100 amino acids. The Zn$\alpha_2$gp gene must have diverged early from the primordial MHC multigene family, and each of the three Zn$\alpha_2$gp domains is likely encoded by a separate exon.

Zn$\alpha_2$gp Is a Secretory MHC-Related Molecule. The Zn$\alpha_2$gp protein probably represents a soluble secretory class I molecule rather than a membrane glycoprotein. A similar truncated transplantation-like antigen in mouse serum is secreted as the soluble product of a class I (H-2)-related gene (clone pH16) that is expressed only in liver cells (24). Although the secreted antigen has ≈80% protein sequence similarity to the extracellular domains of the classical H-2 antigens, it is distinct in having many nonconservative substitutions in the transmembrane domain and in lacking the two cytoplasmic domains. Furthermore, Zn$\alpha_2$gp is as homologous to this mouse serum protein as it is to human class I antigens. This structural relationship has special interest because of the suggestion that the secreted mouse H-2-like serum protein has a potential role in mediating active immunological tolerance (24).

Polymorphism of Zn$\alpha_2$gp. Whereas the α chains of the HLA and H-2 class I and class II antigens are highly polymorphic, the amino acid sequence of Zn$\alpha_2$gp gave no clear evidence of polymorphism. The latter finding also contrasts with the early report of the polymorphism of Zn$\alpha_2$gp in starch gel electrophoresis despite its apparent homogeneity in other physicochemical characteristics (1, 3). The electrophoretic polymorphism of Zn$\alpha_2$gp persisted after removal of sialic acid by neuraminidase, but the number of bands did decrease. A rare genetic polymorphism of Zn$\alpha_2$gp has been reported (25), but this does not appear to correlate in frequency with the electrophoretic polymorphism. One possible explanation of the finding of electrophoretic polymorphism in desialated Zn$\alpha_2$gp despite the failure to identify structural polymorphism by sequence analysis is that the serum Zn$\alpha_2$gp may be the product of a small set of highly homologous genes such as the gene family that encodes the secreted mouse H-2-related serum protein (24). Another explanation would be partial sulfation of the carbohydrate moiety of Zn$\alpha_2$gp as a report (26) indicated that many plasma proteins may contain sulfate residues.

The authors are greatly indebted to Mr. George Crombie for a large series of amino acid analyses, to Dr. John H. Collins for sequencing a carboxyl-terminal peptide of CBI, and to Karen Huss for computer analysis of the sequence data. This work was supported by Grants GM-10374 (to K.S.), DK-19221 and CA-08497 (to F.W.P.), and CA-13402 (to P.H.A.) from the National Institutes of Health and a grant from the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 136) (to R.B.).

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