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Sheep MHC class II molecules

I. IMMUNOCHEMICAL CHARACTERIZATION

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SUMMARY

The physicochemical features, biosynthesis and glycosylation of sheep class II molecules were investigated using a panel of seven monoclonal antibodies. The class II molecules recognized by different monoclonal antibodies could be differentiated using SDS–PAGE. Two monoclonal antibodies, SBU.II 42-20 and 49-1, reacted with dissociated sheep class II molecules and recognized epitopes on class II alpha and beta polypeptides, respectively. The structure of the sheep class II heterodimer differed from that of mouse and man in that it was unstable in the presence of 1% SDS at 20° and, following reduction, sheep beta polypeptides displayed a marked increase in MW, resulting in the apparent co-migration of reduced alpha and beta polypeptides on SDS–PAGE. This phenomenon was not seen using sheep class II molecules synthesized in the presence of tunicamycin. Pulse-chase analyses of biosynthetically labelled sheep class II molecules suggested the rapid association and glycosylation of sheep class II alpha and beta polypeptides during synthesis. Both alpha and beta polypeptides of sheep class II molecules carried *N*-linked oligosaccharides of MW 6,000 and 3,000, respectively. However, unlike human class II oligosaccharides, these were exclusively of the complex or sialylated type.

INTRODUCTION

MHC class II molecules have been studied in a number of mammalian species (Van Dam, 1981; Newman & Antczak, 1983; Kaufman *et al.*, 1984) by serological, biochemical or molecular analyses, with the products of the class II genetic region of man (HLA-D) and mouse (I region) being the best characterized. MHC class II molecules consist of two non-covalently associated glycoprotein subunits of MW 32,000–36,000 (alpha chain) and 25,000–28,000 (beta chain), and are found typically on B lymphocytes, activated T cells and antigen-presenting cells such as monocytes and macrophages (Klareskog & Forsum, 1986). MHC class II molecules are involved in mixed lymphocyte reactions and graft-versus-host reactions, as well as in T cell–macrophage and T–B cell interactions as restricting elements for T-helper ($CD4^+$) cells. At least three distinct types of class II molecules are co-expressed on mature human B cells, while two types are expressed on mature murine B cells (Kaufman *et al.*, 1984).

Mammalian class II molecules display considerable overall structural homology, presumably due to constraints imposed by their common functional roles. However, as a consequence of evolutionary divergence, variations in structure may exist between the class II molecules of different species. Differences in the structure of the class II molecules of man and mouse have been documented (Shackelford *et al.*, 1982; Mengle-Gow & McDevitt, 1985). Some of these differences are apparent during biosynthesis, both in the spectrum of class II-associated intracellular polypeptides and in post-translational modification involving the number and type of *N*-linked oligosaccharides. The importance of these and other species' differences in structure–function relationships remains to be addressed.

Until recently (Puri, Mackay & Brandon, 1985; Hopkins, Dutia & McConnell, 1986), no serological reagents were available for the characterization of sheep class II molecules. Consequently, little is known about the number of expressed sheep class II molecules or their distinguishing structural features. Using a panel of monoclonal antibodies produced in our laboratory, the results of a comprehensive biochemical analysis of the structure of sheep class II molecules, comprising of their physicochemical features, biosynthesis, glycosylation and the antigenicity of the dissociated sheep class II heterodimer, are reported here.

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MATERIALS AND METHODS

Monoclonal antibodies

The preparation and characterization of the monoclonal antibodies SBU.II 28-1, 37-68, 38-64, 38-27, 38-30 and 42-20 to

sheep MHC class II molecules has been described previously (Puri *et al.*, 1985). An additional monoclonal antibody, SBU.II 49-1, directed against sheep class II molecules (produced by M. D. Gorrell), was also used.

Cell preparation

Spleens were removed from 6 to 12-month-old sheep and single-cell suspensions prepared by gentle teasing in PBS containing 0·05% EDTA. Splenocytes were separated from red blood cells by Ficoll–Isopaque centrifugation. Efferent lymph cells were collected by cannulation of the efferent lymphatic duct of the popliteal lymph node (Hall & Morris, 1962).

Radiolabelling of cells and preparation of cell lysates

Ficoll–Isopaque-purified splenocytes were used for all biosynthetic labellings. Briefly, 1×10^8 pelleted cells were resuspended in 10 ml of methionine-deficient DME (Flow Laboratories, Sydney) supplemented with 5% dialysed FCS, 2 mM L-glutamine, $2{\cdot}5 \times 10^{-5}$ M 2-mercaptoethanol (2-ME), 50 μg/ml streptomycin, and incubated for 90 min at 37° in a humidified atmosphere containing 5% CO_2. Cells were harvested by centrifugation (500 *g*, 5 min), resuspended in 2 ml of the above media containing 1 mCi of ^{35}S-methionine (>800 mCi/ml; Amersham Co., Sydney) and incubated for a further 6–8 hr. For inhibition of *N*-linked glycosylation, tunicamycin (Calbiochem, La Jolla, CA), at a final concentration of 10 μg/ml, was included throughout the labelling procedure.

Cell-surface iodination of splenocytes or efferent lymph-derived lymphocytes ($5–10 \times ^7$ cells) was catalysed using lactoperoxidase (Goding, 1980). Subsequent procedures were as described elsewhere (MacKay *et al.*, 1985), except that cells were washed three times in PBS and then lysed on ice for 30 min in 0·50–0·75 ml of 20 mM Tris-HCl, pH 8·0, containing 75 mM NaCl, 1% NP-40 and 5 mM iodoacetamide (Sigma, St Louis, MO). Subcellular debris was removed by centrifugation (10,000 *g*, 5 min) and the lysates used immediately or stored at 4°.

Pulse-chase labelling

Splenocytes (5×10^7) were preincubated as above, pelleted and incubated in 0·5 ml of methionine-deficient DME containing 1·5 mCi ^{35}S-methionine and 1·5 mCi of ^{35} S-cysteine for 5 min at 37° in a humidified atmosphere containing 5% CO_2. Cells were then harvested by centrifugation (500 *g*, 5 min) and diluted in 32 ml of complete DME supplemented with 10% FCS, 2×10^{-5} M 2-ME and 75 μg/ml of methionine and 75 μg/ml cysteine (Sigma). Cells were then reincubated at 37° in 5% CO_2 and 4-ml samples removed at 0, 5, 10, 20, 30, 60, 90 and 120 min after cold chase. These samples were immediately diluted into 50 ml of the DME media, pre-chilled to 4° and placed on ice. Cells were pelleted, washed three times with ice-cold PBS and lysed in 0·5 ml of lysis buffer.

In vitro *cleavage of class II molecules*

Samples of ^{35}S-methionine or ^{125}I surface-labelled cell lysates were treated with 0·2 M HCl (Cohen *et al.*, 1984) prior to being used for immunoprecipitation analyses.

Immunoprecipitation

The method used was as described previously (MacKay *et al.*, 1985), except sheep anti-mouse IgG Sepharose 4B (SAM Sepharose) was used in place of Protein A, and immune complexes were washed in 25 mM Tris-saline, pH 8·0, containing 0·1% NP-40 and 0·1% SDS.

Endoglycosidase digestions

Briefly, radiolabelled proteins were eluted from SAM-Sepharose by boiling in 50 μl of 10 mM Tris-HCl, pH 8·0, containing 1% SDS and 50 μg BSA. For Endoglycosidase H (Endo H) digestion, supernatants were mixed with 450 μl of 0·1 M sodium phosphate buffer, pH 6·0, containing 50 mM EDTA, 1% NP-40 and 10 μl Endo H (74 μg/ml; NEN Research Products, Boston, MA). For Endoglycosidase F (Endo F) digestion, supernatants were mixed with 0·1 M sodium citrate buffer, pH 5·5, and 10 μl of Endo F (500 μg/ml; NEN Research Products). Samples were incubated overnight at 37°, precipitated with trichlororoacetic acid (15% v/v, 30 min 4°), the precipitates pelleted, washed with $3 \times$ 1-ml volumes of −20° acetone, air dried and analysed by SDS–PAGE (Laemmli, 1970).

RESULTS

Physicochemical features of sheep class II molecules

Class II molecules from ^{125}I-surface-labelled cells were analysed by SDS–PAGE following various pre-treatments (Fig. 1). As is evident from Fig. 1a, sheep class II molecules reactive with the SBU class II monoclonal antibodies were not stable after pre-incubation in 1% SDS at room temperature (20°). At least 75% of the class II molecules appeared as dissociated alpha and beta polypeptides of MW 32,000–35,000 and 25,000–29,000, respectively, while the intact portion of the class II heterodimer migrated with a MW of about 55,000–60,000. In the presence of SDS at 100°, the alpha and beta subunits were fully dissociated without prior reduction (Fig. 1b), demonstrating that the two subunits were not disulphide linked. There were also differences in the number and electrophoretic mobilities of the alpha and beta polypeptides immunoprecipitated with the different SBU class II monoclonal antibodies (Fig. 1b). The class II molecules recognized by SBU.II 37-68, 38-27 and 42-20 (Fig. 1b, lanes 2, 4 and 6, respectively) displayed two or more beta chain polypeptides associated with alpha chains of varying MW. Interestingly, the heterogeneous alpha and beta polypeptides of these class II molecules appeared to be subsets of the broad alpha and beta polypeptide bands immunoprecipitated using the monoclonal antibody SBU.II 49-1 (Fig. 1b, lane 7).

Following reduction, the apparent molecular weight of the sheep class II beta polypeptides appeared to undergo a shift to a MW of just over 30,000. Consequently, under reduced conditions (Fig. 1c) it was not possible to distinguish between the alpha and beta subunits, as these co-migrated within a broad band of MW about 31,000–33,000.

From biosynthetically labelled splenocytes (Fig. 2a), the SBU class II monoclonal antibodies recognized major non-reduced species of MW 33,000–36,000 (alpha polypeptides), 31,000–32,000, and 26,000–29,000 (beta polypeptides). The 31,000–32,000 polypeptide(s) most probably correspond to the invariant (Ii or gamma) polypeptides as described for man and mouse (Shackelford *et al.*, 1982). Class II molecules recognized by SBU.II 38-27 and 38-30 (indicated by an arrow in Fig. 2a, lanes 4 and 5) were distinguished by a poorly labelled alpha chain of MW 2000–3000 greater than that evident using the

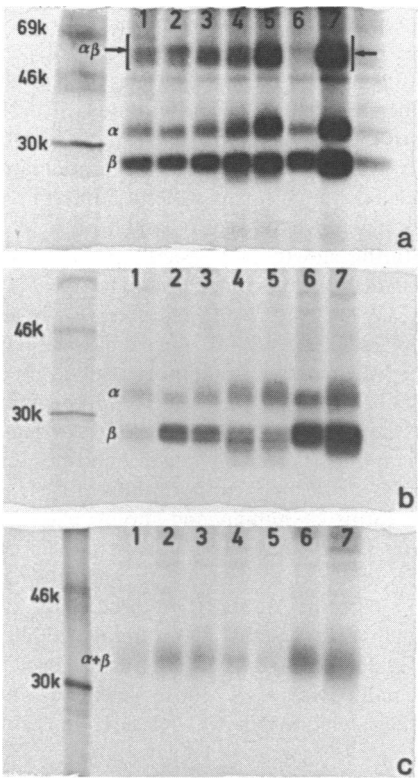

Figure 1. Autoradiograph showing SDS–PAGE analyses of ^{125}I-labelled class II molecules immunoprecipitated from splenocytes. SAM Sepharose-bound immune complexes were in each instance diluted into Laemmli sample buffer containing 1% SDS, and prior to electrophoresis, either: (a) left at 20° for 30 min, (b) boiled for 30 min or (c) boiled for 3 min in the presence of 2·5% 2-ME. Monoclonal antibodies used were: lane (1) SBU.II 28-1, (2) 37-68, (3) 38-64, (4) 38-27, (5) 38-30, (6) 42-20 and in lane (7) 49-1. Samples were run on a 12% polyacrylamide gel. The relative positions of the class II alpha and beta polypeptides, the alpha/beta heterodimer and ^{14}C-labelled molecular weight markers are shown.

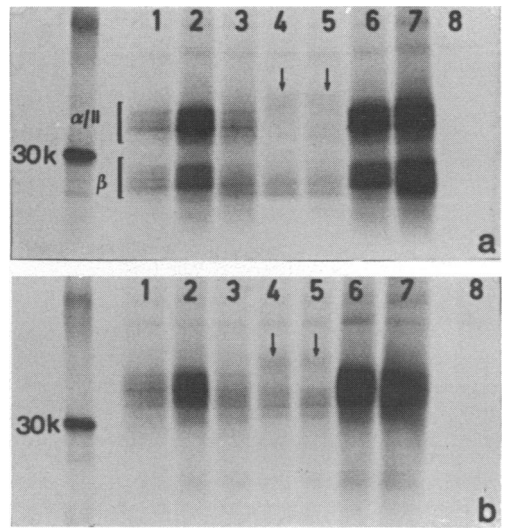

Figure 2. Autoradiograph showing SDS–PAGE analyses of class II molecules immunoprecipitated from ^{35}S-methionine-labelled splenocytes. In part (a) immunoprecipitates boiled in SDS sample buffer and (b) in sample buffer containing 2·5% 2-ME are shown. Monoclonal antibodies used were: lane (1) SBU.II 28-1, (2) 37-68, (3) 38-64, (4) 38-27, (5) 38-30, (6) 42-20, (7) 49-1 and in lane (8) no antibody. The positions of the alpha, putative invariant (Ii) and beta polypeptides are shown. In parts (a) and (b), lanes 4 and 5, the arrows indicate alpha chains of the class II molecule(s) recognized by SBU.II 38-27 and 38-30, respectively.

other SBU class II antibodies. Like ^{125}I-labelled class II molecules, the biosynthetically labelled alpha, beta and the putative Ii/gamma polypeptides also appeared to co-migrate after reduction (Fig. 2b), except for class II molecules recognized by SBU-II 38-27 and 38-30 (Fig. 2b, lanes 4 and 5) where the reduced alpha chains could be distinguished from reduced beta and/or Ii polypeptides.

Dissociation of the sheep class II heterodimer

By mild acid treatment, HLA class II molecules can be irreversibly dissociated in solution into free alpha and beta polypeptides, which are otherwise non-denatured (Cohen *et al.*, 1984). Similar treatment of intact sheep class II molecules resulted in complete dissociation of the sheep class II alpha/beta heterodimers, as identified by the different SBU class II antibodies (Fig. 3a). Such complete dissociation of the alpha/beta heterodimer could otherwise only be accomplished by boiling in SDS (Fig. 1a and b). When radiolabelled lysates were acid-treated prior to being used for immunoprecipitation, only monoclonal antibodies SBU.II 42-20 and 49-1 remained reactive and immunoprecipitated, respectively, unassociated alpha

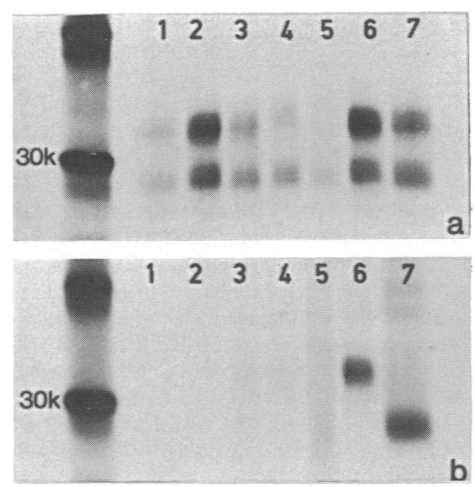

Figure 3. Autoradiograph showing SDS–PAGE analyses of class II molecules immunoprecipitated from ^{125}I surface-labelled efferent lymph cells. For part (a) sheep anti-mouse IgG Sepharose-bound immune complexes were treated prior to electrophoresis with 0·2 M HCl, pH 2·5, for 10 min at 20°, neutralized with 0·2 M NaOH and then incubated at 20° in SDS–sample buffer containing 0·2% SDS. For part (b), class II molecules were immunoprecipitated from lymphocyte lysates which prior to use had been treated with 0·2 M HCl and maintained at pH 2·5 for 10 min, then neutralized with 0·2 M NaOH. Prior to electrophoresis these immunoprecipitates were boiled in standard Laemmli sample buffer without 2-ME. Monoclonal antibodies used in parts (a) and (b) were: lane (1) SBU.II 28-1, (2) 37-68, (3) 38-64, (4) 38-27, (5) 38-30, (6) 42-20, (7) 49-1.

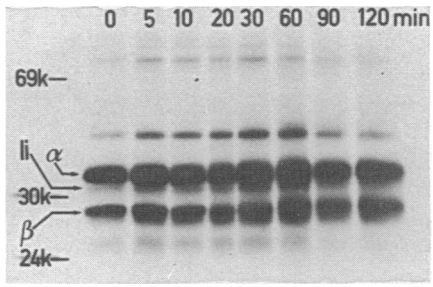

Figure 4. Autoradiograph showing SDS–PAGE analyses of class II molecules from splenocytes pulse labelled for 5 min with ^{35}S-methionine and ^{35}S-cysteine and cold chased for the time periods indicated. In each instance class II molecules were immunoprecipitated using SBU.II 49-1. The relative positions of the alpha, putative invariant (Ii) and beta polypeptides together with standard molecular weight markers are shown.

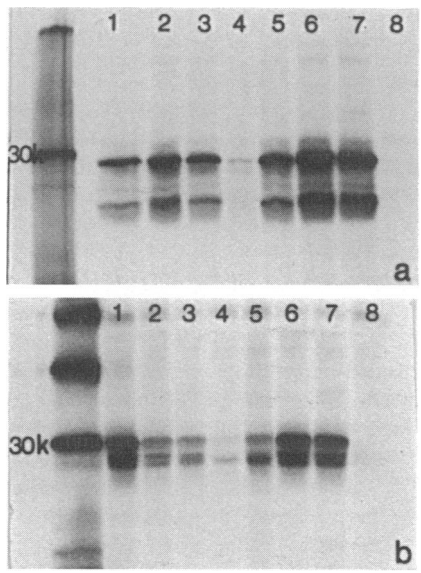

Figure 5. Autoradiograph showing SDS–PAGE analyses of non-glycosylated class II molecules immunoprecipitated from splenocytes labelled with ^{35}S-methionine in the presence of 10 μg/ml tunicamycin. In part (a) non-reduced and in (b) reduced class II molecules were used. Monoclonal antibodies used were lane (1) SBU.II 28-1, (2) 37-68, (3) 38-64, (4) 38-27, (5) 38-30, (6) 42-20, (7) 49-1 and in lane (8) no added antibody. The position of the 30,000 molecular weight marker is indicated.

and unassociated beta polypeptides (Fig. 3b, lanes 6 and 7). The other SBU class II antibodies were non-reactive, and presumably did not recognize their appropriate antigenic epitopes on unassociated alpha and beta polypeptides.

Biosynthesis and maturation of sheep class II molecules

To study the biosynthesis of class II molecules, the monoclonal antibody SBU.II 49-1 was used to immunoprecipitate class II molecules from splenocytes pulse labelled for 5 min and cold chased for different time periods (Fig. 4). After 5 min of pulse labelling, sheep class II molecules appeared to exist as heterodimers, since SBU.II 49-1 recognized beta polypeptides associated with the appropriate alpha species. The same result was seen using the alpha chain-specific antibody, SBU.II 42-20 (not shown). Both the class II alpha and beta polypeptides underwent slow conversion after synthesis to more mature, slightly higher molecular weight forms. After a 5-min pulse, the alpha chain was visualized as a broad band with a MW of about 33,000, maturing slowly to a species of MW 34,000–35,000 following 60–90 min of cold chase. The beta polypeptides, initially of MW 26,000–27,000, matured slowly to a MW of about 28,000–29,000 after 60–90 min of cold chase. For both the alpha and beta chains, significant molecular weight changes did not appear to occur during the period of pulse-chase examined. As in Fig. 2, the 31,000–32,000 polypeptide(s) located below the alpha chain(s) most probably correspond to the invariant glycoprotein(s) associated early in biosynthesis with intracytoplasmic forms of human and mouse class II molecules (Shackelford *et al.*, 1982). These latter 31,000–32,000 MW polypeptides appeared to decrease in intensity after a chase period of 30–60 min. Additional molecules of MW 72,000–74,000, 45,000–47,000 and 27,000 were associated with sheep class II molecules during biosynthesis, as has been observed in mouse and man (Owen *et al.*, 1981; Shackelford *et al.*, 1982). However, their relationship with class II molecules remains unclear.

Inhibition of *N*-linked glycosylation

Non-glycosylated class II molecules were obtained for analysis by biosynthetically labelling splenocytes in the presence of tunicamycin. These class II molecules (Fig. 5a) consisted of alpha and beta subunits of MW about 28,000–29,000 and 25,000–26,000, respectively, corresponding to a decrease in MW of about 6000 for the alpha and 3000 for the beta chain. Moreover, in comparison with the results in Figs 1a and 2a, there appeared to be much less heterogeneity in the relative mobilities of the alpha and beta polypeptides of class II molecules recognized by the SBU class II monoclonal antibodies.

After reduction (Fig. 5b), a shift in the mobility of the beta polypeptides to a MW of about 28,000, and for the alpha polypeptides to a MW of about 30,000 was observed. This presumably reflects cleavage of intra-chain disulphide linkages present in sheep alpha and beta polypeptides. Unlike untreated molecules, non-glycosylated and reduced sheep alpha and beta polypeptides did not co-migrate on SDS–PAGE.

Endoglycosidase sensitivity of sheep class II oligosaccharides during processing

The nature of the *N*-linked glycans acquired by sheep class II molecules during biosynthesis was investigated using Endo H, which cleaves high mannose (non-sialylated) and Endo F, which cleaves both high mannose and complex (sialylated) oligosaccharides. The monoclonal antibodies SBU.II 42-20 and 49-1 were used to immunoprecipitate, respectively, dissociated alpha (Fig. 6a) and beta polypeptides (Fig. 6d) from acid-treated pulse-chase labelling, and the class II alpha (Fig. 6b) and beta polypeptides (Fig. 6e) treated with Endo H. After an initial 5-

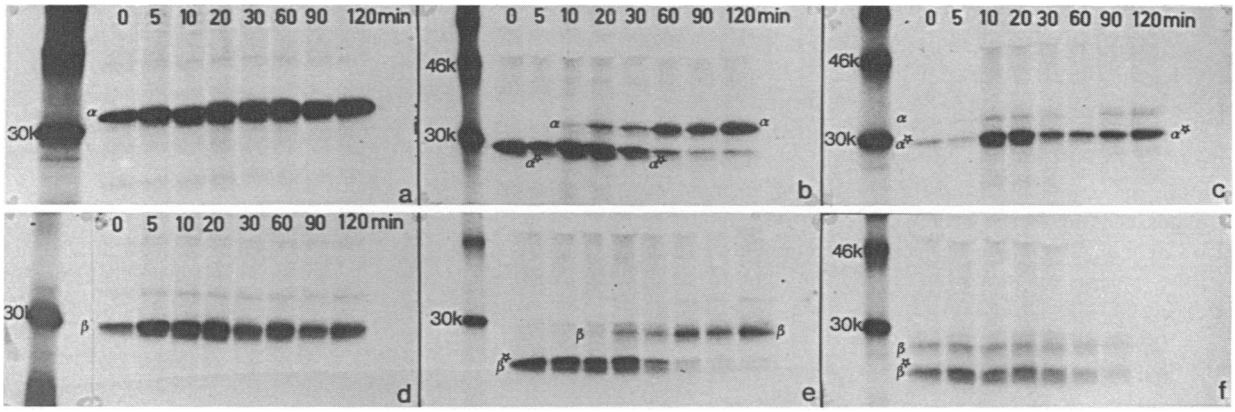

Figure 6. Autoradiographs showing SDS–PAGE analysis of immunoprecipitated, endoglycosidase digested class II alpha and beta polypeptides. Lysates from pulse-chase labellings (as in Fig. 5) were treated with 0·2 M HCl (as in Fig. 4) to dissociate class II molecules into free alpha and beta polypeptides prior to immunoprecipitation analysis. In part (a) undigested alpha polypeptide(s) recognized by SBU.II 42-20, part (b) Endo H-digested alpha polypeptides, (c) Endo F-digested alpha polypeptides, (d) undigested beta polypeptides recognized by SBU.II 49-1, (e) Endo H-digested beta polypeptides, and in part (f) Endo F-digested beta polypeptides are shown. In each instance the endoglycosidase digested alpha or beta species are designated as α^* or β^*. The positions of ^{14}C-labelled molecular weight markers are shown.

min pulse labelling and for up to 90 min following cold chase, both alpha and beta polypeptides were Endo H sensitive, which suggests they were associated with high mannose oligosaccharides. The Endo H-treated alpha chain (α^*) had a MW of about 28,000–29,000, while the beta chain (β^*) had a MW of about 25,000–26,000, corresponding in each instance to the sizes of non-glycosylated alpha and beta polypeptides synthesized in the presence of tunicamycin (Fig. 5a). Based on the relative proportions of the digested and non-digested species, class II alpha polypeptides became insensitive to Endo H after 90–120 min of cold chase (Fig. 6b), but remained sensitive to Endo F digestion (Fig. 6c) throughout the duration of the cold chase. Similarly, beta polypeptides became Endo H insensitive after 90 min of cold chase but were always Endo F sensitive (Fig. 6e and f). Digestion of alpha and beta polypeptides with Endo F resulted in a decrease in MW of about 6000 and 3000, respectively, similar to deglycosylated human class II molecules. However, unlike human class II alpha chains, the oligosaccharides of mature sheep alpha chains did not display any Endo H sensitivity (Shackelford & Strominger, 1983).

DISCUSSION

We have previously shown (Puri *et al.*, 1985) that sheep class II molecules exist as non-covalently associated glycoproteins of MW 32,000–36,000 (alpha chain) and 24,000–29,000 (beta chain). In this study, a more detailed examination of the structure of sheep class II molecules has shown that these molecules display a number of species-specific structural features, but otherwise are similar to human and mouse class II molecules.

The human HLA-DR class II heterodimer is extremely stable (Shackelford *et al.*, 1982). It is not dissociated in the presence of SDS at temperatures of up to 38° and it migrates as a complex having a MW of 55,000 on SDS–PAGE. Only above 80° are the alpha and beta subunits dissociated and the antigenic activitiy of the complex lost. In contrast, the sheep class II heterodimer is relatively unstable and is significantly dissociated after incubation in SDS at room temperature (20°). The significance of such SDS-labile bonding is unknown. With regard to the antigenicity of dissociated sheep class II alpha and beta polypeptides, as opposed to that of the intact alpha/beta heterodimer, only two of the seven SBU class II monoclonal antibodies (SBU.II 42-20, alpha-chain reactive and SBU.II 49-1, beta-chain reactive) retained activity following acid cleavage of class II molecules. This would suggest the majority of sheep class II monoclonal antibodies recognize epitopes dependent on the association of the alpha and beta polypeptides. A similar situation exists for most monoclonal antibodies to human class II molecules (Igarashi *et al.*, 1986). Moreover, monoclonal antibodies specific for human alpha and, to a lesser extent, beta chains are particularly rare, suggesting SBU.II 42-20 and 49-1 will be extremely useful for structural and *in vitro* functional (antibody-blocking) studies involving sheep class II molecules.

The rate of biosynthesis of sheep class II molecules appears similar to, or slightly faster than that in man (Owen *et al.*, 1981; Shackelford & Strominger, 1983). Pulse-chase and endoglycosidase analyses using the two SBU class II monoclonal antibodies reactive with free alpha and free beta polypeptides suggest that rapid association and glycosylation of sheep alpha and beta polypeptides occurs. Rapid association is thought to be necessary for subsequent intracellular transport and/or glycosylation (Owen *et al.*, 1981). The existence of a sheep invariant (Ii/or gamma-like) species, associated with intracellular forms of class II molecules during biosynthesis, is still to be unequivocally demonstrated. However, the 31,000–32,000 MW polypeptide observed to be transiently associated with sheep class II molecules during biosynthesis appears to be a likely candidate.

During post-translational intracellular processing, both human and mouse alpha polypeptides (as determined from tunicamycin and endoglycosidase treatment) acquire two *N*-linked glycans of MW 3000 each, while the beta polypeptide acquires one *N*-linked glycan. In a similar fashion, sheep class II alpha and beta polypeptides were found to have *N*-linked oligosaccharides of MW 6000 and 3000, respectively. In man, one of the alpha chain oligosaccharides is of complex, sialylated (Endo H-insensitive) type, while the other is of Endo H-

sensitive, high mannose type (Shackelford & Strominger, 1983). In contrast, Endo H/Endo F analyses of sheep class II alpha polypeptides showed all *N*-linked oligosaccharides are of complex, presumably sialylated type. As a consequence, the terminally processed cell-surface form of the sheep alpha chain is Endo H insensitive. Consequently, sheep alpha chain oligosaccharides are more similar to those of mouse where most *N*-linked oligosaccharides have been reported to be of complex type (Cowan *et al.*, 1985), while sheep beta chain oligosaccharides are the same as for mouse and man. The significance of altered patterns of glycosylation remains unknown, since no functional role has been assigned to class II oligosaccharides, although their presence has been shown to effect the confirmation of the class II polypeptide backbone (Shackelford & Strominger, 1980; Shackelford *et al.*, 1982).

Sheep class II molecules also differ from those of mouse and man in the behaviour of their reduced beta subunits. Sheep class II alpha and beta polypeptides both contain intra-chain disulphide bonds, as suggested by an alteration in MW following reduction. In mouse and man this alteration in MW is most apparent for the beta chain, which presumably reflects the additional cleavage of its *N*-terminal, disulphide-bonded domain (Kaufman & Strominger, 1983). However, the alteration in MW for the sheep beta polypeptides was more dramatic (apparent increase in MW of 6000–7000 as opposed to 2000–3000 in man) and resulted in the co-migration of reduced alpha and beta polypeptides on SDS–PAGE. Interestingly, using non-glycosylated class II molecules (from tunicamycin-treated cells) sheep beta polypeptides, following reduction, did not co-migrate with alpha polypeptides. In fact, non-glycosylated and reduced sheep and human beta polypeptides both display a MW of about 27,000–28,000 (Shackelford & Strominger, 1980). Consequently, the post-reduction differences in MW of sheep beta polypeptides on SDS–PAGE are only apparent using glycosylated class II molecules. The reasons for this are not clear from the present study.

Considerable variation was found in the electrophoretic mobilities of the alpha and beta polypeptides of class II molecules recognized by different SBU class II monoclonal antibodies. Similar variation has been observed between the human HLA-DR, DQ and DP molecules, and between mouse H-2A and H-2E molecules on SDS–PAGE (Giles & Capra, 1985; Mengle-Gow & McDevitt, 1985). However, in sheep these differences are much less apparent when non-glycosylated class II molecules are analysed, suggesting that at least part of the variation between different class II molecules is due to glycosylation heterogeneity. It is interesting that in man micro-heterogeneity in the oligosaccharides of the alpha and beta polypeptides of HLA-DR and DQ molecules has been directly demonstrated and, moreover, this heterogeneity has been proposed by some investigators to at least partially account for some of the observed functional differences between class II gene products (Iturbe *et al.*, 1986a,b). Whether the SBU class II monoclonal antibodies recognize different sheep MHC class II gene products remains to be established.

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