Curry, M. D., Alaupovic, P., & Suenram, C. A. (1976a) *Clin. Chem. (Winston-Salem, N.C.)* *22*, 315–322.
Curry, M. D., McConathy, W. J., Alaupovic, P., Ledford, J. H., & Popovic, M. (1976b) *Biochim. Biophys. Acta 439*, 413–425.
Davis, B. J. (1964) *Ann. N.Y. Acad. Sci. 121*, 404–427.
Fielding, C. J., Shore, V. G., & Fielding, P. E. (1972) *Biochim. Biophys. Acta 270*, 513–518.
Kostner, G. (1972) *Biochem. J. 130*, 913–917.
Kostner, G., & Alaupovic, P. (1972) *Biochemistry 11*, 3419–3428.
Kostner, G. M., & Holasek, A. (1977) *Biochim. Biophys. Acta 488*, 417–431.
Laurell, C. B. (1965) *Anal. Biochem. 10*, 358–361.
Lee, D. M., & Alaupovic, P. (1974) *Biochem. J. 137*, 155–167.
McConathy, W. J., & Alaupovic, P. (1976) *Biochemistry 15*, 515–520.
Norfeldt, P.-I. P., Olofsson, S.-O., Fager, G., & Bondjers, G. (1981) *Eur. J. Biochem. 118*, 1–8.
Olofsson, S.-O., & Gustafson, A. (1974) *Scand. J. Clin. Lab. Invest. 33*, Suppl. No. 137, 57–62.
Olofsson, S.-O., Gustafson, A., & Svanberg, U. (1975) *Scand. J. Clin. Lab. Invest. 35*, 363–371.
Olofsson, S.-O., McConathy, W. J., & Alaupovic, P. (1978) *Biochemistry 17*, 1032–1036.
Osborne, J. C., Jr., & Brewer, H. B., Jr. (1977) *Adv. Protein Chem. 31*, 253–337.
Privett, O. S., & Blank, M. L. (1962) *J. Am. Oil Chem. Soc. 39*, 520–524.
Suenram, A., McConathy, W. J., & Alaupovic, P. (1979) *Lipids 14*, 505–510.
Weech, P. K. (1981) *Clin. Chem. (Winston-Salem, N.C.) 27*, 357–358.
Weisgraber, K. H., & Mahley, R. W. (1978) *J. Biol. Chem. 253*, 6281–6288.
Wille, L. E. (1978) *Artery (Fulton, Mich.) 4*, 330–347.

Purification and Partial Amino Acid Sequence of Papain-Solubilized Class II Transplantation Antigens†

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ABSTRACT: Papain-solubilized human class II (HLA-DR) antigens have been purified from cadaveric spleens by ion-exchange chromatography, gel chromatography, and immunosorbent purification. The isolated papain-solubilized antigens comprised two subunits with apparent molecular weights of 23 000 and 30 000, respectively. The circular dichroism spectrum for the isolated class II antigens was similar to spectra recorded for HLA-A, -B, and -C antigens, immunoglobulins, and immunoglobulin fragments. Thus, class II antigens contain a considerable amount of β structure. The small subunit (β chain) exhibited extensive charge heterogeneity on two-dimensional isoelectric focusing polyacrylamide gel electrophoresis, whereas the large subunit (α chain) was more homogeneous. The structural heterogeneity of β chains remained after neuraminidase treatment. The NH_2-terminal amino acid sequence of the β chains displayed multiple residues in several positions in accordance with the genetic polymorphism displayed by this chain. The α chain also displayed multiple residues in some positions, suggesting either that some of the genetic polymorphism of the class II antigens may be endowed in this chain or that multiple loci control the expression of several α chains. Papain-solubilized class II antigen subunits were homologous in their amino acid sequences with HLA-DR antigens of defined antigenic specificity as well as with murine I-E/C antigens.

The HLA-D/DR locus of the human major histocompatibility complex (MHC),[1] which was originally defined as the main locus controlling the stimulation in the mixed leucocyte culture reaction, contains genes expressed as polymorphic cell surface glycoproteins (Wernet, 1976). Recently, several groups using monoclonal antibodies have demonstrated that adjacent to the DR locus one or more loci occur that control the expression of cell surface proteins similar in structure to those coded for by the DR locus [see Shackelford et al. (1981) and Accolla et al. (1981)]. Henceforth, this group of antigens, including the DR antigens, will be called class II antigens. The human class II antigens and their murine counterparts, the Ia antigens, have been implicated in a variety of cell recognition phenomena within the immune system, including T-cell–B-cell collaboration (Katz & Benacerraf, 1976), interaction between T suppressor and T helper cells (Miller et al., 1977), and antigen presentation by macrophages (Thomas & Shevach, 1978). These types of antigens consist of two noncovalently associated polypeptides, designated α and β chains, with molecular weights of approximately 35 000 and 29 000, respectively [see Cullen et al. (1976)]. During intracellular transport of newly synthesized α and β chains they are associated with a third invariant chain (Jones et al., 1979; Kvist et al., 1982). Until recently the cell surface expression of the invariant chain was in doubt (Jones et al., 1979; Charron & McDevitt, 1980; Moosic et al., 1980). However, the invariant chain occurs on the cell surface but not in association with α and β chains.[2]

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[1] Abbreviations: MHC, major histocompatibility complex; NaDodSO$_4$, sodium dodecyl sulfate; DEAE, diethylaminoethyl; EDTA, ethylenediaminetetraacetate; DTT, dithiothreitol; CD, circular dichroism; Tris, tris(hydroxymethyl)aminomethane.

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The α and β chains are primarily expressed on the surface of cells belonging to the immune system (Sachs & Cone, 1973; Hämmerling et al., 1975), but in addition, they occur on several types of epithelial cells (Wiman et al., 1978). It is anticipated that structural variability among class II antigens is the basis for the MHC-linked genetic regulation of the immune response [see Uhr et al. (1979)]. Therefore, a thorough knowledge of the structural properties of the class II antigens is a prerequisite for an understanding of mechanisms by which these antigens may mediate cellular recognition events in the immune system.

There are several reports describing the isolation of milligram amounts of HLA-DR antigens, but progress in the characterization of these antigens has been slow (Humphreys et al., 1976; Springer et al., 1977; Snary et al., 1977; Cresswell, 1977; Klareskog et al., 1979). Therefore, we have initiated an attempt to unravel the *common* chemical characteristics of class II antigens. It seemed advantageous to explore the use of pooled class II antigens, comprising a mixture of allelic forms, since our previous structural studies of HLA-A, -B, and -C antigens derived from cadaveric spleens and peripheral blood lymphocytes yielded useful information (Trägårdh et al., 1979b, 1980). The present report outlines the purification of pooled, papain-solubilized class II antigens from cadaveric spleens and demonstrates that the pooled class II antigens are suitable for chemical characterization. At the level of amino acid sequence we provide evidence that most of the structural polymorphism resides in the class II antigen β chain. Furthermore, the isolated class II antigens are homologous in amino acid sequence to the I-E/C antigens of the mouse and display no apparent homology with the murine I-A antigens.

Materials and Methods

Antisera. The xenoantiserum against highly purified, detergent-solubilized HLA-DR antigens has been characterized in detail (Klareskog et al., 1978).

Special Materials. Papain (60 units/mg) was from Kebo AB (Stockholm, Sweden). DEAE-Sephadex G-200, Sepharose 4B, and Pharmalytes were from Pharmacia Fine Chemicals (Uppsala, Sweden). Iodo[^{14}C]acetic acid (54 Ci/mol) was the product of Amersham (Buckinghamshire, England). Neuraminidase (*Vibriae cholerae*; 1 unit/mL) was obtained from Behringwerke AG (Marburg, West Germany). All other chemicals used were of analytical grade or better.

Membrane Preparation. Crude membrane fractions were prepared from cadaveric spleens within 3 days post-mortem. The spleen tissue (750 g wet weight) was repeatedly freeze–thawed and minced in 1000 mL of 0.02 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl and subjected to homogenization in a Virtis homogenizer. Nuclei, unbroken cells, and debris were removed by centrifugation at 5000*g* for 10 min. The supernatant fraction was then subjected to centrifugation at 105000*g* for 60 min. The pellet was resuspended in 500 mL of the Tris-HCl buffer, and the centrifugation procedure was repeated twice. The resulting pellet comprised the crude membrane fraction.

Papain Solubilization of Class II Antigens. The crude membrane fraction was adjusted to 10 mg of total protein/mL in 0.02 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl, 0.05 M cysteine, and 0.05 M EDTA. Papain was added to a final concentration of 3 mg/mL. Digestion was carried out at 37 °C for 60 min. After the digestion had been terminated by the addition of 0.055 M iodoacetic acid (final concentration) and the pH had been adjusted to 8 with NaOH, insoluble material was removed by centrifugation at 105000*g* for 60 min. The supernatant, containing the solubilized class II antigens, was used as the starting material for the isolation of the proteins.

Immunological Techniques. Class II antigens in column fractions were determined with a radioimmunoassay technique, and immunoprecipitations were carried out as described elsewhere (Östberg et al., 1976). A Sepharose 4B immunosorbent column was prepared with antibodies against highly purified detergent-solubilized HLA-DR antigens (Cuatrecasas, 1970). The immunosorbent column was equilibrated with 0.02 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl. After an extensive washing with the equilibrating buffer, protein that had bound to the antibodies was eluted with the equilibrating buffer containing 3 M $MgCl_2$.

Neuraminidase Digestion. Freshly isolated class II antigens (20 μg) were dissolved in 25 μL of 50 mM sodium acetate buffer, pH 5.5, containing 154 mM NaCl and 9 mM $CaCl_2$. A 10-μL aliquot of neuraminidase (1 unit/mL) was added at times 0, 0.5, 1, and 2 h during a 4-h incubation period at 37 °C. The reaction was stopped by dialysis against distilled water at 4 °C.

Electrophoresis and Isoelectric Focusing. Polyacrylamide gel electrophoresis in $NaDodSO_4$ was performed as described by Maizel (1969). Two-dimensional isoelectric focusing and $NaDodSO_4$–polyacrylamide gel electrophoresis was performed as outlined by O'Farrell (1975). In the isoelectric focusing step, 6% polyacrylamide gels and a pH interval of 4–9 were used.

Separation of Class II Antigen Chains. The two class II antigen polypeptide chains were separated by polyacrylamide slab gel electrophoresis in $NaDodSO_4$ on 5 mm thick gels consisting of 15% acrylamide. For avoidance of glycine contamination of samples intended for amino acid analysis and NH_2-terminal sequence determination, 0.2 M Tris–borate buffer, containing 0.1% $NaDodSO_4$, was used as the electrode buffer. Protein zones were detected by staining with dilute Coomassie Brilliant Blue in acetic acid–methanol. Stained protein zones were cut out, and the protein was recovered by electrophoretic elution.

Reduction and Alkylation. The separated class II antigen chains were dissolved in 0.2 M Tris-HCl buffer, pH 8.0, containing 0.5% $NaDodSO_4$ and 10 mM DTT, and heated to 100 °C. After 30 min in the dark at room temperature 25 μCi of iodo[^{14}C]acetic acid/mg of protein was added. After another 15 min, unlabeled iodoacetic acid was added to a final concentration of 23 mM. The reaction was allowed to continue for 30 min. Finally, excess reagents were removed by dialysis, and the samples were lyophilized.

Amino Acid Analysis. The amino acid compositions of the α and β class II antigen chains were determined on a Beckman 121 M automatic amino acid analyzer. Samples containing 35–60 μg of protein, extensively reduced and alkylated, were hydrolyzed in vacuo for 24, 48, and 72 h, respectively, in 6 M hydrochloric acid containing 0.1% phenol. Tryptophan was quantitated after prior hydrolysis in 4 N methanesulfonic acid (Penke et al., 1974).

NH_2-Terminal Sequence Determination. Automatic sequencing was carried out in a Beckman 890 C sequencer by using the Beckman 122974 fast protein Quadrol program with 0.5 M Quadrol. All reagents and solvents for the sequencer were obtained from Beckman Instruments. Prior to application of the sample, 2.5 mg of Polybrene (Aldrich-Europe, Belgium; Tarr et al., 1978) dissolved in 0.1 M NH_3 was added to the sequenator cup. After drying, three complete coupling and

[2] L. Claesson and P. A. Peterson, unpublished results.

degradation cycles were performed to extract possible contaminants from the Polybrene. The reduced and alkylated class II antigen polypeptide chains, each consisting of 35–40 nmol, were dissolved in anhydrous trifluoroacetic acid and applied to the sequenator cup by using the Beckman sample application subroutine 92772. A blank cycle without the cleavage acid was then run to condition the protein film and to extract any remaining extraneous materials such as buffer salts and free amino acids that increase the background accompanying the first cycle.

The anilinothiazolinone derivatives maintained under nitrogen in the refrigerated fraction collector of the sequencer were dried under a stream of nitrogen and converted to the corresponding phenylthiohydantoins in 0.2 mL of 1 N HCl containing 0.1% ethanethiol at 80 °C and subsequently evaporated. The dried phenylthiohydantoins were dissolved in 50 μL of methanol, and aliquots were analyzed on a Waters high-pressure liquid chromatograph in a previously described solvent system (Fohlman et al., 1980). The recovery was calculated for each step.

Analytical Ultracentrifugation. All analytical ultracentrifugations were performed in a MSE Centriscan 60 analytical ultracentrifuge. The sedimentation equilibrium experiments were performed in 4 mM KH_2PO_4 and 15 mM Na_2HPO_4 buffer, containing 134 mM NaCl, by the meniscus depletion technique of Yphantis (1964) and by the modification of the Yphantis technique as described by Chervenka (1970). Calculations of apparent weight average molecular weights were made with the value for the partial specific volume obtained from the amino acid composition (Edsall, 1943).

Determinations of Sedimentation Constants, Diffusion Coefficients, Molecular Weights, and Frictional Ratios. The sedimentation constant was determined by sucrose gradient ultracentrifugation. ^{125}I-Labeled, highly purified papain-solubilized class II antigens were applied to linear sucrose gradients from 5 to 20% in 0.02 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl. The centrifuge was operated at 60 000 rpm for 14 h. Immunoglobulin G, bovine serum albumin, and retinol-binding protein were used as the standards. The calculation of diffusion coefficients, molecular weights, and frictional ratios was carried out as described previously (Karlsson et al., 1972).

Measurements of CD Spectra. CD spectra were measured with a Jasco Model J-41A spectropolarimeter. The proteins studied were dissolved in 4 mM KH_2PO_4 and 15 mM Na_2HPO_2 buffer, containing 134 mM NaCl. The spectra were recorded at room temperature at a protein concentration of 0.5 mg/mL. Cells varying from 10 to 0.25 mm in path length were used. The results are given as reduced mean residue ellipticity or molar ellipticity vs. wavelength. Each experiment represents the average of at least three measurements. The parameter $[\theta]$ was computed in the usual manner (Björk & Tanford, 1971). The mean residue weight for class II antigens, 111, was calculated from the amino acid composition. Calculations of the amounts of α helix and β structure were made according to Greenfield & Fasman (1969) and Chen et al. (1972).

Other Methods. Radioactive labeling with ^{125}I was performed with the chloramine T method (Hunter & Greenwood, 1962). Protein was determined with the Folin–Lowry method (Lowry et al., 1951).

Results

Purification of Papain-Solubilized Class II Antigens. Table I summarizes a typical isolation procedure. The 105000*g* supernatant containing the papain-solubilized membrane proteins was subjected to chromatography on a column of DEAE-Sephadex. As can be seen in Figure 1A, most of the protein was eluted as a major, relatively broad peak. The class II antigens, which emerged slightly later, were pooled and concentrated as indicated in the figure. The class II antigen containing fraction was subjected to gel chromatography on a column of Sephadex G-200. Figure 1B shows that whereas most of the protein was eluted at a K_{av} of about 0.15, the class II antigens were eluted at a K_{av} of approximately 0.48. The class II antigen containing fractions were pooled and concentrated. Further purification of the class II antigens was achieved by affinity chromatography on a column of Sepharose 4B containing antibodies against highly purified, detergent-solubilized HLA-DR antigens. The desorbed material was concentrated after dialysis against the equilibrating buffer and represented highly purified class II antigens.

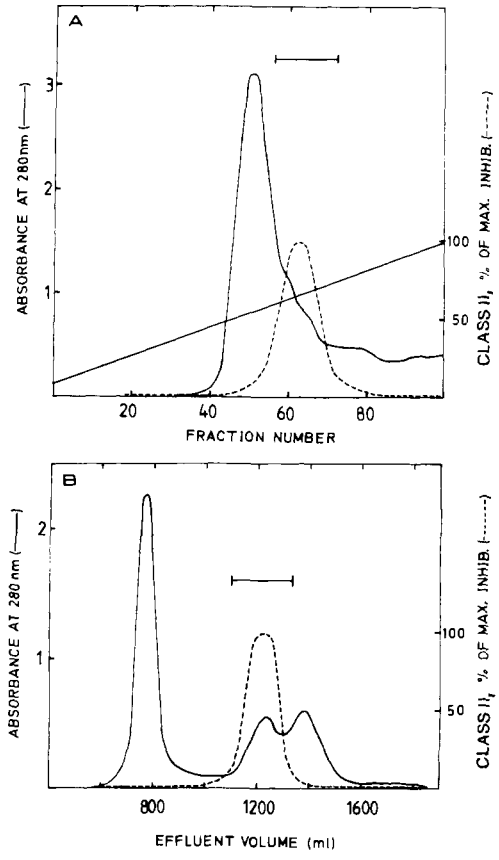

FIGURE 1: Purification of papain-solubilized class II antigens. (A) DEAE-Sephadex chromatography of papain-solubilized spleen cell membrane molecules. The column (20 × 6 cm) was equilibrated with 0.02 M Tris-HCl buffer, pH 8.0, containing 0.05 M NaCl. After application of the sample (containing 9600 mg of total protein and 17.6 mg of class II antigens), which had been dialyzed exhaustively against the equilibrating buffer, elution was performed first with 800 mL of the equilibrating buffer, followed by a 1500-mL linear NaCl gradient from 0.05 to 0.6 M. Fractions of 15 mL were collected at a flow rate of 60 mL/h. (B) Gel chromatography on Sephadex G-200 of the class II antigen containing fraction obtained from the DEAE chromatography step. The column (162 × 4 cm) was equilibrated with 0.02 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl. The sample, containing 550 mg of total protein and 9.6 mg of class II antigens, was eluted at a flow rate of 40 mL/h and fractions of 20 mL were collected. The distribution in the effluent of class II antigens was monitored by a radioimmunoassay procedure. The fractions containing class II antigens were pooled as indicated by the bars.

Purity and Homogeneity of Isolated Class II Antigens. The isolated class II antigens were subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis under reducing conditions. Figure 2, lane A, shows that the isolated papain-solubilized

Table I: Purification of Papain-Solubilized Class II Antigens[a]

fractionation step	total protein (mg)	class II antigens[b] (mg)	yield (%)	purity (%)
papain-solubilized protein	9600[c]	17.6	100	0.18
DEAE-Sephadex chromatography	550[c]	9.6	55	1.75
Sephadex G-200 chromatography	61.7[c]	8.9	51	14.4
immunosorbent chromatography	7.1[d]	7.3	41	103

[a] Crude membrane protein (12 500 mg) was subjected to papain solubilization. [b] Determined with a class II antigen radioimmunoassay. [c] Determined by the Folin–Lowry method. [d] Estimated from the optical density at 280 nm under the assumption that 1 mg/mL class II antigens have an optical density of 2.0 at 280 nm. Amino acid analyses of class II antigens were in agreement with this value.

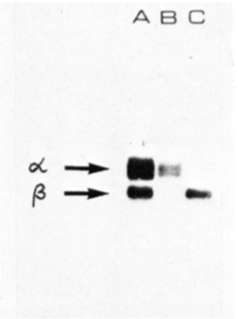

FIGURE 2: $NaDodSO_4$–polyacrylamide gel electrophoresis of highly purified papain-solubilized class II antigens (lane A), isolated α chains (lane B), and isolated β chains (lane C).

class II antigens consisted of two types of polypeptide chains with apparent molecular weights of 23 000 and 30 000, respectively. Henceforth, the subunits will be termed β and α chains, respectively. Sometimes the β chains could be resolved into two bands on $NaDodSO_4$–polyacrylamide gel electrophoresis. In addition, a faint band corresponding to an apparent molecular weight of 27 500 was occasionally seen. The analytical run recorded in Figure 2, lane A, was reproduced in preparative scale. The two protein zones were separately isolated, and lanes B and C demonstrate that the two chains were recovered without apparent cross-contamination. The yields of the subunits ranged from 30 to 50%.

Two-dimensional isoelectric focusing $NaDodSO_4$–polyacrylamide gel electrophoresis revealed extensive charge heterogeneity of the β chains (Figure 3A). In contrast, the α chains gave rise to a single spot, which, however, was large enough to conceal some heterogeneity (Figure 3A). Thus, the α chain spot seemed to contain material that exhibited differences in charge as well as in apparent molecular weights.

Class II antigens were digested with neuraminidase in order to determine to what extent the observed charge heterogeneity was due to differences in content of sialic acid. After dialysis against water and lyophilization, the class II antigens were subjected to two-dimensional isoelectric focusing $NaDodSO_4$–polyacrylamide gel electrophoresis. Figure 3B shows that the neuraminidase-digested material displayed reduced charge heterogeneity both for β and α chains. However, whereas the α chains only appeared as a single spot, most probably containing material of different molecular weights, the β chains gave rise to three major spots with apparently identical molecular weights. Incubation of the class II antigens in the absence of the neuraminidase did not change the two-dimensional pattern from that shown in Figure 3A.

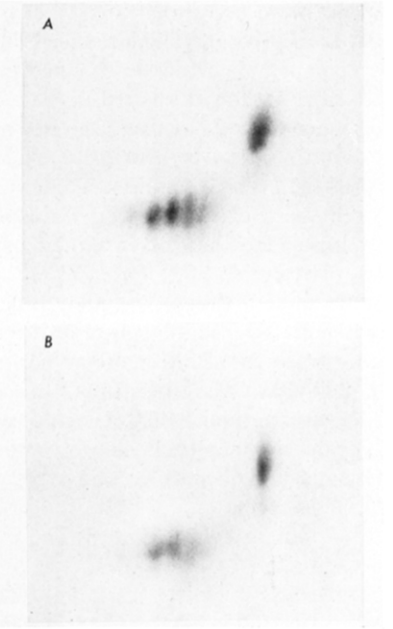

FIGURE 3: Two-dimensional isoelectric focusing $NaDodSO_4$–polyacrylamide gel electrophoresis of highly purified papain-solubilized class II antigens without (A) and with (B) prior digestion with neuraminidase. The acidic end of the isoelectric focusing gel (horizontal) is to the right. The pH gradient ranged from pH 4.5 to pH 7.5.

Table II: Physical–Chemical Characteristics of Papain-Solubilized Class II Antigens

sedimentation constant, $s^0_{20,w}$ (S)	3.85
Stokes molecular radius (Å)	35
diffusion constant X 10^7 (cm^2 s^{-1})[a]	5.9
frictional ratio, f/f_0	1.40
molecular weight	
sedimentation equilibrium[b]	55 800
sedimentation Stokes radius	52 800
electrophoresis[c]	23 000, 30 000
partial specific volume (mL/g)[d]	0.71
ellipticity at 213 nm (deg cm^2 $dmol^{-1}$)	−4950

[a] Estimated by analytical gel chromatography. [b] Average from four determinations at a speed of 10 000 rpm. [c] Data from $NaDodSO_4$–polyacrylamide gel electrophoresis. [d] The partial specific volume for the polypeptide portion of the class II antigens is 0.72.

Immunological Characteristics of Isolated Class II Antigens. Immunodiffusion analyses with antibodies against highly purified, detergent-solubilized HLA-DR antigens (Klareskog et al., 1979) clearly demonstrated complete immunological identity between papain-solubilized, highly purified class II antigens and detergent-solubilized, highly purified HLA-DR antigens (not shown). This information is in keeping with the view that the papain-solubilized class II antigen fragments display the same immunologically recognizable conformation as the intact, detergent-solubilized molecules.

Some Physical–Chemical Properties of Papain-Solubilized Class II Antigens. Some physical–chemical properties of highly purified, papain-solubilized class II antigens are summarized in Table II. The sedimentatioin constant for papain-solubilized class II antigens is 3.85, as measured by sedimentation velocity ultracentrifugation with ^{125}I-labeled class II antigens. The Stokes radius for the papain-solubilized class II antigens was determined to be 35 Å by gel chromatography on calibrated columns of Sephadex G-200.

The frictional ratio (f/f_0) for papain-solubilized class II antigens was calculated from the data given in Table II. The value obtained, 1.40, suggests that the class II antigens have

Table III: Amino Acid Composition of Papain-Solubilized Class II Antigens[a]

amino acid	β chains			α chains		
	residues/ molecule[b]	to nearest integer	residues/ 100 residues	residues/ molecule[b]	to nearest integer	residues/ 100 residues
Lys	6.7	7	3.9	11.8	12	5.6
His	4.8	5	2.8	4.1	4	1.9
Arg	13.1	13	7.3	9.5	10	4.6
Asp	16.3	16	9.0	21.4	21	9.7
Thr[c]	12.4	12	6.7	10.3	10	4.6
Ser[c]	12.1	12	6.7	19.0	19	8.8
Glu	25.1	25	14.0	28.4	28	13.0
Pro	9.0	9	5.1	8.4	8	3.7
Gly	13.6	14	7.9	29.3	29	13.4
Ala	6.9	7	3.9	12.0	12	5.6
CM-Cys[d]	3.9	4	2.2	4.2	4	1.9
Val[e]	16.4	16	9.0	11.7	12	5.6
Met	1.8	2	1.1	2.4	2	0.9
Ile[e]	4.6	5	2.9	7.7	8	3.7
Leu	13.2	13	7.3	18.6	19	8.8
Tyr	7.9	8	4.5	7.3	7	3.2
Phe	8.4	8	4.5	9.8	10	4.6
Trp[f]	1.7	2	1.1	1.4	1	0.5

[a] Except where noted, all figures are average values of 24-, 48- and 72-h hydrolyses. [b] Calculated on the assumption that the polypeptide portions of the class II antigen chains have molecular weights of 20 500 and 24 000, respectively. [c] Values were obtained by extrapolation to zero-time hydrolysis. [d] Determined after extensive reduction and alkylation in 0.5% $NaDodSO_4$. [e] Seventy-two hour hydrolysis value only. [f] Determined after hydrolysis in 4 N methanesulfonic acid.

a rather extended conformation. However, this value is also compatible with a globular structure, particularly in view of the fact that these antigens contain carbohydrate (Springer et al., 1977).

Molecular weights were estimated by sedimentation equilibrium ultracentrifugation at four different concentrations. The values obtained were independent of the protein concentration. That the isolated papain-solubilized class II antigens were homogeneous was suggested by linear relationships between ln C and r^2. It was reassuring to find that the molecular weight estimated by sedimentation equilibrium ultracentrifugation and that calculated from the sedimentation constant and analytical gel chromatography data agreed well (Table II).

The conformation of highly purified, papain-solubilized class II antigens was investigated by circular dichroism measurements. The CD curve for class II antigens has a major negative band at 217 nm with a reduced mean residue ellipticity of about −4950° (Figure 4). The amounts of α helix, β structure, and random coil were calculated, and according to the methods used (Greenfield & Fasman, 1969; Chen et al., 1972) papain-solubilized class II antigens contain approximately 1% α helix, 43% β structure, and 56% random coil. The CD curve recorded in the aromatic region displays two positive bands at 259 and 292 nm and one negtive band at 278 nm (data not shown).

Amino Acid Composition and NH_2-Terminal Sequence Determination of Papain-Solubilized Class II Antigens. Table III presents the amino acid composition of the two papain-solubilized class II antigen subunits. Methionine and tryptophan are the least frequent amino acids in both class II antigen chains. Whereas glutamic acid is the most frequent amino acid in β chains, constituting 14% of the total amino acid content, glycine, comprising 13.4% of the total amino acid content, is the most prominent amino acid in α chains.

Automated amino acid sequence analyses were carried out separately on β and α chains (Figure 5A,B). The NH_2-terminal sequences obtained for the two fragments are given in Figure 6. As the analyses were performed on material comprising a great number of allelic and pseudoallelic forms, it was expected that multiple amino acid residues should occur

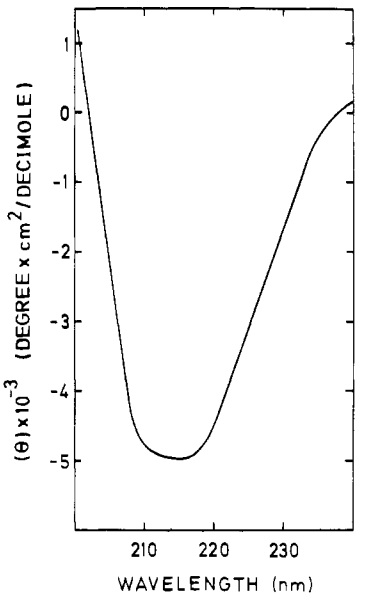

FIGURE 4: Circular dichroism spectrum for papain-solubilized class II antigens.

in several positions. Both types of class II antigen chains exhibited such sequence heterogeneity. However, a quantitatively dominating amino acid residue could easily be identified in all cycles but two. In the β chains more than one residue occurred in positions 1, 5, 6, 7, 11, 13, 16, and 31. More than one residue was also found in positions 4, 7, 8, 9, 13, 19, and 33 of the α chains.

Figure 7 shows a comparison of the sequences obtained with sequences of HLA-DR molecules of defined antigenic specificities (Springer et al., 1977; Allison et al., 1978) and of murine I-A (Uhr et al., 1979; Cecka et al., 1979) and I-E/C (McMillan et al., 1977; Uhr et al., 1979) antigens. The papain-cleaved β chain and intact β chains (p29 and DRw6β) are identical in 6 out of 9 and in 6 out of 11 positions, respectively, available for comparison (Springer et al., 1977; Allison et al., 1978). The corresponding α chains (p34 and DRw6α) are identical with the α chains examined here in 13 out of 14 and in 9 out of 9 positions, respectively. Papain-

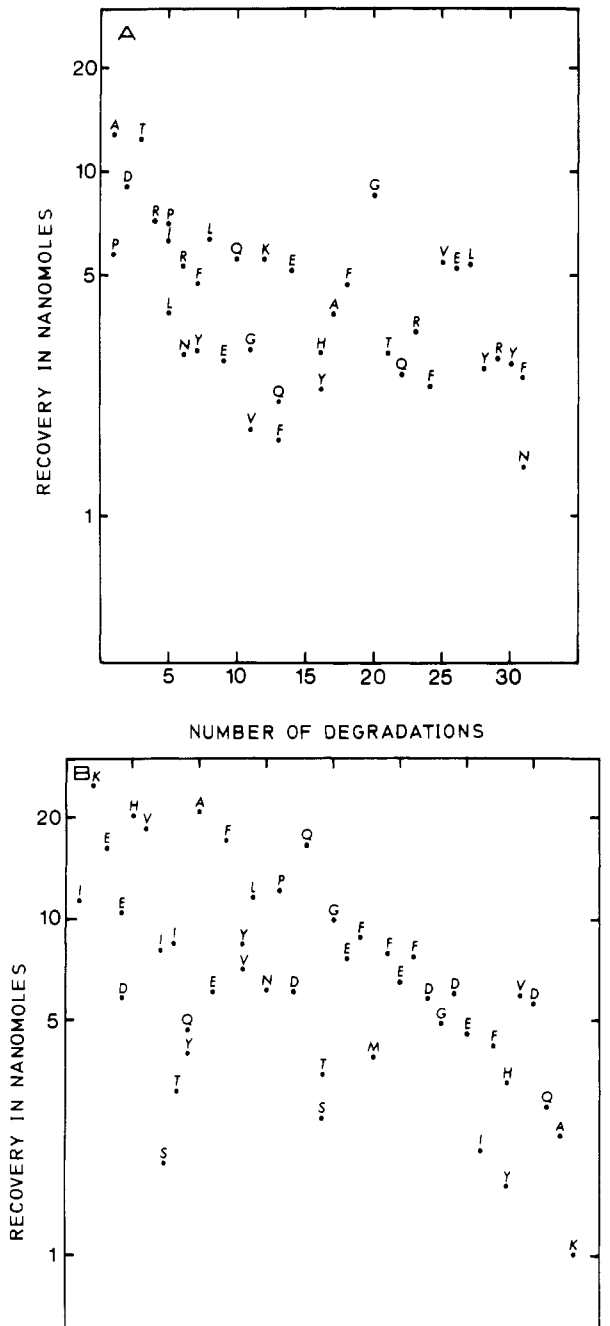

FIGURE 5: Yields of phenylthiohydantoin derivatives for each degradation cycle from the NH_2-terminal sequence of β (A) and α chains (B). In the depicted experiments approximately 40 nmol of each chain was subjected to automated sequence analysis.

solubilized class II antigens and murine I-E/C antigens (McMillan et al., 1977; Uhr et al., 1979) are also homologous (Figure 7). The I-E/C β chain of the k allele and the human β chain are identical in 5 positions among the 12 positions that can be compared. The sequence of the I-E/C^k β chain has an NH_2-terminal extension of one amino acid residue that has no correspondence in human β chains. The I-E/C^k α chain and the human α chains are even more homologous, differing only in 3 out of the 14 positions compared. I-E/C molecules of other alleles (Allison et al., 1978; Cecka et al., 1979) display about the same degree of homology to the class II antigen chains. However, no sequence homology is apparent between murine I-A antigens (Uhr et al., 1979; Cecka et al., 1979) and the class II antigens examined here.

Discussion

The fractionation procedure adopted for papain-solubilized class II antigens is simple, reproducible, and allows the isolation of highly purified class II antigens in a reasonable yield. There are previous reports on the purification of detergent-solubilized (Springer et al., 1977; Snary et al., 1977; Cresswell, 1977; Klareskog et al., 1979) and papain-solubilized (Humphreys et al., 1976) HLA-DR antigens derived from lymphoblastoid cell lines. In these investigations HLA-DR antigens of defined antigenic specificities have been used. The present report seems to be the first one describing the purification of pooled class II antigens, comprising a mixture of allelic and pseudoallelic forms.

In accordance with the results obtained by Humphreys et al. (1976), the papain-solubilized class II antigen consisted of two types of polypeptide chains. The β chains could be resolved into two species differing slightly in mobility upon high-resolution analytical $NaDodSO_4$–polyacrylamide gel electrophoresis. This size heterogeneity may be due to allotypic variations or reflect the presence of several pseudoallelic forms. Alternatively, the β chains may display two adjacent papain-cleavage sites. The latter may also be the case for the α-chain fragments since two molecular species of this chain were apparent after $NaDodSO_4$–polyacrylamide gel electrophoresis. However, the class II antigens appeared homogeneous in the analytical ultracentrifuge and by gel chromatography.

The class II antigen subunits were easily separated by $NaDodSO_4$–polyacrylamide gel electrophoresis. The isolated chains were highly immunogenic, and subunit-specific antisera could be obtained [see Kvist et al. (1982)]. It is interesting to note that such antisera precipitate not only the expected class II antigen chains but also coprecipitate an additional polypeptide chain,[2] which possibly corresponds to the "invariant spot" previously observed by Jones (1979) and others (Charron & McDevitt, 1980; Moosic et al., 1980; Kvist et al., 1982).

The physical–chemical properties of the isolated class II antigens are compatible with the proteins being largely globular. The CD curve for the class II antigens, which has as its main feature a negative band at 217 nm, is similar to CD curves recorded for papain-solubilized HLA-A, -B, and -C antigens (Trägårdh et al., 1979a), immunoglobulins, and various immunoglobulin fragments (Dorrington & Tanford, 1970; Björk et al., 1971). The CD results are consistent with a considerable content of β structure in the class II antigens, but the α-helix content seems to be low.

Previous examinations of class II antigens have demonstrated that β chains display extensive genetic polymorphism (Kaufman et al., 1980; Charron & McDevitt, 1980; Silver & Ferrone, 1980) whereas the α chains are invariant (Kaufman et al., 1980) or, at most, moderately polymorphic (Charron & McDevitt, 1980; Silver & Ferrone, 1980). Two-dimensional isoelectric focusing and electrophoresis confirmed that papain-solubilized β chains were heterogeneous, also after neuraminidase digestion. The α-chain material gave rise to a large spot prior to the neuraminidase treatment. After sialic acid had been removed, this spot was reduced in size in the charge separation direction but remained extended in the size separation direction. It seems reasonable to conclude that α chains of two size classes had been isolated. Whether this reflects identical gene products that have been cleaved at different sites

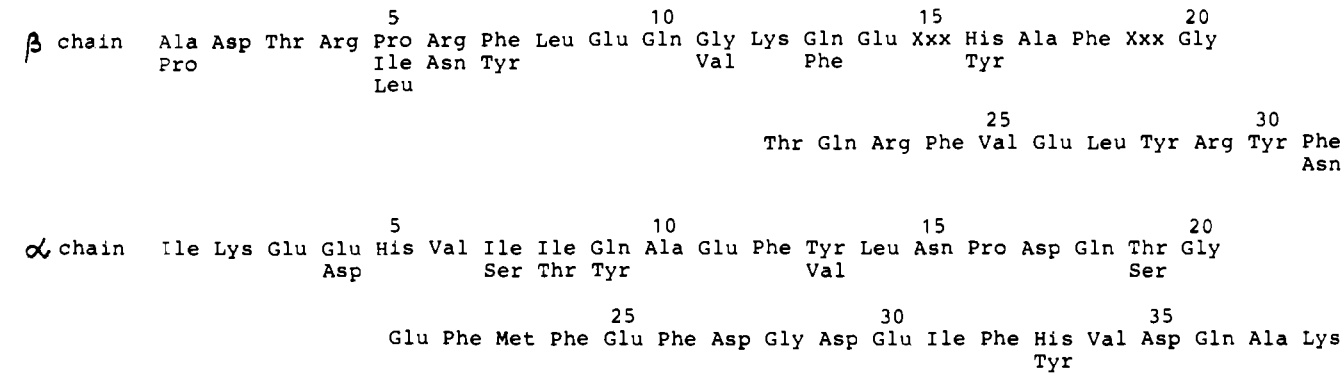

FIGURE 6: NH_2-Terminal amino acid sequence of class II antigen subunits as determined by automated sequence analyses. The sequences represent the amino acid residues obtained in the highest amounts for each degradation cycle. When multiple amino acid residues were found, those present in minor amounts are given below the main sequence.

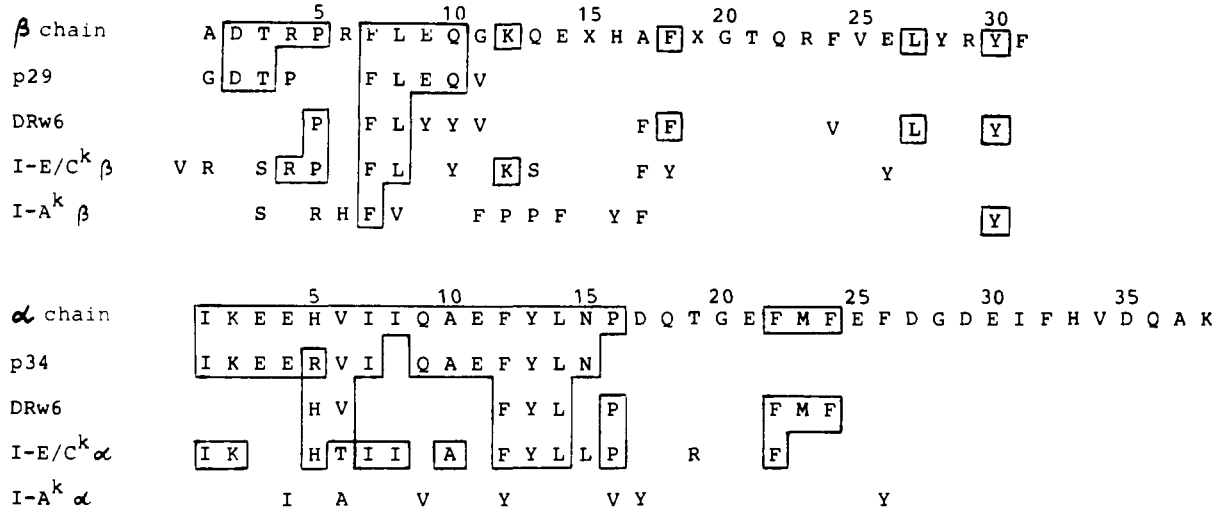

FIGURE 7: Comparisons between NH_2-terminal amino acid sequences of papain-solubilized class II antigen chains, detergent-solubilized HLA-DR antigen chains, and murine I-E/C^k and I-A^k alloantigens. The sequences denoted p29 and p34 are from Springer et al. (1977), the DRw6 sequences from Allison et al. (1978), the I-E/C^k sequences from McMillan et al. (1977) and Uhr et al. (1979), and the I-A^k sequences from Uhr et al. (1979) and Cecka et al. (1979). Identical residues are within boxes.

or different gene products remains to be established.

The NH_2-terminal amino acid sequences of the class II antigen chains displayed a high degree of homogeneity despite the fact that they comprised a mixture of several allelic forms. This is in keeping with data obtained for pooled papain-solubilized HLA-A, -B, and -C antigens (Trägårdh et al., 1980). Likewise, in the NH_2-terminus murine I-A and I-E/C antigens of various haplotypes (Uhr et al., 1979; Cecka et al., 1979) exhibit only minor amino acid sequence variations between different allelic products. In our analyses both β chains and α chains of the class II antigens displayed amino acid sequence variability. Thus, both chains may display allelic polymorphism, although most investigators have not been able to find such polymorphism for the α chain. However, Markert & Cresswell (1980) recently provided evidence for the existence of additional B-cell alloantigens coded for by genes closely linked to the HLA-DR locus. The β and α chains sequenced here may accordingly comprise products of more than one locus. Therefore, the sequence heterogeneity of the class II antigen chains could also be locus dependent, rather than reflecting true allelic polymorphism.

The amino acid sequences of the two class II antigen chains clearly demonstrated that they are the human counterparts to the murine I-E/C antigens. Taken together with published information on other class II antigen chains, which also display I-E/C antigen homology, the sequence analyses reinforce the notion that the β chains display most of the genetic polymorphism. Thus, the β-chain mixture sequenced here only has about 60% homology with previously analyzed β chains (Springer et al., 1977; Allison et al., 1978) whereas the α chain is identical in all positions available for comparison but one with α-chain sequences published elsewhere (Springer et al., 1977; Allison et al., 1978).

Despite the fact that multiple residues were obtained in several positions neither the β nor the α chain displays any obvious homology with murine I-A antigens [see Benacerraf (1981)]. It was surprising that we could not detect human homologues to the murine I-A antigens among the isolated class II antigens, since such antigens seem to exist (Wiman et al., 1982; Larhammar et al., 1981, 1982).

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References

Accolla, R. S., Gross, N., Carrel, S., & Corte, G. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4549–4551.

Allison, J. P., Walker, L. E., Russel, W. A., Pellegrino, M. A., Ferrone, S., Reisfeld, R. A., Frelinger, J. A., & Silver, J. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3953–3956.

Benacerraf, B. (1981) *Science (Washington) 212*, 1229–1238.
Björk, I., & Tanford, C. (1971) *Biochemistry 10*, 1271–1280.
Björk, I., Karlsson, F. A., & Berggård, I. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 1707–1710.
Cecka, J. M., McMillan, M., Murphy, D. B., McDevitt, H. O., & Hood, L. (1979) *Eur. J. Immunol. 9*, 955–963.
Charron, D. J., & McDevitt, H. O. (1980) *J. Exp. Med. 152*, 18s–36s.
Chen, Y.-H., Yang, J. T., & Martinez, H. M. (1972) *Biochemistry 11*, 4120–4131.
Chervenka, C. H. (1970) *Anal. Biochem. 34*, 24–29.
Cresswell, P. (1977) *Eur. J. Immunol. 7*, 636–639.
Cuatrecasas, P. (1970) *J. Biol. Chem. 245*, 3059–3065.
Cullen, S., Freed, J., & Nathenson, S. G. (1976) *Transplant. Rev. 30*, 236–270.
Dorrington, K. J., & Tanford, C. (1970) *Adv. Immunol. 12*, 333–381.
Edsall, J. T. (1943) in *Proteins, Amino Acids and Peptides* (Cohn, E. J., & Edsall, J. T., Eds.) pp 375–377, Reinhold, New York.
Fohlman, J., Rask, L., & Peterson, P. A. (1980) *Anal. Biochem. 106*, 22–26.
Greenfield, N., & Fasman, G. C. (1969) *Biochemistry 8*, 4108–4116.
Hämmerling, G. J., Mauve, G., Goldberg, E., & McDevitt, H. O. (1975) *Immunogenetics (N.Y.) 5*, 428–437.
Humphreys, R. E., McCune, J. M., Chess, L., Herrman, H. C., Malenka, D. J., Mann, D. L., Parkman, P., Schlossman, S. F., & Strominger, J. L. (1976) *J. Exp. Med. 144*, 98–112.
Hunter, W. M., & Greenwood, F. C. (1962) *Nature (London) 194*, 495–496.
Jones, P. P., Murphy, D. B., Hewgill, D., & McDevitt, H. O. (1979) *Mol. Immunol. 16*, 51–60.
Karlsson, F. A., Peterson, P. A., & Berggård, I. (1972) *J. Biol. Chem. 247*, 1065–1073.
Katz, D. H., & Benacerraf, B. (1976) in *The Role of Products of the Histocompatibility Gene Complex in Immune Responsе* (Katz, D. H., & Benacerraf, B., Eds.) pp 355–385, Academic Press, London.
Kaufman, J. F., Andersen, R. L., & Strominger, J. L. (1980) *J. Exp. Med. 152*, 37s–53s.
Klareskog, L., Trägårdh, L., Lindblom, J. B., & Peterson, P. A. (1978) *Scand. J. Immunol. 7*, 199–208.
Klareskog, L., Trägårdh, L., Rask, L., & Peterson, P. A. (1979) *Biochemistry 18*, 1481–1489.
Kvist, S., Wiman, K., Claesson, L., Peterson, P. A., & Dobberstein, B. (1982) *Cell (Cambridge, Mass.) 29*, 61–69.
Larhammar, D., Wiman, K., Schenning, L., Claesson, L., Gustafsson, K., Peterson, P. A., & Rask, L. (1981) *Scand. J. Immunol. 14*, 617–622.
Larhammar, D., Schenning, L., Gustafsson, K., Wiman, K., Claesson, L., Rask, L., & Peterson, P. A. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 3687–3691.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Maizel, J. V. (1969) in *Fundamental Techniques in Virology* (Habel, K., & Salzman, N. P., Eds.) pp 334–362, Academic Press, New York.
Markert, M. L., & Cresswell, P. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 6101–6104.
McMillan, M., Cecka, J. M., Murphy, D. B., McDevitt, H. O., & Hood, L. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5135–5139.
Miller, S. D., Sy, M., & Claman, H. N. (1977) *J. Exp. Med. 145*, 1071–1076.
Moosic, J. P., Nilsson, A., Hämmerling, G. J., & McKean, D. J. (1980) *J. Immunol. 125*, 1463–1469.
O'Farrell, P. H. (1975) *J. Biol. Chem. 250*, 4007–4021.
Östberg, L., Sege, K., Rask, L., & Peterson, P. A. (1976) *Folia Biol. (Prague) 22*, 372–375.
Penke, B., Ferenczi, R., & Kovacz, K. (1974) *Anal. Biochem. 60*, 45–50.
Sachs, D. H., & Cone, J. L. (1973) *J. Exp. Med. 138*, 1289–1304.
Shackelford, D. A., Mann, D. L., van Rood, J. J., Ferrara, G. B., & Strominger, J. L. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4566–4570.
Silver, J., & Ferrone, S. (1980) *Immunogenetics (N.Y.) 10*, 295–298.
Snary, D., Barnstable, C. J., Bodmer, W. F., Goodfellow, P. N., & Crumpton, M. J. (1977) *Scand. J. Immunol. 6*, 439–452.
Springer, T. A., Kaufman, J. F., Terhorst, C., & Strominger, J. L. (1977) *Nature (London) 268*, 213–218.
Tarr, G. E., Beecher, J. F., Bell, M., & McKean, D. J. (1978) *Anal. Biochem. 84*, 622–627.
Thomas, D. W., & Shevach, E. M. (1978) *J. Immunol. 121*, 1152–1156.
Trägårdh, L., Curman, B., Wiman, K., Rask, L., & Peterson, P. A. (1979a) *Biochemistry 18*, 2218–2226.
Trägårdh, L., Rask, L., Wiman, K., Fohlman, J., & Peterson, P. A. (1979b) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5839–5842.
Trägårdh, L., Rask, L., Wiman, K., Fohlman, J., & Peterson, P. A. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1129–1133.
Uhr, J. W., Capra, J. D., Vitetta, E. S., & Cook, R. G. (1979) *Science (Washington, D.C.) 206*, 292–297.
Wernet, P. (1976) *Transplant. Rev. 30*, 271–298.
Wiman, K., Curman, B., Forsum, U., Klareskog, L., Malmnäs-Tjernlund, U., Rask, L., Trägårdh, L., & Peterson, P. A. (1978) *Nature (London) 276*, 711–713.
Wiman, K., Larhammar, D., Claesson, L., Gustafsson, K., Schenning, L., Bill, P., Böhme, J., Denaro, M., Dobberstein, B., Hammerling, U., Kvist, S., Servenius, B., Sundelin, J., Peterson, P. A., & Rask, L. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1703–1707.
Yphantis, D. A. (1964) *Biochemistry 3*, 297–317.