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STUDIES OF THE STRUCTURE OF THE HUMAN Ia-LIKE ANTIGEN[1]

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A two-chain glycoprotein complex from human B lymphoblastoid cell lines has been purified following solubilization by detergent (p29,34). The two chains are of unequal size, tightly associated and both contain carbohydrate. The amino terminal sequences suggest homology to Ia antigens of other species. The tyrosine tryptic peptide maps suggest homology between the small and large subunit.

I. INTRODUCTION

The isolation of the antigens determined by the loci HLA-A and -B from human B lymphoblastoid cell lines led to the unexpected purification of another glycoprotein complex composed of two chains. This complex, when solubilized from the membrane by papain, is composed of polypeptides of apparent molecular weights 23,000 and 30,000 (1); the detergent-solubilized complex (p29,34) is composed of polypeptides of apparent molecular weights 29,000 and 34,000 (2). By various criteria, these antigens are thought to be the human equivalent of Ia antigens in other species. Immunochemical evidence supporting this notion is presented elsewhere in this volume (9).

This report concerns itself with the structural aspects of p29,34. The purification is discussed, and experiments are presented which are consistent with the description of the antigen as a complex of two different polypeptides, each containing carbohydrate, which are tightly and non-covalently associated. Tyrosine tryptic peptide maps, which suggest considerable homology between the two subunits, and amino terminal sequences, which are completely different for p29 and p34, are also presented.

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II. RESULTS AND DISCUSSION

p29 and p34 were initially observed as polypeptides which co-purified with HLA-A,B antigens during detergent solubilization from membranes and subsequent lens culinaris lectin affinity chromatography. They could be separated from HLA-A and HLA-B antigens by several nondenaturing methods, including Agarose A 5M gel filtration in Brij 99:97 detergent or anti-β_2 microglobulin affinity chromatography (2).

p29 and p34 could not be separated from each other by a variety of nondenaturing methods. The following experiments demonstrated that they are tightly and non-covalently associated in a complex (p29,34) which is remarkably resistant to SDS (2). Samples containing these proteins were incubated at various temperatures in the presence of SDS and then analyzed by SDS polyacrylamide gel electrophoresis (Fig. 1). At 100°C

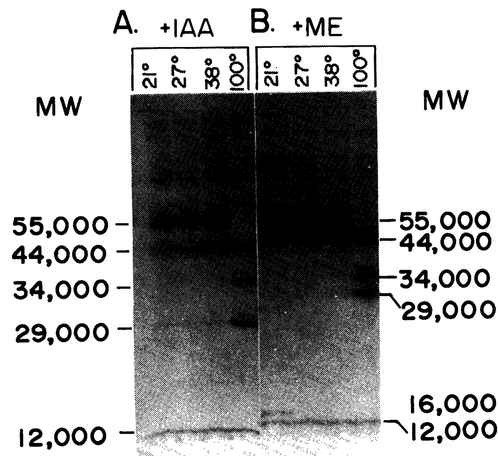

Fig. 1. SDS polyacrylamide gel electrophoresis of aliquots from an Agarose A5M pool containing p29,34 which were treated at various temperatures with SDS and either β-mercaptoethanol or iodoacetamide.

only p29 and p34 were present in the sample, but at lower temperatures a band at the approximate MW 55,000 was present without bands p29 and p34. This 55,000 MW band was isolated, boiled, analyzed and found to contain p29 and p34. The presence of the reducing agent, β-mercaptoethanol (Fig. 1B) or the alkylating agent iodoacetamide (Fig. 1A) in the samples

prior to electrophoresis made no difference in the result. This suggests that the two chains are tightly associated in a complex, but not linked by disulfide bonds.

The properties of p29,34 in isoelectric focusing (IEF) also demonstrate this tight association (3). Two dimensional gel electrophoresis was performed using IEF in a tube gel as the first dimension and standard SDS polyacrylamide slab gel electrophoresis as the second dimension. In Figure 2C IEF was performed in 8 M urea and the resulting tube gel was boiled in sample buffer containing SDS before SDS gel electrophoresis. It is clear that the denatured chains have very different isoelectric points. In the other two figures, IEF was performed in 10% sucrose (and no urea). Whether the gel was boiled in SDS (Fig. 2B) or merely perfused with SDS sample buffer at room temperature (Fig. 2A) after IEF, the SDS gel electrophoresis shows that p29 and p34 focus as a complex under nondenaturing conditions.

p29 and p34 from cell line 4265 were separated for chemical studies by SDS polyacrylamide tube gel electrophoresis. The bands were identified and eluted. SDS and other impurities were removed by Sephadex G50 gel filtration chromatography(2).

Sialic acid content was determined and both chains appeared to be glycoproteins (Table 1), consistent with evidence from SDS polyacrylamide gels analyzed with the carbohydrate stain PAS (2).

TABLE 1
Amino acid and sialic acid determinations of proteins p29 and p34 from cell line 4265 purified by SDS gel electrophoresis.

Amino Acid	*Residues/Molecule*		*Amino Acid*	*Residues/Molecule*	
	p29	*p34*		*p29*	*p34*
CM-Cys	3.1(3)	3.0(3)	Met	3.1(3)	3.6(4)
Asp	16.2(16)	22.3(22)	Ile	6.9(7)	12.2(12)
Thr	13.8(14)	16.6(17)	Leu	17.7(18)	22.9(23)
Ser	14.2(14)	13.2(13)	Tyr	7.8(8)	6.8(7)
Glu	26.0(26)	29.0(29)	Phe	10.9(11)	14.9(15)
Pro	9.9(10)	15.6(16)	His	5.8(6)	6.7(7)
Gly	16.4(16)	17.2(17)	Lys	8.0(8)	11.1(11)
Ala	10.0(10)	13.1(13)	Arg	14.5(14)	13.8(14)
Val	16.8(17)	21.1(21)	Trp	5.2(5)	6.1(6)
sialic acid	2.2	2.6			

The amino acid compositions of p29 and p34 from 4265 cells were determined (2) and are presented in Table 1. There does not appear to be an unusually high representation of either hydrophobic or hydrophilic amino acids, but p29 and p34 appear to be rather similar.

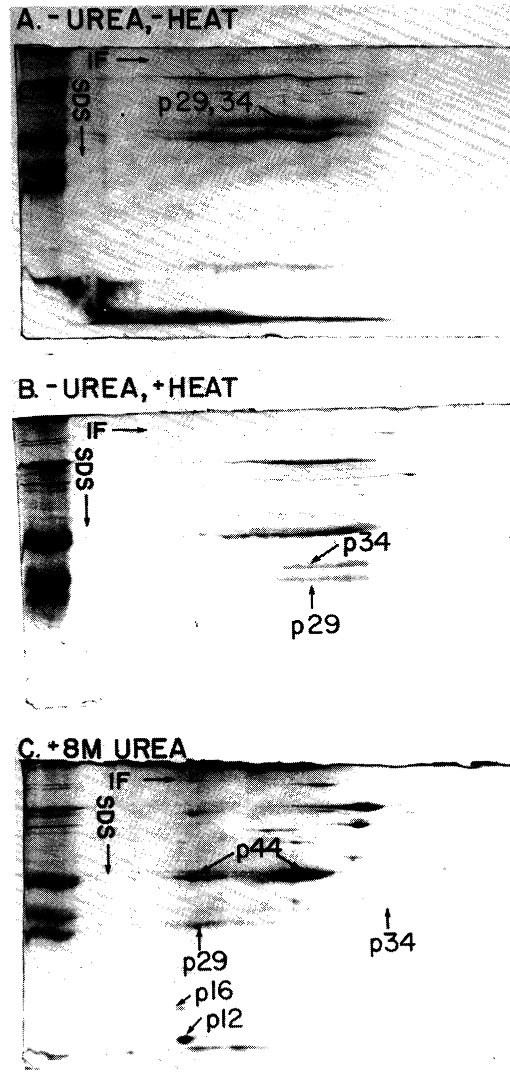

Fig. 2. Two dimensional gel electrophoresis of samples from an Agarose A5M pool containing p29,34. Isoelectric focusing (IEF) was the first dimension and was performed in either 8M urea (C) or 10% sucrose (A and B). SDS polyacrylamide gel electrophoresis was the second dimension and was performed after boiling the IEF tube gel in sample buffer (B and C) or merely perfusing the IEF tube gel with sample buffer (A).

TABLE 2.
Amino terminal sequences of proteins p29 and p34 from cell line JY purified by SDS gel electrophoresis.

	1 5 10
p29	GLY-ASP-THR-PRO-GLU-ARG-PHE-LEU-GLU-GLN-VAL
	1 5 10
p34	ILE-LYS-GLU-GLU-ARG-VAL-ILE-(ILE/LEU)-GLN-ALA-
	11 15 20
	GLU-PHE-TYR-LEU-ASN-TYR-ASP-PHE-GLN-GLY

To further investigate the structural similarities between the two subunits, ^{125}I-tyrosine tryptic peptides were analyzed (2). The proteins were iodinated by chloramine T in the presence of SDS and digested by trypsin. Chromatography on silica gel G in one dimension showed that the pattern for these peptides was very similar for p29 and p34, but was very different from the patterns for p44 (the heavy chain of HLA) and p16 (another purified protein)(Fig. 3). Electrophoresis

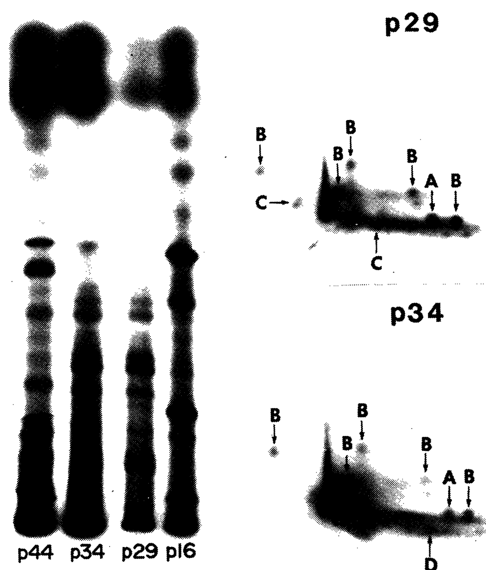

Fig. 3. ^{125}I-tyrosine tryptic peptide maps of proteins purified by SDS gel electrophoresis. Origin is on the bottom for the one dimensional maps and the lower left for the two dimensional maps; see text for additional detail.

in the second dimension confirmed the similarity of the patterns of tyrosine tryptic peptides for p29 and p34 from cell line 4265 (Fig. 3). Peptide mapping thus suggests extensive structural similarities between the two chains.

Amino terminal sequencing was performed on p29 and p34 of both cell lines 4265 and JY using ^{35}S-PITC. The sequence of JY is presented in Table 2, and it appears identical with the published sequence of 4265 (2). This is consistent with immunological data presented in this volume (9). By contrast there is no striking similarity between the sequence of p29 and p34. The sequences of p29 and p34 are not similar to published sequences of HLA (4,5,6), β_2 microglobulin (7) or Ia from the I-A subregion of the mouse (8). However, intriguing possibilities of homology between p34 and the large subunit of murine Ia from subregion I-C or I-E and between p29 and the small subunit of guinea pig Ia are presented elsewhere in this volume. These homologies strengthen further the analogy suggested by comparison of the structural descriptions of p29,34 with those for Ia of other species.

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