THE K-CHAINS OF THE IMMUNOGLOBULIN FROM A CONTINUOUS CULTURE OF HUMAN LYMPHOCYTES (DAUDI) HAVE AN UNUSUAL MOLECULAR SIZE*

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Daudi is a continuously growing lymphoblast cell line derived from a patient with Burkitt’s lymphoma (1). It has been used to study plasma membrane-associated immunoglobulin (M-Ig) (2, 3), complement component receptors (4), β2 microglobulin,1 as well as other problems in cell biology (5). We report here that M-Ig of Daudi has a molecular size of about 330,000 daltons. The most common M-Ig is an IgM monomer molecule (6) with a molecular size on SDS polyacrylamide gel electrophoresis (SDS-PAGE) of about 265,000 daltons (7). Daudi M-Ig has μ-chains slightly larger than normal (approximately 75,000 daltons) and also has large K-light chains (approximately 33,000 daltons). The large K-chain is probably due to a longer than normal polypeptide, rather than extra polysaccharide or lipid.

Materials and Methods

Daudi, kindly provided by Dr. J. W. Uhr (University of Texas Southwestern Medical School, Dallas, Texas), was grown in MEM-Autopow (Flow Laboratories, Inc., Rockville, Md.), supplemented as described for growth of Wil2-A3 cells (7). Cell surface iodination, plasma membrane purification, membrane protein solubilization, and immunoprecipitation were done as previously described (7, 8).

For incorporation of [14C]amino acids, log phase cells were concentrated to 5 X 10⁴/ml in medium composed of 100 ml of MEM and 800 ml of Earle’s balanced salt solution supplemented with 10% fetal bovine serum, 10 μCi/ml of NEC-445 L-U-[14C]amino acids MIX (New England Nuclear, Boston, Mass.) were added to the medium and the cells were incubated in 10% CO₂ for 4 h at 37°C.

For incorporation of tritiated monosaccharides, log phase cells were concentrated to 5 X 10⁴/ml in complete medium. Labeling was conducted in a 10% CO₂ incubator at 37°C for 18 h using 10 μCi/ml of either NET-281n-[6-3H(N)]galactose > 150 mCi/mmol (New England Nuclear) or NET-190 α-glucosamine-6-3H(N)S-15 Ci/mmol (New England Nuclear).

SDS-PAGE and determination of radioactivity was as described (7). 3H, 14C, or radioiodinated marker proteins were included in every gel to insure accurate molecular size determinations.

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1 H. M. Grey, personal communication.
RESULTS

Molecular Size of Radioiodinated Daudi M-Ig.—Radioiodinated whole cells and purified plasma membranes were solubilized, and M-Ig was precipitated by the addition of carrier immunoglobulins and the appropriate antiserum. A major labeled component with a molecular size of 330,000 daltons was resolved in 3% SDS-PAGE (Fig. 1). When precipitates were reduced before electrophoresis, the label was found in two peaks, one of approximately 75,000 daltons and one of about 33,000 daltons (Fig. 2). Experiments using specific antisera confirm reports (1, 5) that Daudi M-Ig is of the \( \mu \)-, K-type. To rule out disulfide linkage or light-chain aggregation in this gel system, extreme conditions of reduction and dissociation were employed. Samples for SDS-PAGE were incubated in 1% SDS, 10 M urea containing 10 mM dithiothreitol, and 10 mM thioglycolic acid for 1 h at 60°C before electrophoresis on gels containing 1% SDS rather than the normal 0.1%. These conditions failed to alter the molecular sizes for Daudi \( \mu \)- and K-chains.

![Figure 1: 3% SDS-PAGE of \( ^{125}\)I Daudi M-Ig.](image)

Fig. 1. 3% SDS-PAGE of \( ^{125}\)I Daudi M-Ig. Upper panel, M-Ig from solubilized purified plasma membranes (○—○). Lower panel, M-Ig from solubilized cells (●—●). Marker proteins are IgM and IgG labeled with \( ^{131}\)I and they contain small amounts of dimerized material (○—○). Plots of \( \log_{10} \) molecular weight versus migration are superimposed on each panel (□—□).
FIG. 2. 6% SDS-PAGE of reduced Daudi M-Ig. Upper panel, M-Ig from solubilized cells (○—○). Lower panel, M-Ig from solubilized purified plasma membranes (■—■). Marker proteins are μ-chains, γ-chains, and light chains labeled with 125I (O—O).

Further Characterization of K-Chains of Daudi M-Ig.—Since Daudi μ-chains were within 10% of the normal molecular size, we concentrated our studies on the nature of the unusual K-chain (about 50% larger than normal).

Analysis of Daudi M-Ig on sucrose gradients confirmed reports (5) that no lipid was present on the molecule after solubilization with NP40. Since the large K-chain could not be explained by dimerization or lipid association, Daudi immunoglobulin (Ig) was tested for the presence of carbohydrate on the light chain. We were unable to isolate enough Ig for chemical characterization and so experiments with radioactive metabolic precursors were necessary.

Cells were allowed to incorporate [3H]glucosamine or [3H]galactose, and Ig was isolated and analyzed from these labeled cells. μ-chains but not K-chains were found to contain glucosamine while both μ- and K-chains were labeled with [3H]galactose (Fig. 3).

Since glycoproteins may exhibit an erroneously large molecular weight in SDS-PAGE (10), Daudi M-Ig samples were treated with neuraminidase or with neuraminidase plus a complex preparation of glycosidases from the culture fluid of the fungus Aspergillus niger (Rhozyme, Rohm and Haas Co., Philadelphia, Pa.). No change in mobility in SDS-PAGE was noted in either sample. Segrest et al. (9) report that molecular weights of glycoproteins can be estimated by conduction SDS-PAGE at different acrylamide concentrations. Daudi K-chain mol wt is between 34,200 to 32,400 ± 2,000 daltons on gels.
Isolation of Ig from Cells Labeled with $^{14}$C Amino Acids.—In order to determine if Daudi Ig was associated with “extra” proteins which were not iodinated by the lactoperoxidase method, cells were labeled with $^{14}$C mixed amino acids. Daudi Ig was resolved into two major peaks of radioactivity on 3% SDS-PAGE (Fig. 4); peak A at 330,000 daltons corresponds to the mobility of iodinated M-Ig, while peak B was smaller than $\gamma$G. Both peaks were extracted from the gels, reduced, and rerun on 5% acrylamide gels (Fig. 4). Peak B contained mostly K-chain material with a small amount of $\mu$-chain. Peak A corresponding to IgM monomer molecules was dissociated by reduction into proteins of 75,000 and 33,000 daltons. This suggests that the IgM monomer molecule is made up of only $\mu$- and K-chains since no other major protein peak was resolved.
Finally, if we assume (a) that these light and heavy chains have the same specific activity, (b) that there are equal numbers of light and heavy chains in the 330,000 dalton molecule, and (c) that the protein mol wt of Daudi μ-chains is about 70,000 daltons, we can calculate the protein molecular weight of the light chains. Integration of μ- and K-peaks gives the relative amounts of amino acids in each species. Thus, the expression cpm (K)/cpm (μ) × 70,000 daltons gives an estimated K-chain protein mol wt of 45,000 daltons. This is somewhat higher than SDS-PAGE estimations but is certainly consistent with the hypothesis that Daudi K-chains are longer polypeptides than normal K-chains.

**DISCUSSION**

Some studies on the Ig and M-Ig of Daudi have shown that they are composed of normal molecular weight μ- and K-chains (2, 3, 5). Other workers were unable to find normal light chains on the M-Ig of these cells (10). We confirm that Daudi M-Ig is composed of μ- and K-chains and that intracellular Ig is similar in molecular size to M-Ig. We also confirm that Daudi K-chains can be labeled with [3H]galactose, but contrary to previous studies (2, 5), we report that Daudi K-chains have an unusual mobility on SDS-PAGE consistent with a molecular size of 33,000 daltons. This finding has now been confirmed in the laboratory of Dr. H. M. Grey.

Several lines of evidence indicate that this large K-chain is a single polypeptide about 1.5 times as long as normal light chains. (a) It cannot be broken down by reduction of disulfide bonds in denaturing solvents, i.e., urea-SDS. (b) It contains little if any lipid. (c) Carbohydrate, if present, does not completely account for its increased size.

The combination of 2 μ-chains and 2 K-chains to give an IgM monomer molecule predicts a mol wt of 216,000 daltons. Molecular size determinations of 330,000 daltons for M-Ig are inconsistent with the predicted molecular weight, and indicate that if M-Ig is an IgM monomer molecule it must have a peculiar conformation to give such a large size on SDS-PAGE. This is in accordance with data for M-Ig of another human cell line, Wil2, in which the predicted monomer weight is 184,000 daltons, while M-Ig has a molecular size of 265,000 daltons (7).

Finally, the biochemical reason for Daudi’s large K-chains is not known. Daudi may contain a mutation such as a partial gene duplication, or the messenger RNA coding for K-chains may be improperly translated. Alternatively, the translation product of this RNA could be a precursor K-chain containing an extra sequence of amino acids (11) and Daudi may be deficient in the enzyme(s) necessary to cleave this precursor to produce a mature K-chain. If so, Daudi would be an excellent source of material for studies of the processing of Ig. The additional protein on Daudi K-chains may be the reason that Daudi secretes no Ig even though it has large amounts of M-Ig (reference 2 and footnote 1). The answers to these questions await primary amino acid sequence analysis of the molecule.
Membrane-associated immunoglobulin (M-Ig) was isolated from Daudi cells which had been radioiodinated using lactoperoxidase. This M-Ig was found to have a molecular size on SDS-PAGE of 330,000 daltons. The component protein chains (μ and K) have molecular sizes of 75,000 and 33,000 daltons, respectively. Further studies showed that [3H]galactose can be incorporated into K-chains, but that extra protein and not carbohydrate was responsible for the increased molecular size of this molecule.

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