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Immunology

λ2 light chains in normal mouse immunoglobulins

(synthetic peptides/peptide maps/inbred mice/myeloma proteins)

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ABSTRACT **Light (L) chains of the λ type are rare in mouse immunoglobulins. One λ chain, the L chain of myeloma protein 315 (L^{315}), differs in amino acid sequence at many positions in the COOH-terminal domain from all other λ chains whose sequences have been determined (called λ1 chains). To determine whether chains of the L^{315} type (called λ2) occur in normal mouse immunoglobulins, we synthesized the COOH-terminal peptides expected in tryptic digests of κ, λ1, and L^{315} and developed procedures to separate the *S*-carboxymethyl derivatives of these peptides. Peptide maps of tryptic digests of [^{14}C]carboxymethyl-labeled L chains from normal serum immunoglobulins showed that about 1% of mouse 7S immunoglobulins have L chains of the L^{315} or λ2 type.**

In many vertebrate species the two types of immunoglobulin (Ig) light (L) chains, κ and λ, are expressed unequally (1). About 95% in the mouse are throught to be κ (2); of several hundred mouse myeloma proteins (3), only 21 with λ chains have been identified (4). The existence of at least two types of L chain was suggested by the L chain made by mouse myeloma tumor MOPC-315 (5): this chain (L^{135}) is λ-like but differs from the other λ chains at 29% of the COOH-terminal 110 positions ("constant" domain; 6,7,8). The more prevalent λ chains were designated λ1, and L^{315} was tentatively designated a λ2 chain (7, 8), implying that there are other L chains with the same constant domain.

To search for λ2 chains in normal mouse Igs we exploited the expectation that a tryptic digest of a mixture of κ, λ1, and λ2 chains would yield the respective COOH-termini as lysine- and arginine-free peptides with the sequences shown in Fig. 1. In the present study these peptides were synthesized (9) and procedures were developed for separating their *S*-carboxymethyl (CM) derivatives. From peptide maps of tryptic digests of [^{14}C]CM-labeled L chains from normal Igs, [^{14}C] peptides corresponding to the COOH-termini of κ, λ1, and L^{315} chains were recovered in amounts suggesting that about 1–2% of mouse Igs have L chains of the L^{315} or λ2 type.

MATERIALS AND METHODS

Preparation of [^{14}C]CM-Labeled L Chains. 7S Igs were isolated from normal mouse sera (yields, 1–3 mg/ml) by starch block electrophoresis and gel filtration on Sephadex G-200 (10). Myeloma proteins (M315 and M460) were isolated from sera of tumor-bearing mice by adsorption at about 23° on Sepharose coupled with ε-Dnp-L-lysine and desorption at 45° (yields, 3 and 1.6 mg/ml, respectively).

The purified Igs were reduced and alkylated as follows. Ten milligrams of Ig in 1.0 ml of 0.10–0.55 M Tris (pH 8.0) was treated first with 2 μmol of dithiothreitol and then with 5.15 μmol of iodo[^{14}C]acetate (New England Nuclear) (11). Residual

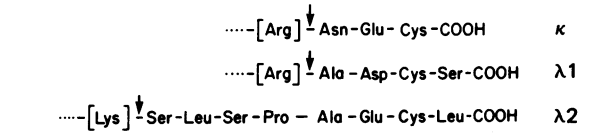

FIG. 1. COOH-terminal peptides expected in tryptic digests of κ, λ1, and λ2 (L^{315}) chains. Arrows mark proteolytic cleavage. Based on published sequences for κ (1), λ1 (4, 6), and L^{315} (7, 8) chains.

iodo[^{14}C]acetate was eliminated by adding 4 μmol of dithiothreitol. To cleave the remaining S-S bonds, we again reduced and alkylated the proteins, this time with unlabeled iodoacetate: 37.6 μmol of dithiothreitol and 540 mg of urea were added and after 120 min (about 20° under N_2) 75 μmol of iodoacetate was introduced. The reaction was stopped 35 min later with 20 μl of 2-mercaptoethanol (286 μmol). The fully reduced and alkylated protein was dialyzed against 0.2 M acetic acid and freeze-dried; it contained about 12% of the iodo[^{14}C]acetate added initially, and it separated completely into H and L chains on electrophoresis in polyacrylamide gel containing sodium dodecyl sulfate. No Coomassie blue-staining impurities were detected.

L chains were isolated by gel filtration of the reduced-alkylated Igs on Sephadex G100 (5). From their ^{14}C content there appeared to be 0.8 CM groups per L chain and 4.2 per H chain, assuming extinction coefficients and molecular weights for L and H chains, respectively, of 1.1 and 22,500 and of 1.5 and 55,000 (12).

Peptide Maps. The isolated [^{14}C]CM-labeled L chains were dialyzed against 0.25 M acetic acid, freeze-dried, dispersed at 10 mg/ml in 0.2 M NH_4HCO_3, pH 8.2, and digested with trypsin (diphenylcarbamyl chloride-treated, Sigma) at 1:50 (wt/wt; for 18 hr at 37°). The digest was freeze-dried and taken up in the first buffer of the peptide map (Fig. 2). Then a volume containing 0.25–1.0 mg of digested L chains was spotted on a 20 × 20-cm Eastman thin-layer cellulose plate (no. 13225). The plate was equilibrated for 1 hr with the lower phase and then developed with the upper phase of 1-butanol/acetic acid/water, 4:1:5 (vol/vol). After the plate was dried it was subjected to electrophoresis in the perpendicular direction (130 min, 800 V) with pyridine/acetic acid/water (25:25:950), pH 4.8, as buffer.

[^{14}C]Peptides [detected by contact radioautographs with Kodak XR-1 film (X-omat R) for 40 hr at −80°] were eluted from the maps with 0.5 ml of 0.3% NH_4OH; after freeze-drying they were subjected to additional electrophoresis on thin-layer cellulose at pH 6.5 with pyridine/acetic acid/water (100:4:896) and at pH 1.9 with acetic acid/formic acid/water (80:20:900).

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Abbreviations: Boc, *t*-butyloxycarbonyl; CM, carboxymethyl; Ig, immunoglobulin; L, light chain; V region, variable region.

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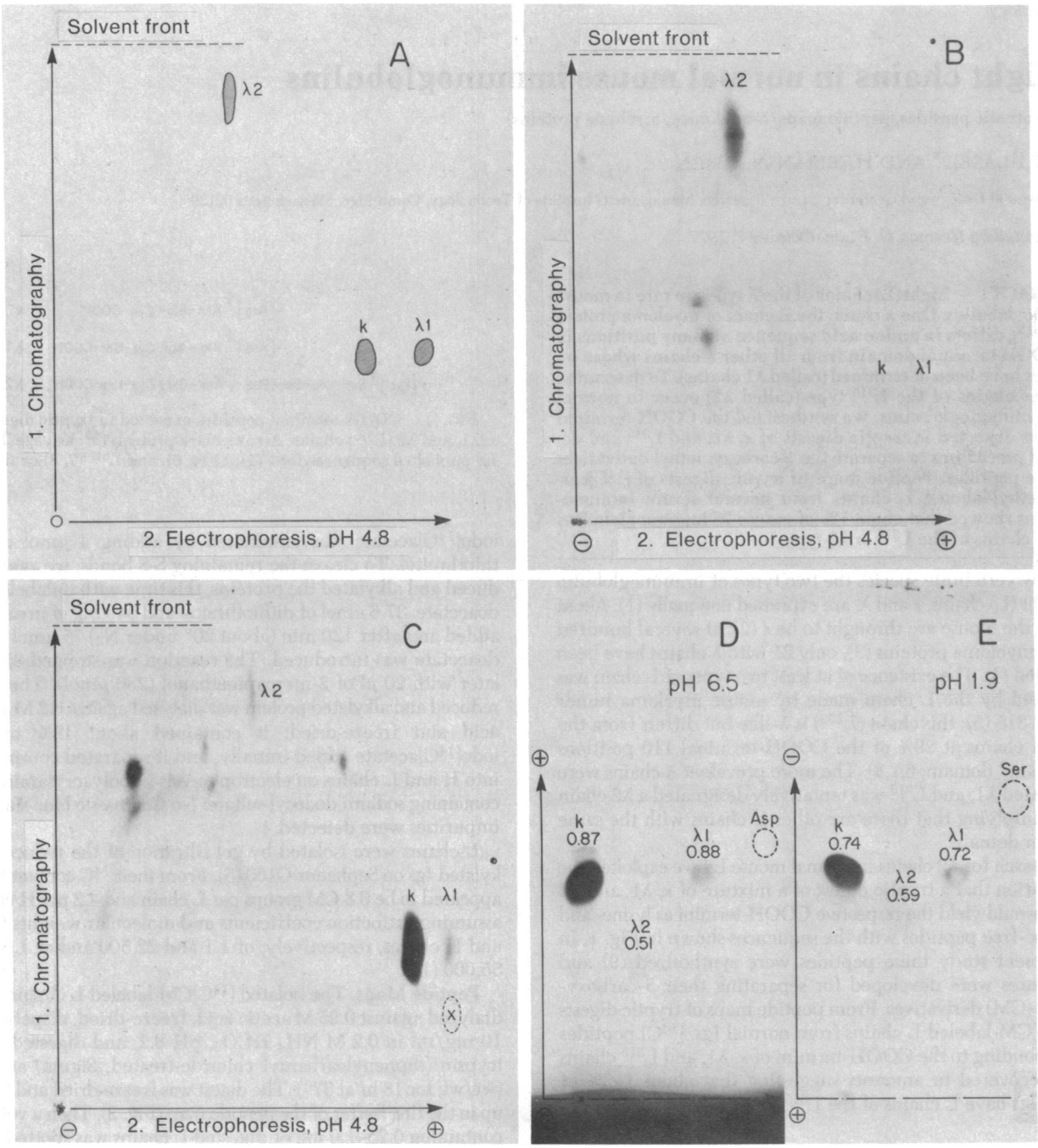

FIG. 2. (*A*) Separation of CM derivatives of the COOH-terminal peptides shown in Fig. 1 and synthesized as in Tables 1–3. Chromatography on thin-layer cellulose sheets was followed, in the perpendicular direction, by electrophoresis at pH 4.8. Peptides were visualized with ninhydrin. (*B*) Radioautograph of peptide map of tryptic digest of [^{14}C]CM-labeled L^{315}, prepared as in *A*. Note the prominent [^{14}C]peptide at the position expected for the CM-λ2 octapeptide. The faint spots at the positions of CM-κ and CM-λ1 peptides probably reflect contamination of purified myeloma protein 315 by a trace of normal Igs. (*C*) Radioautograph of peptide map of tryptic digest of [^{14}C]CM-labeled L chains of 7S Igs from normal serum, prepared as in *A*. X marks a [^{14}C]peptide of variable intensity, probably a sulfone or sulfoxide derivative of the CM-κ-tripeptide (see footnote to Table 4). (*D*) Radioautograph of thin-layer electrophoresis at pH 6.5 of the presumptive κ, λ1, and λ2 [^{14}C]CM-peptides eluted from a peptide map of a digest of [^{14}C]CM-labeled L chains from normal serum 7S Igs. The synthetic CM-peptides (not ^{14}C-labeled) were added to the ^{14}C-labeled eluates before the pH 6.5 electrophoresis. The synthetic peptides, localized with ninhydrin, had the same mobilities as the corresponding [^{14}C]peptides, shown by radioautography. Numbers refer to mobility relative to aspartic acid (Asp). (*E*) Same as *D* except that the presumptive κ, λ1, and λ2 [^{14}C]peptides eluted from the two-dimensional map were subjected to electrophoresis at pH 1.9. As in *D*, the synthetic peptides, localized by ninhydrin, had the same mobilities as the corresponding [^{14}C]peptides, identified by radioautography. Numbers refer to mobility relative to serine (Ser).

For measurement of ^{14}C in eluted peptides, the samples (0.5 ml) were decolorized with 0.1 ml of 1% H_2O_2 (when ninhydrin had been used) and mixed with 10 ml of Aquasol-2 (New England Nuclear), and radioactivity was determined in a Packard Tri-carb Scintillation Spectrometer (model 8330).

Peptide Syntheses. Peptides were synthesized by the two-

Table 1. Synthesis of COOH-terminal tripeptide of mouse κ chains: Yield and some properties of intermediates

Product of step	Reaction conditions: Mole excess of N-hydroxy-succinimidester,	Reaction conditions: Reaction time, hr	Yield of product*: mmol	Yield of product*: %	R_F†	Some properties
1. Boc-Cys(Me_2Bz)-OBz‡	—	48	2.8	87	0.91	Yellow oil
2. TFA·H-Cys(Me_2Bz)-OBz§	—	0.5	—	—	0.80	Yellow oil
3. Boc-Glu(OBz)-Cys(Me_2Bz)-OBz	1.5	2	2.3	99	0.78	Yellow oil
4. TFA·H-Glu(OBz)-Cys(Me_2Bz)-OBz§	—	0.5	—	—	0.63	Slightly yellow oil
5. Boc-Asn-Glu(OBz)-Cys(Me_2Bz)-OBz	1.2	18	2.2	95	0.56	mp 171°–172°¶

Analyses of protected tripeptide (step 5; $C_{40}H_{50}N_4O_9S$; molecular weight 762.93):
Elementary analysis: Anal.: C, 63.05; H, 6.63; N, 7.43; S, 4.30; Calc.: C, 62.97; H, 6.61; N, 7.34; S, 4.20.
Amino acid analysis: Asp 1.02, Glu 0.98, Cys—. NMR spectra: ratio of Boc protons to aromatic protons, 9:13.

Analysis of deprotected, carboxymethylated tripeptide. (*a*) Amino acid analysis: Asp 1.00, Glu 0.99, CM-Cys 1.00. (*b*) Leucineaminopeptidase digestion yielded three ninhydrin-positive spots upon thin-layer chromatography on cellulose in ethanol/concentrated NH_3 (4:1), identified by markers as Asn, Glu, and CM-Cys.

* Based on weight of purified product, dried from organic solvent in the two-phase method (9).

† Retardation factor of product upon thin-layer chromatography on silica gel G. Solvent: $CHCl_3$/methanol (9:1).

‡ Prepared by reacting 1.1 g (3.2 mmol) of Boc-Cys (Me_2Bz)-OH, obtained as a colorless oil, with 0.85 g (5.0 mmol) of α-bromotoluene in 5 ml of dimethylformamide containing 0.71 ml (5.1 mmol) of trimethylamine for 48 hr at room temperature and 2 hr at 60°; purified by extraction as in the two-step synthesizing system (9). Yield, 1.2 g (87%). Me_2Bz, 3,4-dimethylbenzyl; OBz, benzyloxy.

§ Trifluoroacetate salt of the partially deprotected peptide.

¶ Recrystallized from CH_2Cl_2/ethanol/ether (8:2:10).

phase method (9; also K. Blaser and C. H. Schneider, unpublished data). The benzylester of the COOH-terminal amino acid was reacted successively with the *N*-hydroxysuccinimidesters of α-*N*-butyloxycarbonyl (Boc) amino acids with protected side-chains (serine hydroxyl as benzylester, aspartic and glutamic ω-carboxyls as benzylesters, and cysteine SH as 3,4-dimethylbenzylthioether or as *p*-methoxybenzylthioether). Before the next amino acid in sequence was added, the NH_2-terminal Boc was removed from the intermediate peptide with cold trifluoroacetic acid. Elongation reactions were in 450 ml of CH_2Cl_2, and progress at each step was monitored by chromatography on silica gel G-plates. Peptides were identified with I_2 vapor, UV light (254 mm), ninhydrin, or fluorescamine. After a reaction had gone to completion (free NH_2 groups were no longer detectable), excess amino acid *N*-hydroxysuccinimidester was eliminated with 4-picolylamine.

Trivial products and partially unblocked peptides were removed by sequential extractions with 1 liter each of 0.1 M HCl, H_2O, 0.3 M K_2CO_3, and H_2O. Emulsions that formed during extraction were broken up by an alternating current field (electrodes in the upper and lower phases were connected directly to house current; 110 V, 60 cycles). The fully blocked peptides were dried after each step and reacted as above with the next amino acid in sequence.

Completed peptides were freed of all blocking groups either by reaction with HF (13), or in two steps: benzyl groups were first removed with Na in liquid NH_3 (14) and Boc was then removed with cold trifluoroacetic acid. Both procedures gave similar results and yields. Liberated cysteine SH was carboxymethylated with iodoacetate (15). When protecting groups were eliminated in two steps, SH was carboxymethylated just before the NH_2-terminal Boc was removed. The CM-peptides were finally purified by Sephadex chromatography in 0.5 M acetic acid, the κ and λ1 peptides on G15 and λ2 peptide on G25.

Amino acid derivatives were obtained from Bachem AG, Switzerland, cellulose thin-layer plates from Eastman, Rochester, NY, and silica gel thin-layer plates from Merck, Darm-

Table 2. Synthesis of COOH-terminal tetrapeptide of mouse λ1 light chains: Yield and some properties of intermediates

Step	Reaction conditions: Mole excess of N-hydroxy-succinimidester,	Reaction conditions: Reaction time, hr	Yield of product*: mmol	Yield of product*: %	R_F*	Some properties
1. Boc-Cys(MOBz)-Ser(Bz)-OBz	1.1	18	3.3	88	0.81	Colorless oil
2. TFA·H-Cys(MOBz)-Ser(Bz)-OBz*	—	0.5	—	—	0.68	Colorless ppt.
3. Boc-Asp(OBz)-Cys(MOBz)-Ser(Bz)-OBz	1.1	18	2.7	84	0.81	Colorless ppt.
4. TFA·H-Asp(OBz)-Cys(MOBz)-Ser(Bz)-OBz*	—	0.5	—	—	0.54	Colorless ppt.
5. Boc-Ala-Asp(OBz)-Cys(MOBz)-Ser(Bz)-OBz	1.2	18	2.3	87	0.73	mp 127.5°–129.5°†

Analysis of protected tetrapeptide (step 5; $C_{47}H_{56}N_4O_{11}S$; molecular weight 885.04):
Elementary analysis: Anal.: C, 63.87; H, 6.55; N, 6.39; S, 3.70. Calc.: C, 63.78; H, 6.38; N, 6.33, S, 3.62.
Amino acid analysis: Ala 1.00, Asp 0.89, Ser 1.03, Cys—. NMR spectra: ratio of Boc protons to aromatic protons, 9:19.

Analysis of deprotected, carboxymethylated tetrapeptide. Amino acid analysis: Ala 1.06, Asp 1.10, Ser 0.82, CM-Cys 1.00.

* See footnotes *, †, and § of Table 1. MOBz, *p*-methoxybenzyl.

† Recrystallized from CH_2Cl_2/ether/petroleum ether (1:10:50).

Table 3. Synthesis of COOH-terminal octapeptide of mouse λ2 light chains: Yields and some properties of intermediates

Step	Reaction conditions: Mole excess of *N*-hydroxy-succinimidester,	Reaction time, hr	Yield of product*: mmol	%	R_F*	Some properties
1. Boc-Cys(MOBz)-Leu-OBz	1.0	5.5	5.06	99	0.90	Colorless oil
2. TFA·H-Cys(MOBz)-Leu-OBz*	—	0.5	—	—	0.71	Colorless ppt.
3. Boc-Glu(OBz)-Cys(MOBz)-Leu-OBz	1.05	18	4.3	89	0.89	Colorless resin
4. TFA·H-Glu(OBz)-Cys(MOBz)-Leu-OBz*	—	0.5	—	—	0.36	Colorless oil
5. Boc-Ala-Glu(OBz)-Cys(MOBz)-Leu-OBz	1.1	18(4°)	4.05	96	0.80	Colorless oil
6. TFA·H-Ala-Glu(OBz)-Cys(MOBz)-Leu-OBz*	—	0.5	—	—	0.42	Colorless ppt.
7. Boc-Pro-Ala-Glu(OBz)-Cys(MOBz)-Leu-OBz	1.2	18(4°)	3.65	86	0.66	Colorless ppt.
8. TFA·H-Pro-Ala-Glu(OBz)-Cys(MOBz)-Leu-OBz*	—	0.5	—	—	0.34	Colorless ppt.
9. Boc-Ser(Bz)-Pro-Ala-Glu(OBz)-Cys(MOBz)-Leu-OBz	1.2	24	2.98	87	0.77	Colorless oil
10. TFA·H-Ser(Bz)-Pro-Ala-Glu(OBz)-Cys(MOBz)-Leu-OBz*	—	0.5	—	—	0.45	Colorless ppt.
11. Boc-Leu-Ser(Bz)-Pro-Ala-Glu(OBz)-Cys(MOBz)-Leu-OBz	1.2	18	2.70	92	0.92	Colorless oil
12. TFA·H-Leu-Ser(Bz)-Pro-Ala-Glu(OBz)-Cys(MOBz)-Leu-OBz*	—	0.5	—	—	0.40	Colorless ppt.
13. Boc-Ser(Bz)-Leu-Ser(Bz)-Pro-Ala-Glu(OBz)-Cys(MOBz)-Leu-OBz	1.2	18	2.1	94	0.70	mp 97°–99°†

Analysis of protected octapeptide (step 13; $C_{75}H_{98}N_8O_{16}S$, molecular weight 1399.87):
Elementary analysis: Anal.: C, 64.32; H, 7.28; N, 8.16; S, 2.35. Calc.: C, 64.34 H, 7.06; N, 8.01 S, 2.29.
Amino acid analysis: Ser_2 1.91, Pro 1.01, Glu 1.04, Ala 1.01, Leu 2.00, Cys-.

Analysis of deprotected, carboxymethylated octapeptide. Amino acid analysis: Ser_2 1.94, Pro 1.02, Glu 1.04, Ala 1.02, Leu_2 2.04, CM-Cys 0.89.

* See footnotes *, †, and § of Table 1.

† Recrystallized from acetone/petroleum ether (1:1).

stadt, West Germany. Other chemicals were from Sigma Chemical Corp., St. Louis, MO. Normal mouse sera were obtained by tail bleeding 9- to 11-week-old mice of both sexes from various inbred strains.

RESULTS

Yields and properties of the synthetic peptides are summarized in Tables 1–3. Fig. 2*A* shows that the three CM-substituted peptides were well separated by the two-dimensional map. Their mobilities at pH 1.9 and 6.5 agreed with their charge and calculated molecular weights (Fig. 2 *D* and *E* and ref. 16). Maps prepared from [^{14}C]CM-labeled L^{315} (representing λ2) and [^{14}C]CM- labeled L^{460} [from myeloma protein M460 (17) and representing κ chains] showed in both cases that the natural and synthetic CM-peptides were indistinguishable. (See Fig. 2*B* for L^{315} results.)

Tryptic digests of [^{14}C]CM-labeled L chains from 7S Igs of normal serum had many faintly radioactive peptides, suggesting that the [^{14}C]CM groups were not totally confined to the COOH-terminal peptides. Nevertheless, [^{14}C]peptides corresponding to κ, λ1, and λ2 were evident (Fig. 2*C*). Of the three, the predominant one corresponded to the κ peptide and the next most intense to the λ1 Peptide. At the position of the λ2 peptide there was a faint but distinct [^{14}C]peptide. When this was eluted, mixed with the synthetic peptides, and electrophoresed at pH 1.9 and at pH 6.5, it migrated under both conditions precisely with the synthetic L^{315} octapeptide (Fig. 2 *D* and *E*). The κ and λ1 [^{14}C]CM-peptides also migrated at pH 1.9 and at pH 6.5 with the corresponding synthetic peptides (Fig. 2 *D* and *E*). Amino acid analyses of eluates from the peptide maps had high background levels of amino acids; significant analyses could be obtained only for the [^{14}]CM-labeled κ peptide, which was present in large amounts.

Recoveries of the CM-peptides were determined by adding a mixture of the three synthetic peptides (20–50 nmol each) to a digest of [^{14}C]CM-labeled L chains and subjecting the [^{14}C]peptides eluted from the peptide map to electrophoresis at pH 6.5; amino acid analysis of eluates from the second electrophoresis showed that for all three peptides the recoveries were 6–9%. Because recoveries were roughly the same for the three synthetic peptides, radioactivity of the [^{14}]CM-peptides eluted from the second electrophoresis (pH 6.5) was used to estimate the relative proportions of κ, λ1, and λ2 chains in serum Igs from various mouse strains (Table 4).

Table 4. Relative abundance of L chain isotypes in normal mouse 7S immunoglobulins

Mouse strain	% of total L chains*: κ	λ1	λ2
BALB/c AnN	91.4	6.7	1.7
	91.5	6.7	1.7
	91.5	6.7	1.7
DBA/2J	89.9	7.7	2.4
	90.0	7.7	2.3
A/J	93.1	5.8	1.1
SWR/J	91.4	6.4	2.2
	92.7	5.4	1.9

* Calculated as follows: ^{14}C in the individual COOH-terminal peptides eluted from the second electrophoresis (pH 6.5, Fig. 2) was divided by the sum of ^{14}C in all three peptides plus a fourth peptide, called X in Fig. 2*C*. X appeared at low levels (usually 1–3%, but once at 20%); its mobility and composition (Asx_1, Glx_1, CM-Cys 0.2) suggest that it is a sulfone or sulfoxide of the κ peptide. Total ^{14}C in the four peptides was generally 40,000–50,000 cpm per map; λ 2 cpm were usually 20- to 25-fold above background (15–25 cpm). The amounts of 7S Ig recovered per ml of serum differed considerably in different strains. Hence absolute levels could differ substantially in various strains.

DISCUSSION

This study shows that about 1–2% of Igs in normal mouse serum have L chains with the COOH-terminal octapeptide of L^{315} (i.e., λ2 type). Because λ1 chains were also present in all sera tested, λ1 and λ2 are clearly isotypes (i.e., genes for both constant domains are present in all individuals), rather than allelic variants of each other. Only one myeloma protein (M315) has been known until now to have a λ2 chain. However, with an antiserum that is specific for the constant COOH-terminal domain of L^{315} (T. M. D. Cotner and H. N. Eisen, unpublished data) and a radioimmunoassay developed with this antiserum, four additional myeloma proteins with λ2 chains have recently been identified by screening 260 mouse myeloma sera (T. M. D. Cotner, K. Blaser, J. Kriedberg, M. Potter, and H. N. Eisen, unpublished observations). Thus, the frequency of λ2 chains is about the same in normal BALB/c Igs and in BALB/c myeloma proteins, suggesting that transformation of normal to myeloma cells occurs at random in lymphocytes of B lineage.

The variable (V) region sequence of L^{315} differs from the relatively invariant V region sequences of λ1 chains at 11–13 positions (4, 6–8), distributed in "framework" and hypervariable sectors. This suggests that Vλ2 and Vλ1 belong to separate V subgroups and are probably derived from different germline genes (18). This view is strengthened by recent evidence that there are two V genes in mouse embryonic DNA (19) and that one of them corresponds almost exactly to the V segment of L^{315} from the NH_2-terminus to position 98; the only discrepancies are in two framework and three hypervariable region codons (20), and one of the framework differences (Gln for Glu at position 6) probably represents an error in the reported amino acid sequence (7, 8).

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