Cunningham, B. A., Wang, J. L., Berggård, I., & Peterson, P. A. (1973) *Biochemistry 12*, 4811–4822.
Hyafil, F., & Strominger, J. L. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5834–5838.
Isenman, D. E., Painter, R. H., & Dorrington, K. J. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 548–552.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.
Lancet, D., Parham, P., & Strominger, J. L. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3844–3848.
Lopez de Castro, J. A., Orr, H. T., Robb, R. J., Kostyk, T. G., Mann, D. L., & Strominger, J. L. (1979) *Biochemistry 18*, 5704–5711.
Loring, R. H., Jones, S. W., Matthews-Bellinger, J., & Salpeter, M. M. (1982) *J. Biol. Chem. 257*, 1418–1423.
Orr, H. T., Lopez de Castro, J. A., Lancet, D., & Strominger, J. L. (1979) *Biochemistry 18*, 5711–5719.
Parham, P., Albert, B. N., Orr, H. T., & Strominger, J. L. (1977) *J. Biol. Chem. 252*, 7555–7567.
Parker, K. C., & Strominger, J. L. (1982) *Mol. Immunol. 19*, 503–504.
Peterson, P. A., Cunningham, B. A., Berggård, I., & Edelman, G. M. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1697–1701.
Ploegh, H. L., Orr, H. T., & Strominger, J. L. (1981) *Cell (Cambridge, Mass.) 24*, 287–299.
Saul, F. A., Amzel, L. M., & Poljak, R. J. (1978) *J. Biol. Chem. 253*, 585–595.
Seidah, N. G., Dennis, M., Corval, P., Rochemont, J., & Chrêtien, M. (1980) *Anal. Biochem. 109*, 185–191.
Suggs, S. V., Wallace, R. B., Hirose, T., Kawashima, E. H., & Itakura, K. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 6613–6617.
Trägårdh, L., Wiman, K., Rask, L., & Peterson, P. A. (1979a) *Biochemistry 18*, 1322–1328.
Trägårdh, L., Curman, B., Wiman, K., Rask, L., & Peterson, P. A. (1979b) *Biochemistry 18*, 2218–2226.
Yamada, H., Imoto, T., Fujita, K., Okazaki, K., & Motomura, M. (1981) *Biochemistry 20*, 4836–4842.
Yokoyama, K., & Nathenson, S. G. (1983) *J. Immunol.* (in press).

# Amino Acid Sequence of the Light Chain Variable Region from a Mouse Anti-Digoxin Hybridoma Antibody†

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ABSTRACT: A hybridoma cell line (26-10) derived from the A/J strain of mice secretes an immunoglobulin (IgG2a-κ) which binds digoxin with an association constant of 1.2 nM. Such high-affinity antibodies have been utilized in clinical radioimmunoassays as well as in the reversal of toxicity due to excess digoxin. The amino acid sequence of the light chain variable region of this antibody was derived by automated sequencing of the following: the intact chain; a fragment beginning C terminal to the tryptophan residue 40, obtained by cleavage with iodosobenzoic acid; a fragment beginning C terminal to arginine residue 82, obtained by trypsin cleavage on the completely reduced, alkylated, and succinylated chain. Difficulties which had previously prevented the automated Edman sequencing of this chain (and, presumably, similar ones of the same subgroup) were overcome by increasing the duration of the cleavage step at proline residues 8 and 12. The sequences of the first two hypervariable and framework regions of this chain are virtually identical with those of the dinitrophenol- and menadione-binding myeloma light chain MOPC 460 (95% homology). This anti-digoxin hybridoma from the A/J strain makes use of a $V_\kappa$ gene which is similar to that utilized by some BALB/c 2,4-dinitrophenol-binding myelomas.

Mouse hybridoma proteins with anti-digoxin activity are of considerable practical and theoretical interest (Margolies et al., 1981; Mudgett-Hunter et al., 1982a,b). Their practical importance is related to the fact that digoxin, one of a large group of cardiac glycosides, is the drug most frequently prescribed for patients with congestive heart failure. Digoxin intoxication is one of the most prevalent drug reactions encountered in clinical practice, the frequency of digoxin toxicity being related to the narrow margin between therapeutic and toxic doses of the digitalis glycosides. Anti-digoxin antibodies have frequently been used as reagents for radioimmunoassays to monitor levels of circulating digoxin. Fab fragments prepared from sheep anti-digoxin antibodies have been used to rescue victims of otherwise fatal toxicity resulting from digoxin overdose (Smith et al., 1976). In addition to their clinical importance, anti-digoxin antibodies are excellent models for the study of antigen–antibody interactions at the molecular level as digoxin approximates the size of the antigen binding site, and its steroid moiety is conformationally rigid (Figure 1).

We report the complete amino acid sequence of the light chain variable region of an anti-digoxin hybridoma protein with a high binding constant, 1.2 nM (Mudgett-Hunter et al., 1982a,b). The light chain proved to be difficult to sequence because of the presence, at positions 8–9 and 12–13, of prolyl–leucyl and prolyl–valyl bonds, respectively. Some prolyl bonds are known to cleave slowly during Edman degradation (Brandt et al., 1976) and require modification of the cleavage reaction. To our knowledge, the sequence of 26-10 light chain represents the first complete amino acid sequence of the mouse subgroup $V_\kappa 1$ (Potter, 1977) characterized by the $Pro^8$-$Leu^9$ and $Pro^{12}$-$Val^{13}$ residues.

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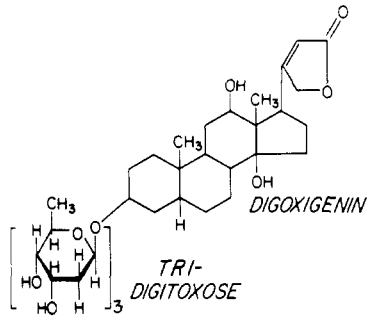


FIGURE 1: Structure of the cardiac glycoside digoxin (digoxigenin tridigitoxoside).

## Materials and Methods

The production of hybridoma cell lines has been reported previously in detail (Mudgett-Hunter et al., 1982a,b). Briefly, spleen cells from A/J mice immunized with digoxin·protein conjugates were fused with cells of the nonsecreting BALB/c myeloma cell line Sp2/0-Ag14. Hybridomas secreting anti-digoxin antibodies, as detected by a solid-phase radioimmunoassay, were cloned by limiting dilution and amplified in the ascites form in pristane-primed BALB/c × A/J mice.

*Antibody Isolation.* Typically, 30 mL of ascites fluid was applied to a 30-mL ouabain–amine-Sepharose column (Zurawski et al., 1978). The column was then washed with phosphate-buffered saline (0.15 M NaCl, 0.01 M sodium phosphate, pH 7.4, and 0.02% $NaN_3$). Bound antibody was eluted either with 1 M acetic acid or with ouabain (Sigma) (0.01 M), extensively dialyzed against 1 M acetic acid, and lyophilized. The yield of antibody was 2–3 mg/mL ascites. Antibody homogeneity was assessed by $NaDodSO_4$[1] gel electrophoresis in 10% acrylamide (Laemmli, 1970), analytical isoelectric focusing (Mudgett-Hunter et al., 1982a), and N-terminal sequence analysis of both the light and heavy chains (see below).

*Preparation of Light Chains.* Purified antibody (15–20 mg) was dissolved in 0.5 M Tris-HCl buffer, pH 8.2 (15 mg/mL), the solution was flushed with nitrogen, and the interchain disulfide bonds were reduced by addition of dithiothreitol to a final concentration of 20 mM under nitrogen. After incubation at 37 °C for 90 min, the solution was chilled to 4 °C, and the sulfhydryl groups were alkylated by the addition of iodoacetic acid to a final concentration of 50 mM and incubated for 15 min. The alkylation was stopped by addition of concentrated acetic acid (10% of the total reaction mixture by volume). The protein was immediately freed from reagents by desalting on a column of Sephadex G-25 (2.5 × 10 cm, total volume 30 mL) and lyophilized.

For separation of light and heavy chains, 30 mg of partially reduced and alkylated antibody was dissolved in 0.1 M sodium acetate and 5.5 M guanidine hydrochloride (Gdn·HCl), pH 5.5, and applied to a column of Sephadex G-100 or AcA44 (LKB) (total volume 500 mL). The column was eluted with the acetate–guanidine buffer, and the light and heavy chain containing fractions were pooled, desalted on a column of Sephadex G-25, and lyophilized.

*Complete Reduction and Alkylation.* Complete reduction and alkylation were performed as described above for the preparation of light chains except that the reaction buffer also contained 7 M Gdn·HCl.

*Succinylation of the Light Chain and Trypsin Cleavage.* Completely reduced and alkylated light chains (15–20 mg) were dissolved in 2 mL of 0.2 M sodium borate, pH 9.0, containing 7 M Gdn·HCl. An equal weight of solid succinic anhydride (Sigma) was added. The pH was maintained in the range 8.0–9.0 by dropwise addition of 2 M NaOH. After 15–20 min, the modified protein was desalted into 0.2% ammonium bicarbonate on a column of Sephadex G-25 (total volume 30 mL) and either treated directly with TPCK–trypsin (Worthington) or lyophilized. For digestion, an enzyme:substrate ratio of 1:100 (w/w) was used; the reaction proceeded for 4 h at 37 °C. Fully reduced, alkylated, succinylated, and trypsin-digested light chains were subjected to gel filtration on an AcA202 column (LKB) (2.0 × 88 cm, total volume 276 mL) in the acetate–guanidine buffer, and the resulting peptide fractions were desalted on Sephadex G-25 in 0.2% ammonium bicarbonate and lyophilized.

*Cleavage at Tryptophan Residues.* Cleavage at tryptophan residues with *o*-iodosobenzoic acid was performed according to Mahoney & Hermodson (1979) and Mahoney et al. (1981). Twenty milligrams (76 μmol) of *o*-iodosobenzoic acid (Pierce) was dissolved in 3 mL of 80% acetic acid and 4 M Gdn·HCl. *p*-Cresol (0.4 mg) was added, and the solution was left at room temperature in the dark for 2.5 h. The mixture was then transferred into a vial containing 500 nmol (10 mg) of light chains, and the reaction proceeded in the dark for 24 h. The reaction mixture was then applied directly on a Sephadex G-75 column (1 × 95 cm, total volume 75 mL) equilibrated with 0.1 M sodium acetate and 5.5 M guanidine hydrochloride. Absorbance of the effluent at 280 nm was measured, and selected fractions were desalted into 1 M acetic acid on the Sephadex G-25 column and lyophilized.

*Ion-Exchange Chromatography.* A DEAE-Sephadex column (1.2 × 15 cm, total volume 17 mL) was equilibrated with 0.1 M Tris-HCl, pH 7.5. Twenty milligrams of a mixture of succinylated tryptic peptides obtained following gel filtration on AcA202 was dissolved in 5 mL of this starting buffer and applied to the column, followed by a linear gradient of NaCl (0–1.0 M NaCl, total volume 500 mL). Fractions (1.5 mL) were monitored by absorbance at 280 nm, desalted in 0.2% ammonium bicarbonate on Sephadex G-25, and lyophilized.

*Automated Edman Degradation.* A Beckman Model 890C sequencer equipped with a Beckman cold trap and a Sequemat Model SC-150 sequential controller (Sequemat, Watertown, MA) was used. The light chain and all peptides were subjected to at least two independent sequencer runs. A 0.1 M Quadrol program (Brauer et al., 1975) which employs a single acid cleavage (3 min) was used. In degradations on the intact light chain, the program was altered at cycles 8 and 12 to include three successive acid cleavages (9 min). Polybrene (Tarr et al., 1978; Klapper et al., 1978) (1–3 mg) was added to the sequencer cup prior to the degradation of peptides. The anilinothiazolinone amino acids were converted automatically to phenylthiohydantoin (Pth) derivatives by using methanol–HCl in a Sequemat Model P-6 autoconvertor (Margolies et al., 1982). The Pth-amino acids as well as the Pth-methyl esters of glutamic acid, aspartic acid, *S*-(carboxymethyl)-cysteine, and ε-succinyllysine were identified by high-pressure liquid chromatography (HPLC) on Zorbax ODS columns (Du Pont) as described previously (Margolies & Brauer, 1978; Margolies et al., 1982). Secondary identification methods included gas–liquid chromatography on 10% SP-400 columns and back-hydrolysis with hydriodic acid (6 h at 150 °C) with subsequent amino acid identification on a Dionex D-500 analyzer (Smithies et al., 1971). At certain cycles *o*-phthal-

[1] Abbreviations: HPLC, high-pressure liquid chromatography; Pth, phenylthiohydantoin; TPCK, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone; DEAE, diethylaminoethyl; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; Gdn, guanidine.

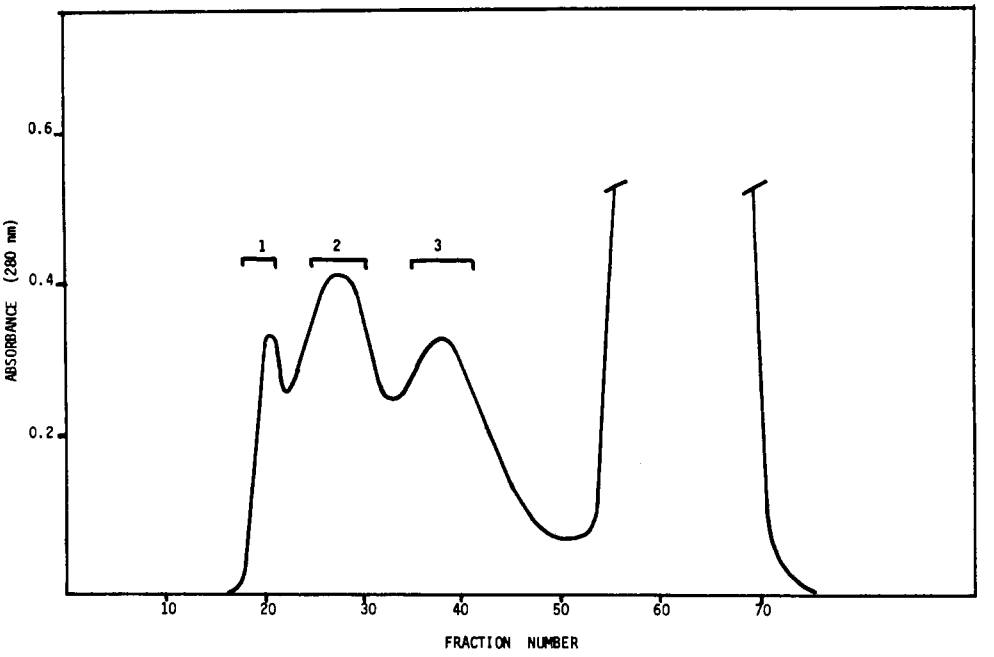


FIGURE 2: Fractionation of iodosobenzoic acid digest of reduced, alkylated, and succinylated 26-10 light chains. The reaction mixture of 10 mg of modified chains and 20 mg of iodosobenzoic acid was applied on a column of Sephadex G-75 (total volume 100 mL) equilibrated with guanidine–acetate buffer. Fractions of 1.5 mL were collected. Peak 2 was pooled, desalted, and lyophilized as indicated.

aldehyde was delivered to the cup in butyl chloride from a separate reagent bottle (R4) prior to coupling, in order to block primary amines, based on a method described by Bhown et al. (1981).

## Results

Preliminary automated degradations of the intact 26-10 light chain invariably resulted in only a partial cleavage of proline residues 8 and 12 (Figure 4) as manifested by a sudden increase in overlap (as much as 30% per cycle) resulting in premature termination of the degradation. Use of a degradation protocol with longer acid cleavage (9 min) at cycles 8 and 12 reduced the overlap to less than 3% at these two cycles and permitted determination of the sequence of the first 43 N-terminal residues (Figure 4), using 30 nmol of light chain (repetitive yield 94%).

Inasmuch as the residue at cycle 40 was found to be tryptophan and the mouse $\kappa$ constant region is known to contain a single tryptophan residue at position 148 (Kabat et al., 1979), specific cleavage at tryptophanyl bonds was attempted to obtain a large overlap fragment. Iodosobenzoic acid cleavage of completely reduced and alkylated chains produced three major fractions of different length as judged by gel filtration on Sephadex G-75 (Figure 2). The native (i.e., unreduced) chain was not cleaved under identical conditions. Each of the three pools was freed from salt, and aliquots were subjected to automated Edman degradation. A total of three sequences were detected in various combinations in these pools (see Figure 4): (1) the N-terminal sequence of the intact light chain (Asp-Val-Val-...), (2) the sequence beginning at position 41 (Tyr-Leu-Glu-...), and (3) the sequence beginning at constant region position 149 (Lys-Ile-Asp-...). Fragments due to cleavage at tyrosine residues were not observed, nor were sequences found which could be ascribed to cleavage at additional variable region tryptophan residues. The sequence results were thus consistent with each of the Sephadex G-75 fractions containing a mixture of peptides arising by a specific, partial cleavage at tryptophan residues.

Further experiments were limited to pool 2, which contained the N-terminal sequence of the light chain as well as a sequence consistent with that expected beginning C terminal to the tryptophan residue 40 in a ratio of approximately 1:1. The iodosobenzoic acid cleavage was repeated on succinylated light chains, the N-terminal amino group of which had been blocked by the reaction with succinic anhydride. Upon automated degradation, pool 2 now yielded a single amino acid sequence starting at tyrosine residue 41 (Figure 4, peptide W-1). The fragment (4 nmol) was degraded for 45 cycles (i.e., to residue 84 of the light chain) with average repetitive yield 96%, thus providing overlap information beyond arginine residue 82 (Figure 4). However, serine residues 72 and 81 were not positively identified in this degradation (Figure 4). The assignment of succinyllysine residues found on HPLC was confirmed by back-hydrolysis and amino acid analysis of liberated lysines. In some experiments, Pth-tyrosines expected in degradations of iodosobenzoic acid cleaved peptides showed on HPLC chromatography as two stable, distinct derivatives [presumably 3-chloro- and 3,5-dichlorotyrosine (cf. Fontana et al., 1981)] eluting at −3.8 min (i.e., between Pth-histidine and Pth-alanine) and −8.3 min (between Pth-dehydrothreonine and Pth-methionine), respectively, relative to the Pth-norleucine standard (Margolies et al., 1982). In other instances, unmodified Pth-tyrosine derivatives were encountered. As the degradation of peptide W-1 (Figure 4) indicated the presence of arginine at position 82, cleavage at arginyl peptide bonds was carried out. Digestion of fully reduced, alkylated, and succinylated chains with trypsin yielded fragments which, after being size fractionated by gel filtration on an AcA202 column (Figure 3A), were resolved by ion-exchange chromatography on DEAE-Sephadex (Figure 3B,C) and screened by automated Edman degradation. The fraction pool R-1 (Figure 3B) yielded an amino acid sequence consistent with that expected C terminal to arginine-59 (i.e., Phe-Ser-Gly-Val-Pro-Asp-Arg-Phe-...) but, in addition, contained smaller amounts of amino acid sequences consistent with peptides beginning C terminal to arginine-82 (Val-Glu-Ala-Glu-Asp-Leu-...) and a constant region peptide beginning C terminal to arginine-113 (Ala-Asp-Ala-Ala-Pro-...). The ratio of the prevalent sequence Phe-Ser-Gly to the major contaminating sequence Val-Glu-Ala was, in nanomoles, 8.0:3.0 as determined by

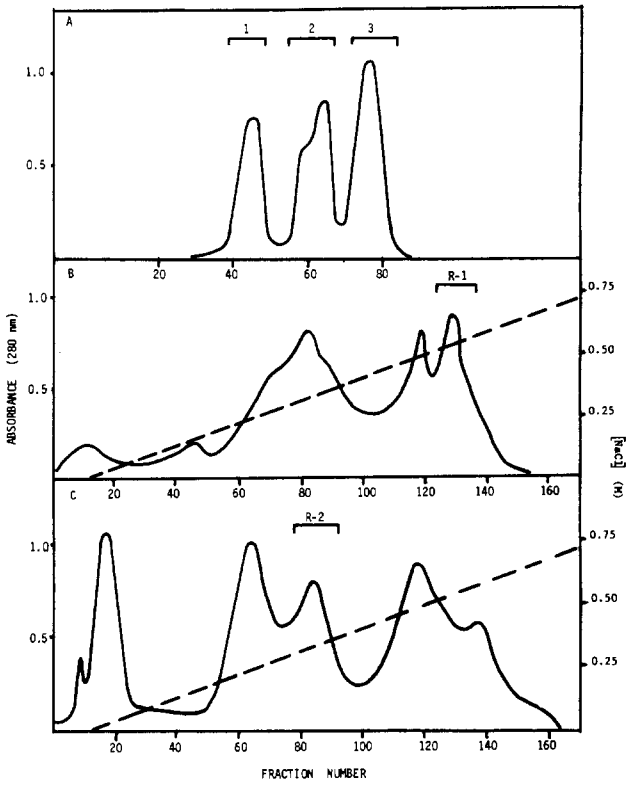


FIGURE 3: Fractionation of tryptic digest of reduced, alkylated, and succinylated light chains. (A) Gel filtration on AcA202 column (total volume 275 mL) in guanidine–acetate buffer. Fractions of 2.5 mL were collected and pooled as indicated. (B) Ion-exchange chromatography of pool 1 (see panel A) on DEAE-Sephadex A-25 column (total volume 17 mL) in 0.1 M Tris-HCl buffer, pH 7.4. A linear gradient of sodium chloride (0.0–1.0 M, 500 mL total volume) was used. (C) Ion-exchange chromatography of pool 2 (see panel A) under the conditions described in (B).

HPLC. For confirmation of the sequence of residues 61–83 and, in particular, identification of residues 72 and 81, the pool R-1 was subjected to a repeat of Edman degradation employing a treatment with *o*-phthalaldehyde at cycle 5. The peptide of interest includes proline at cycle 5 (position 64 in Figure 4), and as the treatment with *o*-phthalaldehyde blocks all other peptides which have N-terminal primary amines exposed in cycle 5, this tactic (Bhown et al., 1981) resulted in substantial decrease of the contaminating sequences (to less than 10%) and allowed an unambiguous identification of serine residues 72 and 81 (Figure 4). The average repetitive yield of this run was 90% (40 nmol of peptide degraded).

The peptide comprising residues 83–113 (Figure 4) was obtained in pure form (see pool R-2 in Figure 3C) and sequenced completely including the C-terminal arginine residue (repetitive yield 94%), thus accounting for the remainder of the variable region sequence.

## Discussion

The technique of somatic cell fusion (Köhler & Milstein, 1975) allows one to prepare large numbers of different, monoclonal antibodies against a single antigen (hapten) in quantity, thus opening a new way for studies of antigen–antibody reactions. Such studies were previously limited to certain hapten-binding myelomas (Potter, 1977) or the occasional production of monoclonal antibodies during immunizations with bacterial vaccines (Haber et al., 1976). Hapten–antibody interactions involving myeloma proteins are usually characterized by relatively low-affinity constants of the order of 10–100 mM. In contrast, mouse anti-digoxin hybridomas bind haptens with an affinity 4 orders of magnitude greater and represent a more useful model for the elicited antibody response. The anti-digoxin hybridoma 26-10, the fine specificity of which has been studied (Mudgett-Hunter et al., 1982a), is directed solely to the steroid moiety (i.e., digoxigenin) of the digoxin molecule (Figure 1). Digoxigenin is an exceptionally rigid structure, its single rotational degree of freedom being around the bond which connects the lactone ring to the sterane nucleus (Figure 1). In view of this conformational rigidity and the fact that the dimensions of the digoxin molecule (30 × 9 × 9 Å; cf. Go & Kartha, 1980) are approximately those of the antibody binding site (34 × 12 × 7 Å; cf. Kabat, 1966), anti-digoxin antibodies promise to be a valuable model for antigen–antibody interactions. We expect that a detailed study of anti-digoxin hybridomas will answer questions about the degree of binding site diversity with respect to a bulky, aliphatic hapten. Correlation of combining site structure with reactivity toward a set of related cardiac glycosides (some 80 digoxin derivatives are now available) may shed light on how changes in amino acid sequence within the

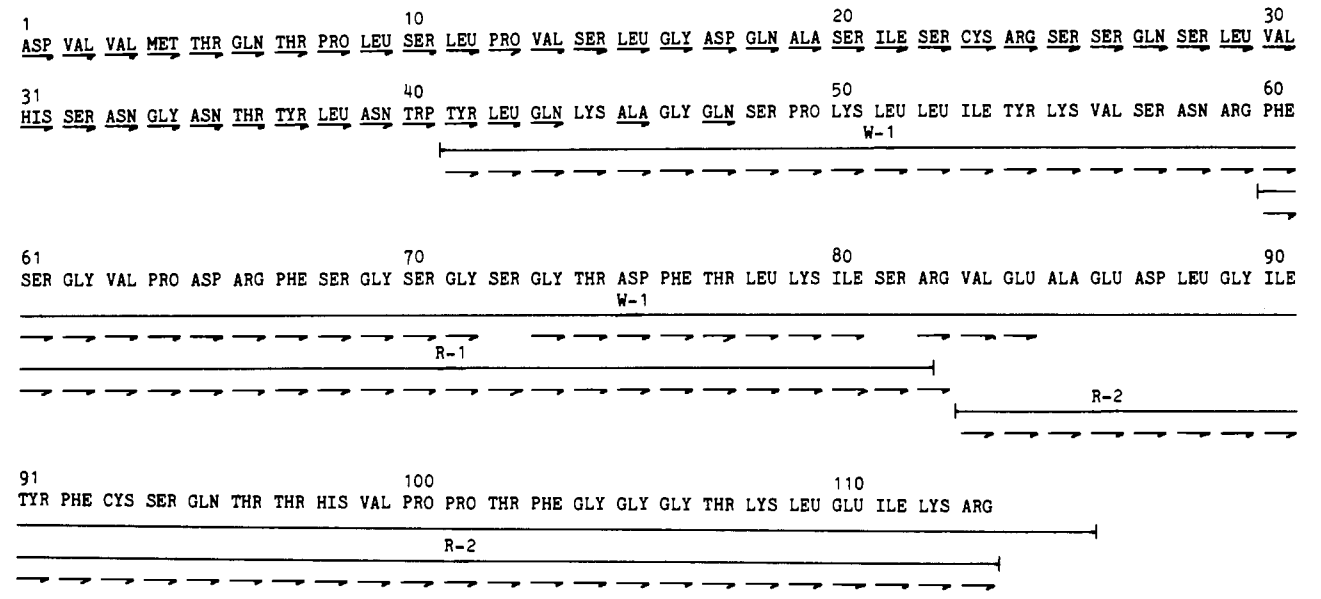


FIGURE 4: Amino acid sequence of the 26-10 light chain variable region. Arrows indicate residues identified by automated Edman degradation.

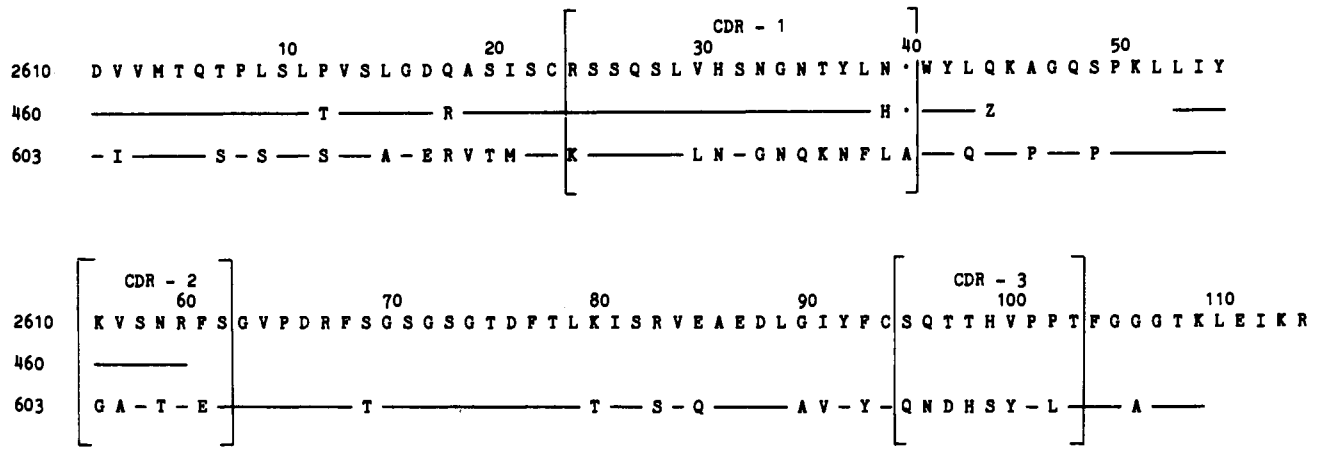


FIGURE 5: Comparison of variable region amino acid sequences of light chains 26-10, MOPC 460 (Barstad et al., 1978), and MCPC 603 (Rudikoff et al., 1981). The three complementarity-determining regions (Kabat et al., 1979) are boxed. Amino acid sequences are given in the one-letter code (IUPAC–IUB Tentative Rules, 1968).

combining site can influence specificity and/or affinity for a given antigen. Twenty-two different anti-digoxin hybridomas are available in our laboratory (M. Mudgett-Hunter, M. N. Margolies, and E. Haber, unpublished data). Fine specificity analysis performed on some of these hybridomas revealed small differences in the probable orientation of digoxin in the antibody combining site (Mudgett-Hunter et al., 1982a,b). Also, each of the hybridomas examined thus far appears to be a unique molecule as judged from their isoelectric spectra, affinity constants, specificity for various cardiac glycosides, and N-terminal sequence of their polypeptide chains.

In deriving the amino acid sequence of the 26-10 light chain variable region, we adopted a fragmentation strategy aimed at obtaining large peptides by specific cleavages. Tryptophan-40 [35 in Kabat et al. (1979) numbering] is an invariant residue (the mouse $\kappa$ constant region contains only one additional tryptophan at position 148), and an arginine residue is always present close to position 61 (Kabat numbering). It was thus possible to complete the variable region sequence with cleavages limited to tryptophan and arginine residues.

Although tryptophan-40 [35 in numbering by Kabat et al. (1979)] is a constant feature of all the immunoglobulin light chains, its usefulness as a cleavage point has not been extensively exploited because of inherent difficulties in chemical methods of tryptophan cleavage. Rudikoff et al. (Rudikoff & Potter, 1978; Rudikoff et al., 1981) reported the use of BNPS–skatole [bromine adduct of 2-(2-nitrophenylsulfenyl)-3-methylindole; Omenn et al., 1970] to obtain a fragment starting at tryptophan-35 [Kabat et al. (1979) numbering] from several phosphorylcholine-binding myeloma light chains. For 26-10 light chain, we found that iodosobenzoic acid cleaves the fully reduced, alkylated, and succinylated light chains specifically and in 40–50% yield. Pretreatment of iodosobenzoic acid with *p*-cresol is reported to be essential for the specificity of the reaction (Mahoney et al., 1981). Tyrosine residues were found to be modified under the conditions of *p*-cresol–iodosobenzoic acid treatment, but this modification [presumably giving rise to 3-chloro- and 3,5-dichlorotyrosine derivatives (cf. Fontana et al., 1981)] is stable, is reproducible, and, in our hands, did not proceed further to peptide bond cleavage. The modified tyrosine residues were readily detectable by HPLC.

When compared to other mouse $V_\kappa$ sequences, the amino acid sequence of the 26-10 anti-digoxin variable region shows unusual similarity with the dinitrophenol- and menadione-binding myeloma MOPC 460. Although only incomplete amino acid sequence data from the MOPC 460 light chain exist at present (N-terminal 51 residues; Barstad et al., 1978), there is 95% homology between the MOPC 460 and 26-10 variable regions. When only the first two hypervariable regions are compared, their homology is equally high, differing in only one position (Figure 5). By contrast, homology between two light chain variable regions of the same antibody specificity is often much lower in both the framework and hypervariable regions; e.g., the phosphorylcholine-binding light chains M197 and MCPC 603 display only 61% homology (Rudikoff et al., 1981). Since the antibody specificities of the 26-10 hybridoma and MOPC 460 myeloma are widely different [26-10 binds the steroid moiety of digoxin, whereas 460 binds the aromatic compounds 2,4-dinitrophenol and menadione with association constants of 10 mM and 200 mM, respectively (Rosenstein & Richards, 1976)], it is possible that the first two hypervariable regions of the light chain 26-10 may not be directly involved in defining the antibody specificity of 26-10. A similar conclusion can be reached from a comparison of 26-10 light chain variable region with that of MCPC 603, a phosphorylcholine-binding myeloma protein whose tertiary structure is known (Segal et al., 1974). The overall homology of these two sequences amounts to 60%. Their first two hypervariable regions are, on the average, 40% homologous, but there is only 22% homology between their third hypervariable regions. The degree of homology being inversely proportional to three-dimensional dissimilarity (Saul et al., 1978), these data imply that the third hypervariable region contributes more strongly to differences in antibody specificities between MCPC 603 and 26-10 than the other two. It is known from X-ray crystallographic data that phosphorylcholine binds to the MCPC 603 Fab, so that the hapten-contacting residues are predominantly provided by the heavy chain hypervariable regions (Segal et al., 1974; Rudikoff et al., 1981). For the light chain, only the third hypervariable region contributes significantly to hapten binding, the other two being screened from the hapten-contacting site by the heavy chain. Thus, the limited functional importance of the first two light chain hypervariable regions may not be an idiosyncratic feature of a single antibody molecule; rather, it may represent a trait common to antibodies of various specificities.

## Acknowledgments

We are grateful to Dr. Edgar Haber for advice and discussion. We thank A. Brauer, E. Locke, and C. Oman for technical assistance and R. Rubin for editorial assistance.

**Registry No.** Immunoglobulin G2a (mouse hybridoma 26-10 $V_\kappa 1$

protein moiety reduced), 84173-72-8; digoxin, 20830-75-5.

References


Barstad, P., Hubert, J., Hunkapiller, M., Goetze, A., Schilling, J., Black, B., Eaton, B., Richards, J., Weigert, M., & Hood, L. (1978) *Eur. J. Immunol. 8*, 497–503.

Bhown, A. J., Bennett, J. C., Morgan, P. H., & Mole, J. E. (1981) *Anal. Biochem. 112*, 158–162.

Brandt, W. F., Edman, P., Henschen, A., & von Holt, C. (1976) *Hoppe-Seyler's Z. Physiol. Chem. 357*, 1505–1508.

Brauer, A. W., Margolies, M. N., & Haber, E. (1975) *Biochemistry 14*, 3029–3035.

Fontana, A., Dalzoppo, D., Grandi, C., & Zambonin, M. (1981) *Biochemistry 20*, 6997–7004.

Go, K., & Kartha, G. (1980) *Acta Crystallogr., Sect. B B36*, 1811–1819.

Haber, E., Margolies, M. N., & Cannon, L. E. (1976) *Cold Spring Harbor Symp. Quant. Biol. 41*, 647–659.

IUPAC–IUB Tentative Rules (1968) *Eur. J. Biochem. 5*, 151–153.

Kabat, E. A. (1966) *J. Immunol. 97*, 1–11.

Kabat, E. A., Wu, T. T., & Bilofsky, H. (1979) *DHEW Publ. (HSA) (U.S.) No. 80-2008.*

Klapper, D. G., Wilde, C. E., & Capra, J. D. (1978) *Anal. Biochem. 85*, 126–131.

Köhler, G., & Milstein, C. (1975) *Nature (London) 256*, 495–497.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Mahoney, W. C., & Hermodson, M. A. (1979) *Biochemistry 18*, 3810–3814.

Mahoney, W. C., Smith, P. K., & Hermodson, M. A. (1981) *Biochemistry 20*, 443–448.

Margolies, M. N., & Brauer, A. W. (1978) *J. Chromatogr. 148*, 429–439.

Margolies, M. N., Mudgett-Hunter, M., Smith, T. W., Novotnỳ, J., & Haber, E. (1981) in *Monoclonal Antibodies and T Cell Hybridomas* (Hämmerling, G., Hämmerling, U., & Kearney, J. F., Eds.) pp 367–374, Elsevier, Amsterdam.

Margolies, M. N., Brauer, A. W., Oman, C. L., Klapper, D. G., & Horn, M. J. (1982) in *Proceedings of the IVth International Conference on Methods in Protein Sequence Analysis* (Elzinga, M., Ed.) pp 189–203, Humana, Clifton, NJ.

Mudgett-Hunter, M., Margolies, M. N., Ju, A., & Haber, E. (1982a) *J. Immunol. 129*, 1165–1172.

Mudgett-Hunter, M., Budzik, G. P., Donahoe, P. K., Khaw, B. A., Margolies, M. N., & Haber, E. (1982b) in *Biological Basis of New Developments in Biotechnology* (Hollaender, A., Laskin, A. I., & Rogers, P., Eds.) Plenum Press, New York (in press).

Omenn, G. S., Fontana, A., & Anfinsen, C. B. (1970) *J. Biol. Chem. 245*, 1895–1902.

Potter, M. (1977) *Adv. Immunol. 25*, 141–211.

Rosenstein, R. W., & Richards, F. F. (1976) *Immunochemistry 13*, 939–943.

Rudikoff, S., & Potter, M. (1978) *Biochemistry 17*, 2703–2707.

Rudikoff, S., Satow, Y., Padlan, E., Davies, D., & Potter, M. (1981) *Mol. Immunol. 18*, 705–711.

Saul, F. A., Amzel, L. M., & Poljak, R. J. (1978) *J. Biol. Chem. 253*, 585–597.

Segal, D. M., Padlan, E. A., Cohen, G. H., Rudikoff, S., Potter, M., & Davies, D. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4298–4302.

Smith, T. W., Haber, E., Yeatman, L., & Butler, V. P. (1976) *New Engl. J. Med. 294*, 797–800.

Smithies, O., Gibson, D., Fanning, E. M., Goodfliesh, R. M., Gilman, J. G., & Ballantyne, D. L. (1971) *Biochemistry 10*, 4912–4921.

Tarr, G. E., Beecher, J. F., Bell, M., & McKean, D. J. (1978) *Anal. Biochem. 84*, 622–627.

Zurawski, V. R., Novotnỳ, J., Haber, E., & Margolies, M. N. (1978) *J. Immunol. 121*, 122–129.