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A new isotype sequence ($V_\kappa 27$) of the variable region of κ-light chains from a mouse hybridoma-derived anti-(streptococcal group A polysaccharide) antibody containing an additional cysteine residue

Application of the dimethylaminoazobenzene isothiocyanate technique for the isolation of peptides

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The first complete sequence of the variable region of a κ-light chain (V_κ) from a mouse anti-(streptococcal group A polysaccharide) antibody (immunoglobulin 7S34.1) is reported. Immunoglobulin 7S34.1 was isolated from the ascitic fluid of hybridoma 7S34.1 previously cloned *in vitro*. A newly developed technique for the isolation of peptides by using pre-column formation of peptide derivatives with dimethylaminoazobenzene isothiocyanate also served to complete the sequence. The sequence of the variable region of the κ-light chain of immunoglobulin 7S34.1 defines a new mouse V_κ isotype ($V_\kappa 27$) and is the first mouse immunoglobulin light-chain variable region to be shown to have an extra cysteine residue at position 48.

Antigen-binding myeloma proteins from mouse plasmocytomas have largely been the probes for studying the diversity of the variable regions of immunoglobulins (Potter, 1977; Rudikoff *et al.*, 1980). More recently, monoclonal antigen-elicited hybridoma-derived antibodies have become available for similar analyses (Alkan *et al.*, 1980; Marshak-Rothstein *et al.*, 1980; Schilling *et al.*, 1980; Gearhart *et al.*, 1981). We have continued to use streptococcal group polysaccharide antigens for such studies, and we have additionally pursued our efforts to develop further micro-sequencing techniques (Chang *et al.*, 1982*b*). Micro-sequencing of protein requires sensitive methods for both sequence determination and peptide isolation. Conventional peptide purification procedures, usually including the successive application of combined chromatographic techniques (Allen, 1981; Hirs & Timasheff, 1977), are inefficient and inadequate in dealing with subnanomole amounts of peptide mixtures.

The development of reversed-phase h.p.l.c. has, however, overcome this inadequacy. By the use of h.p.l.c. we have developed a new method for the isolation and characterization of polypeptide at the picomole level (Chang, 1981*a*; Chang *et al.*, 1982*b*).

Abbreviations used: h.p.l.c., high-pressure liquid chromatography; DABITC, dimethylaminoazobenzene isothiocyanate: DABTC, dimethylaminoazobenzenethiocarbamoyl; DABTH, dimethylaminoazobenzenethiohydantoin; PTH, phenylthiohydantoin; V_κ, variable region of κ-light chains.

Peptides were subjected to pre-column reaction with DABITC to form DABTC-peptides, which were separated on a reversed-phase h.p.l.c. column and then sequenced directly by either automatic or manual Edman degradations. This new technique combines the versatility of the separation mechanism of reversed-phase h.p.l.c. with the high sensitivity of the detection of DABTC-peptides (2–5 pmol) in the visible spectral region. Thus 1–2 nmol of DABTC-peptide mixtures is sufficient for 10–20 analytical runs to determine the optimum conditions for the resolution of a peptide mixture with the consumption of only 100–200 pmol of the total material.

In the present paper the use of the DABITC technique for the isolation and sequence determination of the tryptic peptides together with automated Edman degradation is reported to establish the first complete sequence of the variable region of a light chain from a monoclonal mouse hybridoma-derived anti-(streptococcal group A polysaccharide) antibody, immunoglobulin 7S34.1.

Materials and methods

Monoclonal anti-(streptococcal group A polysaccharide) antibody, and isolation of its heavy chains and light chains

Monoclonal mouse anti-(streptococcal group A polysaccharide) antibody immunoglobulin 7S34.1 was obtained by somatic-cell fusion of spleen cells

from a C×BK mouse that had previously been hyperimmunized with streptococcal group A (strain J17A4) vaccine by established methods (Cramer & Braun, 1974; Herbst *et al.*, 1982). The somatic-cell hybridization, followed by cloning and recloning of streptococcal-group-A-polysaccharide-specific hybridomas, was performed essentially in accordance with the original method described by Köhler & Milstein (1975) (see Galfré *et al.*, 1977; Shulman *et al.*, 1978; Herbst & Braun, 1981). Details about antibody fine specificity, association constants and isolation procedures are published elsewhere (Herbst *et al.*, 1982). Heavy chains and light chain of immunoglobulin 7S34.1 were isolated, after partial reduction and alkylation, on a Sephadex G-100 (Pharmacia) column (2cm×90cm) with 1M-acetic acid as solvent (Huser & Braun, 1979).

Chemical modification of the light chain

The light chain was oxidized with performic acid (Hirs, 1967) or it was completely reduced and alkylated with iodo[2-^{14}C]acetic acid (O'Donnell *et al.*, 1970). Irreversible blocking of lysine residues by reaction with methyl isocyanate was performed in aq. 50% (v/v) pyridine (Chang, 1981*a*). Proteins were cleaved by reaction with CNBr (Gross & Witkop, 1961), and CNBr-cleavage peptides were separated by gel filtration (Sephadex G-75). Tryptic peptides were subjected to pre-column reaction with DABITC followed by separation of the DABTC-peptides on a reversed-phase h.p.l.c. column (see the following subsection).

Isolation of DABTC-peptides by h.p.l.c.

Details of the formation of DABTC-peptides and the h.p.l.c. system used for their isolation have been described previously (Chang, 1981*a*). DABTC-peptides were dissolved in aq. 20–40% pyridine for h.p.l.c. injection. The eluted DABTC-peptides were collected manually and freeze-dried, and those used for sequence analysis (preparative separation) were incubated with 100μl of anhydrous trifluoroacetic acid (for 10min at 52°C), dried and stored at −20°C.

N-Terminal analysis and N-terminal sequence determination of DABTC-peptides

N-Terminal analyses of DABTC-peptides were performed by the method described previously (Chang, 1981*a*). The amino acid sequences of DABTC-peptides were determined either manually (Chang *et al.*, 1978; Chang, 1981*b*) or by use of the automatic Beckman 890C sequenator (Edman & Begg, 1967) with the 0.25M-Quadrol (Fluka 83335) program no. 291274 in accordance with the Beckman manual. The sequenator had been modified by replacing the original pumps with two Alcatel pumps connected with the system via a liquid-N_2 trap (Wittmann-Liebold, 1973). Hexadimethrine bromide (Polybrene) (3mg) was added as a carrier (Tarr *et al.*, 1978). The amino acid PTH derivatives were analysed on a Zorbax-CN column (Dupont) with a Spectra Physic SP 8000 instrument (Chang *et al.*, 1982*b*) connected to the Spectra Physic SP 4000 computing system.

Strategy for the isolation of DABTC-peptides

The outlined strategy is based on the sensitivity limit of the available sequencing techniques. (1) A 2nmol portion of the tryptic-digest peptides was treated with DABITC. About 10–15% of the sample of DABTC-peptides was removed, diluted with aq. 25% pyridine to approx. 100μl and used for analytical runs in order to determine the optimal separation conditions. (2) Once the optimal conditions had been established (e.g. as judged by the total separated peaks), 0.5–1nmol of the DABTC-peptide sample was separated and manually col-

Table 1. *Comparison of the amino acid composition of immunoglobulin 7S34.1 light chain determined by the dimethylaminoazobenzenesulphonyl chloride method and with the amino acid analyser*

Results given for the dimethylaminoazobenzenesulphonyl chloride (DABS-Cl) method (Chang *et al.*, 1981, 1982*a*) were derived from the analysis of approx. 14ng of dabsylated protein hydrolysate (with Pierce 6M-HCl, lot no. 24309). Analyses by the standard method were performed with a Biotronik analyser, and results were derived by the analysis of approx. 5μg of protein hydrolysate (with Merck 6M-HCl). N.D., Not determined.

	Composition of immunoglobulin 7S34.1 (mol of residue/mol)	
Amino acid	DABS-Cl method	Standard method
Asp	22.2	21.0
Glu	21.9	22.0
Ser	27.2	26.2
Thr	18.2	20.8
Gly	14.2	15.5
Ala	10.1	10.9
Pro	12.0	11.9
Val	13.4	12.8
Arg	10.1	10.5
Met	N.D.	N.D.
Ile	9.3	8.5
Leu	15.7	15.3
Phe	8.6	8.3
Lys	10.9	11.1
His	2.5	3.4
Tyr	10.2	8.3*
Cys	N.D.	6.2

* Proteins were oxidized with performic acid for the analysis of cysteic acid, and tyrosine residues were partially destroyed by oxidation.

lected, and the purity and identity of each fraction were examined by *N*-terminal analysis (Chang, 1981*a*). (3) Finally, 6–8nmol of the tryptic peptides was treated with DABITC for preparative isolation. The recovered DABTC-peptides, with yields ranging from 0.5 to 3nmol, were sequenced by either automatic or manual degradation.

Results

Sequence of the variable region of immunoglobulin 7S34.1 light chain

The amino acid composition of the oxidized immunoglobulin 7S34.1 light chain, as determined with a Biotronik LC 6000 analyser, disclosed one additional cysteine residue (Table 1). The sequence was established by using (1) intact light chain, (2) CNBr-cleavage peptides and (3) tryptic peptides (see Fig. 3).

The determination of the *N*-terminal sequence of intact immunoglobulin 7S34.1 light chain provided the sequence of the first 63 amino acids (Fig. 1) with the exception of residue positions 23 (cysteine), 48 (cysteine), 55 (arginine), 57 (serine) and 61 (serine).

CNBr-cleavage peptides of immunoglobulin 7S34.1 light chain (completely reduced and alkylated, and with lysine residues blocked with methyl isocyanate) were separated by gel filtration (Sephadex G-75, fine grade), and two fractions were collected. Purity and identity of the peptides contained in each fraction were assessed by *N*-terminal analysis with the use of DABITC (Chang, 1980). The first fraction contained the peptide spanning residues 95–173, peptide CB1 (see Fig. 3). The second fraction contained two peptides in approximately equal amounts, peptides CB2 (carrying residues 57–94) and CB3 (carrying residues 176–214). The first 26 residues of peptide CB1 were unambiguously sequenced with the sequenator, and the first 19-amino-acid-residue sequence accounted for the *C*-terminal sequence of the variable region. Peptides CB2 and CB3 were sequenced together to their *C*-terminal residues. The sequence of peptide CB2 was established by subtracting the known sequence of peptide CB3 (constant region).

The sequence gaps left from the sequence analysis of the intact light chain and the overlapping of peptides CB1 and CB2 were finally determined by sequence analysis of tryptic-digest peptides, which were isolated by a newly developed technique involving the pre-column formation of peptide derivatives with DABITC (Chang, 1981*a*).

Two preparations of tryptic peptides, both from the performic acid-oxidized immunoglobulin 7S34.1 light chain with lysine residues blocked with methyl isocyanate, were isolated. The first preparation was obtained by using commercial trypsin (lot no. 24581; 1-chloro-4-phenyl-2-tosylamidobutan-2-one-treated) from Merck (Darmstadt, Germany) yielded resolved peaks that exceeded the number of pep-

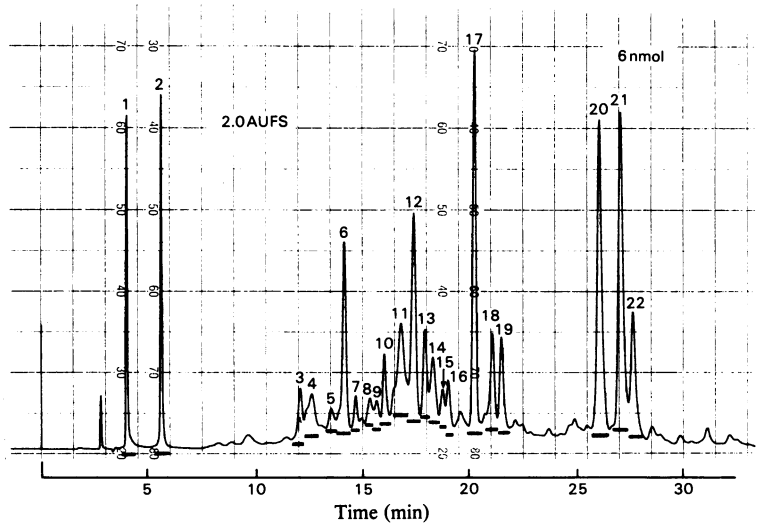

Fig. 1. *Preparative h.p.l.c. isolation of DABITC derivatives of tryptic peptides of immunoglobulin 7S34.1 light chain*
A 6nmol portion of the oxidized light chain was treated with methyl isocyanate and digested with Merck trypsin in 0.1M-NH_4HCO_3 for 24h at 37°C. Chromatographic conditions were as follows: solvent A was 0.5% pyridine (pH 7.0, adjusted with acetic acid) containing 0.005% 2-mercaptoethanol; solvent B was acetonitrile; the gradient was 15–80% solvent B in 40min; the column was μ-Bondapak C-18 (Waters); the column temperature was 22°C. The detector wavelength was 436nm. The chart speed was 40cm/h. AUFS stands for absorption units, full scale.

tides expected from the amino acid composition (Fig. 1). The additional peptides were subsequently identified as chymotryptic split products. Most of the DABTC-peptides, including chymotryptic peptides with leucine, phenylalanine or tyrosine as *C*-terminal residue, were sequenced to their *C*-terminal end. Those that aligned within the variable region are indicated in Fig. 3. A second preparation of tryptic peptides obtained by cleavage with Worthington Biochemical Corp. (Freehold, NJ, U.S.A.) trypsin (cat. no. 3740; 1-chloro-4-phenyl-3-tosylamido-butan-2-one-treated) produced DABTC-peptides selectively cleaved only at arginine residues (Fig. 2; see also Fig. 3).

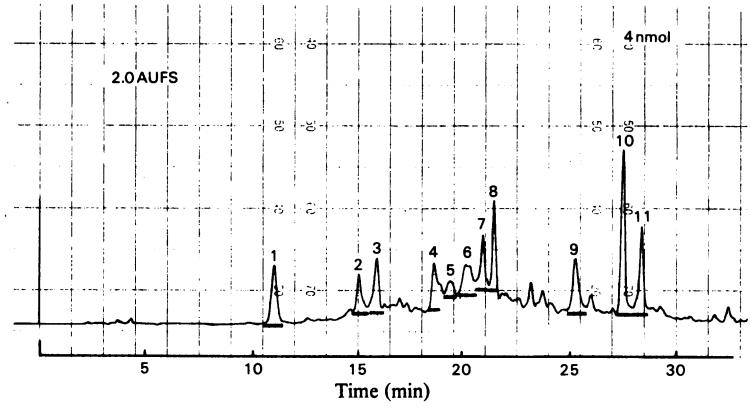

Fig. 2. *Preparative h.p.l.c. isolation of DABITC derivatives of tryptic peptides of immunoglobulin 7S34.1 light chain*
A 4 nmol portion of the oxidized light chain was treated with methyl isocyanate and digested with Worthington trypsin in aq. 0.2% pyridine for 24 h at 37°C. Chromatographic conditions were as follows: solvent A was 10 mM-phosphate buffer, pH 7.2, containing 0.005% 2-mercaptoethanol; solvent B was acetonitrile/tetrahydrofuran (3:1, v/v); the gradient was 15–63% solvent B in 30 min; the column was μ-Bondapak C-18 (Waters); the column temperature was 22°C. The detector wavelength was 436 nm. The chart speed was 40 cm/h.

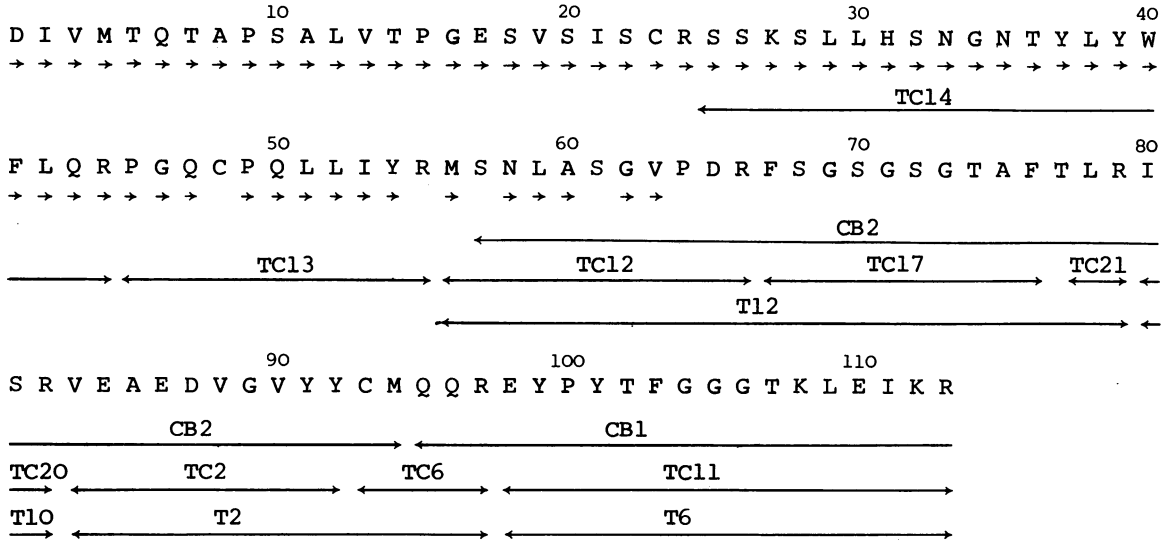

Fig. 3. *Complete sequence of the variable region of immunoglobulin 7S34.1 light chain*
Key: CB, CNBr-cleavage peptide; T, tryptic peptide obtained from the experiment shown in Fig. 2; TC, tryptic peptide (contaminated by non-specific chymotryptic cleavage) obtained from the experiment shown in Fig. 1. The one-letter notation for amino acid sequences (IUPAC–IUB Commission on Biochemical Nomenclature, 1969) is used.

Identification of an extra cysteine residue at position 48 of immunoglobulin 7S34.1 light chain

The amino acid composition of immunoglobulin 7S34.1 light chain indicated the existence of one additional cysteine residue. In the sequencing of both native light chain and tryptic DABTC-peptide TC13, i.e. peak 13 depicted in Fig. 1, comprising residues 45–55 of the native chain, a gap appeared at position 48 and position 4 respectively. It was reckoned that this gap was generated by a hydrophilic residue that remained unextractable by organic solvent after cleavage, or an amino acid residue involved in a disulphide linkage. A modified protocol of the manual Edman degradation was used to give the answer. DABTC-peptide TC13 (1.5nmol) was subjected to the manual DABITC–phenylisothiocyanate degradation (Chang, 1981*b*) for two cycles. After the third coupling and washing out of excess of reagent, the sample was dried *in vacuo* and treated with aqueous acid directly. The acid sample was dried and redissolved in aq. 50% (v/v) acetonitrile (no extraction of amino acid DABTH derivative was required) for identification of the amino acid DABTH derivative. Analysis by both h.p.l.c. (Chang, 1981*b*) and t.l.c. (Chang *et al.*, 1978) (Fig. 4) identified residue 48 as cysteic acid.

Complete sequence of the variable region of immunoglobulin 7S34.1 light chain

Automated and manual Edman degradation of the whole light chain and of the isolated peptides (Fig. 3) revealed that the variable region of this light chain consists of 113 amino acid residues. Residues not commonly found in mouse immunoglobulin V_κ regions, e.g. histidine-31, arginine-44, cysteine-48, arginine-55, methionine-56 and alanine-75, together with a unique amino acid sequence of the *N*-terminal 17 amino acid residues (threonine-7, proline-9, alanine-11, leucine-12, proline-15 and glutamic acid-17), clearly define a prototype sequence of a new subgroup, V_κ27.

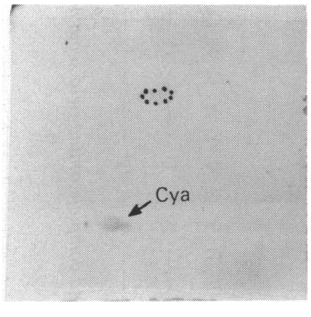

Fig. 4. *T.l.c.(polyamide, Schleicher und Schüll) analysis of the amino acid DABTH derivative released at the third degradation cycle (residue 4) of DABTC-peptide TC13 (Fig. 1)*

DABTH-cysteic acid appeared as a pear-shaped red spot. Internal marker DABTC-diethylamine (dotted cycle) was blue. Solvent 1 (first dimension) was acetic acid/water (1:2, v/v). Solvent 2 (second dimension) was toluene/n-hexane/acetic acid (2:1:1, by vol.). The polyamide sheet size was 2.5cm × 2.5cm.

Discussion

Immunoglobulin 7S34.1 is one of several monoclonal hybridoma-derived mouse anti-(streptococcal group A polysaccharide) antibodies that are being characterized in our laboratory (Herbst *et al.*, 1982). Its reaction with the antigen is fully inhibitible by the addition of *N*-acetyl-D-glucosamine, the immuno-determinant sugar moiety of the streptococcal group A polysaccharide (Krause, 1970).

Immunoglobulin 7S34.1 used for amino acid sequence determination was secreted by the ascites-producing hybridoma 7S34.1, a tumour derived by somatic-cell fusion between the myeloma line Sp2/0 and spleen cells of C × BX mice immunized with the streptococcal group A vaccine J17A4 by established methods (Galfré *et al.*, 1977; Shulman *et al.*, 1978; Herbst & Braun, 1981; Herbst *et al.*, 1983). It was purified from ascites by affinity chromatography on a (streptococcal group A polysaccharide)–Sepharose column for binding and *N*-acetyl-D-glucosamine for elution of the antibody (Eichmann & Greenblatt, 1971). The purity of the immunoglobulin 7S34.1 was verified by sodium dodecyl sulphate/polyacrylamide/gel electrophoresis and subsequently by automated *N*-terminal sequence determination of the isolated heavy chains and light chains.

The *N*-terminal 23 residues of mouse immunoglobulin V_κ regions are particularly diverse, as shown by comparison with those from other species (McKean *et al.*, 1978). These variations are also the basis for the previously defined 26 V_κ subgroups (McKean *et al.*, 1978). All monoclonal hybridoma-derived mouse antibodies conformed to this subgroup or isotype assignment, since these paralleled in structure their homologues previously described as myeloma proteins (Potter, 1977).

Fig. 5 summarizes the comparison of the V_κ region of immunoglobulin 7S34.1 with only a few V_κ regions derived from mouse immunoglobulins known to bind sugar determinants. The V_κ region of immunoglobulin 7S34.1 clearly presents the prototype sequence of a new subgroup, V_κ27. The nearest sequence homologue is that of serum antibody A5A, which is also specific for streptococcal group A polysaccharide (Capra *et al.*, 1976). However, myeloma protein S117, previously described to bind the hapten *N*-acetyl-D-glucosamine (Vicari *et al.*,

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                                                                        ————————CDRI————
                      1                 1                 2                 3 3 a b c d e 3                 4
                                        0                 0                 0 1         2                   0
A 1. 7S34.1  Hy γ3    D I V M T Q T A P S A L V T P G E S V S I S C R S S K S L L H S N G N T Y L Y W F L Q R P
A 2. A5A     Se γ2a   ————————— A ——— L
A 3. S117    My α     E — L — S P A I T A A S L — Q K — T — T — S A — S — V S(           )—M B —
G 4. XRPC 24 My α     E — L — S P A I T A A S L — Q K — T — T — S A — S — V S(           )—M H — Y Q — K S
F 5. UPC  61 My α     — V Q — I — S P S — L S A S L — D I — T M T — Q A — Q G T S(    )———( )— N —— Q — K —
D 6. PC 3858 My α     —— L L —— S P A T L S — S ——— R —— F ——
D 7. W 31 29 My α     — V — V ——— G L — L P — S M — D R ————————

                                       ——CDRII——
                      4                 5                 6                 7                 8
                      1                 0                 0                 0                 0
A 1. 7S34.1  Hy γ3    G Q C P Q L L I Y R M S N L A S G V P D R F S G S G S G T A F T L R I S R V E A
G 4. XRPC 24 My α     — T S — K P W —— E I — K ——————— A ——————————— S Y S — T —— S M ——
F 5. UPC 61  My α     — K A — K ——— G A — I — E D ——— S ——————— R Y —— D ——— T —— S L — D

                                     ——CDRIII——
                                                                1         1
                      8                 9               9       0         0 a
                      1                 0               7       0         6
A 1. 7S34.1  Hy γ3    E D V G V Y Y C M Q Q R E Y P Y T F G G G T K L E I( )K R
G 4. XRPC 24 My α     —— A A I ——— Q — W N Y P L I ——— S ——————— L( )——
F 5. UPC 61  My α     —— M A T — F — L — H S ——————————————————— ( )——
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Fig. 5. *Comparison of partial and complete amino acid sequences of V_κ regions of mouse immunoglobulin molecules with that of immunoglobulin 7S34.1 (for references see Kabat et al., 1979)*

Key: A, streptococcal-group-A-polysaccharide-binding protein; D, 1,6-α-dextran-binding protein; F, 2,1-β-fructosan-binding protein; G, 1,6-β-D-galactan-binding protein; Hy, hybridoma-derived antibody; My, myeloma protein; Se, serum antibody. The one-letter notation for amino acid sequences (IUPAC–IUB Commission on Biochemical Nomenclature, 1969) is used. Continuous lines mark sequence identity with the amino acid sequence of the V_κ region of immunoglobulin 7S34.1 light chain; parentheses identify gaps inserted to match maximal homology. CDRI, CDRII and CDRIII indicate the first, second and third complementarity-determining regions respectively.

1970) and taken as a prototype sequence for this specificity (Berek *et al.*, 1976), has very little in common with the V_κ region of immunoglobulin 7S34.1, since the degree of homology is only 12% over the *N*-terminal 40 positions. It should be remembered, however, that protein S177 is specific for the disaccharide *N*-acetyl-D-glucosamine–β-1,3-galactose, whereas the streptococcal-group-A-polysaccharide determinant comprises the disaccharide *N*-acetyl-D-glucosamine–β-1,3-L-rhamnose (Vicari *et al.*, 1970; Krause, 1970). Like the V_κ region of the 1,6-β-D-galactan-binding myeloma protein, XRPC24, the V_κ region of protein S117 belongs to the subgroup V_κ4. The V_κ region of protein XRPC24 also shows only a 29% degree of identity in the 40 *N*-terminal homology positions, with an overall degree of homology with the V_κ region of immunoglobulin 7S34.1 of 48%. The amino acid sequences of V_κ regions from ten hybridoma-derived 1,6-β-D-galactan-binding antibodies completely conform to these differences (Pawlita *et al.*, 1982).

Similarly, the V_κ regions of the 2,1-β-fructosan-binding myeloma proteins, of which that of protein UPC 61 is a prototype sequence, show only an overall degree of homology of 53%. Also, the partial sequences of the V_κ regions of the light chains from the mouse 1,6-α-dextran-binding myeloma proteins PC3858 and W3129 are, with 52% and 57% homology, significantly different. The V_κ region of immunoglobulin 7S34.1 has a longer first complementarity-determining region (positions 29–35) than for V_κ regions from all other sugar-binding antibodies, and its second (positions 50–55) and third (positions 89–97) complementarity-determining regions are unique, as is the extra cysteine residue in homology position 43. This extra cysteine residue may well possess a functional role, but its exact function remains to be investigated. In addition, methionine-89 is noteworthy. Its presence shifts the dipeptide sequence Tyr-Pro from positions 93–94 to positions 94–95, followed by a Tyr-Thr sequence known for a number of mouse immunoglobulin light-chain V_κ sequences in these positions, and the framework 4 or J-segment (positions 98–107) of the V_κ region of immunoglobulin 7S34.1 conforms to established information (Kabat *et al.*, 1979; Gearhart *et al.*, 1981).

The present description of the V_κ27 sequence of immunoglobulin 7S34.1 is not in conflict with a previous analysis of V_κ regions of mouse immunoglobulins, since that predicted the existence of more than 26 V_κ isotypes (McKean *et al.*, 1978). The overall number of individual V_κ-region genes has been estimated by various approaches to be from 90 up to 700 (Adams *et al.*, 1981).

The complete sequence of the V_κ region of immunoglobulin 7S34.1 light chain was essentially derived from 5mg (approx. 80% was consumed for the CNBr-cleavage peptide) of isolated polypeptide chain by combining the automated sequence determination and the DABITC method for the isolation of peptides. We stress that within the present context the DABITC method has distinct advantages over conventional techniques for the separation of peptides: (1) it allows the isolation of as little as 5–10pmol of pure DABTC-peptide for sequence determination; (2) the quantity of the DABTC-peptide recovered may be directly determined from the peak response; (3) the purity of the collected DABTC-peptide is readily determined by *N*-terminal analysis at the picomole level; (4) pre-column formation of the DABTC-peptide derivative may prevent the blocking of the peptide *N*-terminus during chromatographic separation, e.g. cyclization of glutamine to pyrrolid-2-one-5-carboxylic acid; (5) proline-containing peptides, which are unreactive with florescamine and *o*-phthalaldehyde, are readily identified at the same sensitivity level as are other peptides.

In order to perform the DABITC method at optimal sensitivity, possible oxidation of DABTC-peptides during separation was prevented by the addition of 0.005% 2-mercaptoethanol to solvent A. Fresh NH_4HCO_3 solution was always used for tryptic digestion, and freeze-drying of NH_4HCO_3 solutions was performed for a minimum of 20h. It was found that unremoved ammonia traps DABITC, resulting in incomplete formation of the derivatives. DABTC-peptides collected for sequence determination should be incubated, before storage, with trifluoroacetic acid in order to release the *N*-terminal amino acid DABTH derivative. This is again to prevent oxidation of the DABTC-peptide bond during prolonged storage.

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References

Adams, J. M., Kemp, D. J., Bernard, O., Gough, N., Webb, E., Tyler, B., Gerondakis, S. & Cory, S. (1981) *Immunol. Rev.* **59**, 5–32

Alkan, S. S., Knecht, R. & Braun, D. G. (1980) *Hoppe-Seyler's Z. Physiol. Chem.* **361**, 191–195

Allen, G. (1981) in *Sequencing of Proteins and Peptides* (Allen, G., ed.). pp. 73–134, Elsevier/North-Holland, Amsterdam

Berek, C., Taylor, B. A. & Eichmann, K. (1976) *J. Exp. Med.* **144**, 1164–1174

Capra, J. D., Berek, C. & Eichmann, K. (1976) *J. Immunol.* **117**, 7–10

Chang, J.-Y. (1980) *Anal. Biochem.* **102**, 384–392

Chang, J.-Y. (1981*a*) *Biochem. J.* **199**, 537–546

Chang, J.-Y. (1981*b*) *Biochem. J.* **199**, 557–564

Chang, J.-Y., Brauer, D. & Wittmann-Liebold, B. (1978) *FEBS Lett.* **93**, 205–214
Chang, J.-Y., Knecht, R. & Braun, D. G. (1981) *Biochem. J.* **199**, 547–556
Chang, J.-Y., Knecht, R. & Braun, D. G. (1982*a*) *Biochem. J.* **203**, 803–806
Chang, J.-Y., Knecht, R. & Braun, D. G. (1982*b*) *Proc. Int. Conf. Methods Protein Sequence Analysis 4th, 1981*, in the press
Cramer, M. & Braun, D. G. (1974) *J. Exp. Med.* **139**, 1513–1528
Edman, P. & Begg, G. (1967) *Eur. J. Biochem.* **1**, 80–91
Eichmann, K. & Greenblatt, J. J. (1971) *J. Exp. Med.* **133**, 424–441
Galfré, G., Howe, S. C., Milstein, C., Butcher, G. W. & Howard, J. C. (1977) *Nature (London)* **266**, 550–552
Gearhart, P. J., Johnson, N. D., Donglas, R. & Hood, L. (1981) *Nature (London)* **291**, 29–34
Gross, E. & Witkop, B. (1961) *J. Am. Chem. Soc.* **83**, 1510–1511
Herbst, H. & Braun, D. G. (1981) *Ann. Immunol. (Paris)* **132C**, 87–100
Herbst, H., Chang, J.-Y., Aebersold, R. & Braun, D. G. (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363**, 1069–1076
Hirs, C. H. W. (1967) *Methods Enzymol.* **11**, 197–199
Hirs, C. H. W. & Timasheff, S. N. (1977) *Methods Enzymol.* **47**, 195–246
Huser, H. & Braun, D. G. (1979) *Scand. J. Immunol.* **9**, 105–114
IUPAC–IUB Commission on Biochemical Nomenclature (1969) *Biochem. J.* **113**, 1–4
Kabat, E. A., Wu, T. T. & Bilofsky, H. (1979) *DHEW Publ. (NIH) (U.S.)* no. 80-2008
Köhler, G. & Milstein, C. (1975) *Nature (London)* **256**, 495–497
Krause, R. M. (1970) *Adv. Immunol.* **12**, 1–56
Marshak-Rothstein, A., Siekevitz, M., Mudgett-Hunter, L., Margolies, M. N. & Gefter, M. L. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1120–1124
McKean, D. J., Bell, M. & Potter, M. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3913–3917
O'Donnell, I. J., Frangione, B. & Porter, R. R. (1970) *Biochem. J.* **116**, 216–268
Pawlita, M., Rudikoff, S. & Potter, M. (1982) *J. Immunol.* **129**, 615–618
Potter, M. (1977) *Adv. Immunol.* **25**, 141–211
Rudikoff, S., Rav, D. N., Claudemans, C. P. J. & Potter, M. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4270–4274
Schilling, J., Clevinger, B., Davie, J. M. & Hood, L. (1980) *Nature (London)* **283**, 35–40
Shulman, M., Wilde, C. D. & Köhler, G. (1978) *Nature (London)* **276**, 269–270
Tarr, G. E., Beecher, J. F., Bell, M. & McKean, D. (1978) *Anal. Biochem.* **84**, 622–627
Vicari, G., Sher, A., Cohn, M. & Kabat, E. A. (1970) *Immunochemistry* **7**, 829–838
Wittmann-Liebold, B. (1973) *Hoppe-Seyler's Z. Physiol. Chem.* **354**, 1415–1431