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Biochemistry

Activation of complement by immunoglobulin M is impaired by the substitution serine-406 → asparagine in the immunoglobulin μ heavy chain

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ABSTRACT **We have isolated and analyzed the DNA encoding the μ heavy chain constant region of a mutant IgM that is defective in initiating complement-dependent cytolysis. By assaying the expression of μ-chain genes that were constructed *in vitro* from mutant and wild-type gene segments, we have mapped the mutation into a 555-base-pair segment that spans part of the third and fourth constant region domains. In this segment there is one nucleotide change, such that the mutant μ-chain gene encodes asparagine rather than the normal serine at amino acid position 406 in the third constant domain. We have used site-directed mutagenesis to introduce a comparable mutation into the normal μ-chain gene and confirmed that this substitution causes the production of IgM with the original mutant phenotype. Evidence is also provided that the serine-406 → asparagine substitution might cause the mutant μ chain to be abnormally glycosylated.**

The classical pathway of complement-dependent cytolysis is initiated when the latent protease of the first complement component C1 is activated by multivalent binding to immunoglobulin (reviewed in ref. 1). A single molecule of pentameric IgM bound to the cell surface can activate C1 (2). The inability of free IgM molecules in solution to activate C1 has suggested that the C1 binding site on IgM is normally unavailable but can be exposed when the IgM is distorted by multivalent binding, such as to a cell surface or large molecules (2–4). To identify the structural features of IgM that are important for activating C1, we have isolated mutant hybridoma cell lines that produce noncytolytic, hapten-specific IgM (5, 6). One class of mutants produces pentameric IgM that is normal in its ability to bind to haptenated erythrocytes but does not cause their lysis by complement (5). Here we show that the μ-chain gene of one such mutant encodes asparagine rather than serine at amino acid position 406 in the third constant (C) region domain and that this substitution is responsible for the decreased capacity of the IgM to trigger complement-dependent cytolysis.

MATERIALS AND METHODS

Cell Lines. The PC7 wild-type hybridoma makes IgM(κ) specific for the hapten phosphocholine. The cell line, 42, was selected from PC7 as a mutant making noncytolytic IgM (5). Cells were grown in Dulbecco's modified Eagle's medium (GIBCO H21) supplemented with 15% fetal calf serum and 3.5×10^{-4}% 2-mercaptoethanol. The igm-10 cell line, which makes κ light chain specific for 2,4,6-trinitrophenyl (TNP) and contains no μ gene, is described in refs. 6 and 7.

Gene Transfer. The transfer vectors used here are comparable to pR-Sp6, which bears the genes for the TNP-specific μ heavy chain and for resistance to the drug G418 (7). To facilitate the *in vitro* recombination steps, many of the constructions used a derivative of pSV2-neo, which lacks the *Bam*HI site. As indicated in *Results*, μ gene segments from the mutant hybridoma were substituted for the corresponding region of the pR-Sp6 μ gene. The pR-Sp6 and other μ gene-containing vectors were introduced into igm-10 cells by fusing them with bacterial protoplasts bearing the corresponding vector (7). Protoplasts were prepared as described (8) and pelleted by centrifugation. igm-10 cells (10^7) were then washed in serum-free medium (H21) and centrifuged 10 min at 600 × *g* onto the pellet containing 3×10^8 to 1.5×10^9 protoplasts. The cells were then resuspended together in 0.1 ml of H21 containing 10% sucrose and 0.01 M $MgCl_2$ and then mixed with a solution containing 1 ml of H21, 0.8 g of polyethylene glycol M_r 1000, and 0.2 ml of dimethyl sulfoxide. After 10 sec at 37°C, 10 ml of H21 was added. The cells were centrifuged 10 min at 600 × *g* and resuspended in 40 ml of medium. After incubation overnight, viable cells were counted and distributed to wells at 10^3, 10^4, and 10^5 cells per well in medium supplemented to contain 0.5 mg of G418 per ml. When the transformants had grown to high density, the culture supernatant was tested for TNP-specific IgM.

Analysis of Ig. Ig was biosynthetically labeled in the presence or absence of tunicamycin (5) and analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis ($NaDodSO_4$/PAGE) with (9) or without (10) reduction of disulfide bonds. In some cases, the sensitivity of the autoradiography was enhanced by soaking the gel in salicylate (11). To measure the IgM concentration, culture supernatant was compared to purified IgM (myeloma protein TEPC183, from Litton Bionetics) by using an enzyme-linked immunosorbent assay (ELISA). For this ELISA, affinity-purified goat anti-mouse IgM (Cappel Laboratories, Cochranville, PA) was adsorbed at 1 μg/ml in plastic 96-well dishes. After blocking the wells with 1% albumin, the IgM-containing samples were incubated in the wells for 90 min, and the unbound material was removed. The bound IgM was then detected with alkaline phosphatase-linked goat anti-mouse IgM.

To assess the defect in cytolytic activity, culture supernatants containing different concentrations of IgM from transformants expressing normal and mutant μ-chain genes were incubated in 0.5 ml with ≈2 μl (A_{541} = 1.0) of TNP-coupled sheep erythrocytes (TNP-SRBC) for 10 min at 37°C. Under these conditions, >90% of the IgM binds to the TNP-SRBC. Following this preincubation, 25 μl of guinea pig serum was added as a complement source, and the incubation was continued for 10 min at 37°C. Thereafter, the unlysed cells were removed by centrifugation. Lysis was measured as released hemoglobin—i.e., the absorbance of the supernatant was measured at 541 nm. To maintain comparable incubation conditions over the range of IgMs tested, the culture super-

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Abbreviations: TNP, 2,4,6-trinitrophenyl; C, constant; SRBC, sheep erythrocyte(s); bp, base pair(s).

natants were dialyzed against phosphate-buffered saline (PBS) and the incubation mixtures included 30% dialyzed culture medium.

End-point dilutions for TNP-specific cytolytic and agglutination activity were measured for the IgM in culture supernatants, as described (5, 6). For lysis spot tests, 25 μl of packed TNP-SRBC and 50 μl of guinea pig serum (complement source) were mixed with 2 ml of 0.6% agarose in PBS and poured onto a 10-cm Petri plate. Culture supernatant was serially diluted 1:3 in PBS containing 1% fetal bovine serum. A 2-μl aliquot of each dilution was spotted onto the above plate and after 1 hr at 37°C was scored for lysis of the underlying erythrocytes. The same serial dilutions were also tested for their agglutination titer—i.e., 50 μl of TNP-SRBC (diluted 1:800) was added to each well, and after 2 hr of incubation at room temperature, the wells were scored for agglutination.

DNA Cloning, Sequencing, and Mutagenesis. The segment bearing the genes encoding the μ-chain C region, bounded by the *Eco*RI enzyme sites, was isolated in λgtWES; DNA was subcloned and sequenced in M13mp18, using the dideoxy method, as described (12). Oligonucleotide primers for sequencing and mutagenesis were synthesized on an Applied Biosystems DNA synthesizer (model 380A) and purified by PAGE. Site-directed mutagenesis was done as described (13), except that DNA synthesis reactions were done at 22°C for 16 hr and the alkaline sucrose gradient enrichment was omitted. The C_μ *Bam*HI–*Kpn* I DNA segment (Fig. 3) was inserted into phage M13mp18. The 22-base oligomer indicated in *Results* was used to prime DNA synthesis. The phage DNA was transfected into bacteria JM103 and plated. To distinguish phage bearing the normal and mutant μ gene segments, plaque-lifts onto nitrocellulose paper (14) were hybridized at 65°C with a ^{32}P-labeled 15-base oligomer bearing the mutation (ACCTTCAACGCTAAG) and washed in 0.9 M NaCl/90 mM sodium citrate, pH 7, at room temperature. The presence of the mutation was verified by nucleotide sequencing. By using double-stranded (replicative form) DNA, the mutated segment of the μ gene was then substituted for the corresponding segment of the μ gene transfer vector, as described in *Results*.

RESULTS

Detection of the Mutation Affecting IgM Function. The wild-type hybridoma PC7 secretes normal, cytolytic IgM(κ) specific for the hapten phosphocholine (5). We have described the isolation from PC7 of the mutant cell line 42, which produces pentameric IgM that has the normal affinity for phosphocholine but is unable to lyse phosphocholine-coupled erythrocytes (5). This phenotype suggested a defect in the μ-chain C_μ region. We therefore isolated the DNA segment encoding the C_μ region from the mutant 42 cell line (see *Materials and Methods*). To test whether this segment bears the mutation causing the cytolysis defect, we made use of a gene transfer system for expressing a functional μ gene (Fig. 1). This system derives from the hybridoma Sp6, which produces IgM(κ) specific for the hapten TNP. The TNP-specific μ gene from this cell line has been inserted into the vector pSV2-neo, thus creating the vector pR-Sp6 (7). The capacity of this vector to encode a functional μ heavy chain has been verified by transferring it to the Sp6-derived cell line igm-10, which has lost the μ gene of Sp6 but still makes the TNP-specific κ chain. Transformants of igm-10 produce normal TNP-specific, cytolytic IgM (7). To test the C_μ region of the mutant, we therefore replaced the normal C_μ region of the vector pR-Sp6 with the DNA segment obtained from mutant 42 (Fig. 1). This vector was then transferred to the cell line igm-10, and transformants resistant to the drug G418 were selected. Their culture medium was then tested for

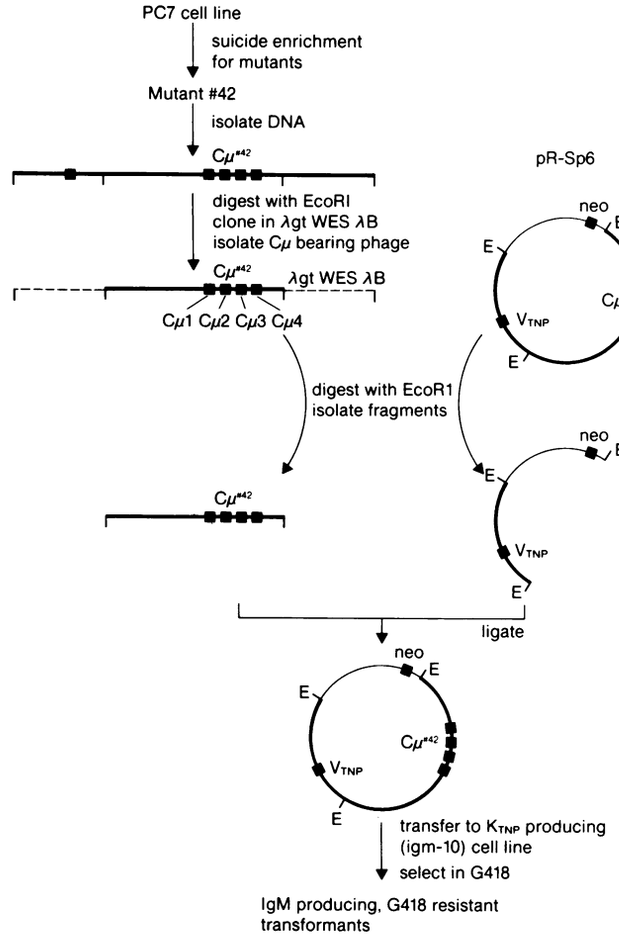


FIG. 1. Isolation and assay of the C_μ gene from mutant 42. The plasmid pR-Sp6 for transferring and expressing the TNP-specific μ gene has been described (7). The DNA segment encoding the μ-chain constant (C_μ) region was isolated from mutant 42 after digestion with *Eco*RI (E) and substituted for the wild-type C_μ DNA segment in pR-Sp6.

TNP-specific IgM by measuring the hemagglutination titer using TNP-SRBC. As reported (7), independent transformants produce different amounts of IgM, and those making the highest amount were subcloned by limiting dilution and analyzed further. The wild-type or mutant origin of the immunoglobulin produced by the transformants is indicated by superscript—i.e., IgM^{wt} or IgM^{42}.

We have tested the cytolytic activity of the IgMs by their capacity to lyse TNP-SRBC, as measured by release of hemoglobin (Fig. 2). The instantaneous rate of lysis is expected to depend linearly on the IgM concentration (15)—i.e., the fraction of unlysed erythrocytes (u) is expected to follow the equation $u = e^{-\alpha I} = 1 -$ fraction lysed, in which I = IgM concentration and α = proportionality constant related to the activity of IgM. The results plotted in Fig. 2*a* indicate that this equation is a fair approximation and that IgM^{wt} is about 100-fold more active than IgM^{42} in promoting hemolysis. We have tested whether lysis by normal IgM can be inhibited by the IgM^{42} preparation by measuring lysis by mixtures of IgM^{wt} and various amounts of IgM^{42}. As shown in Fig. 2*b*, lysis by the mixture approximates the lysis expected if the activities of the IgMs are additive—that is, lysis by IgM^{wt} (20 ng/ml) is not inhibited by the simultaneous addition of IgM^{42} (10–300 ng/ml). Comparable results were obtained when the IgM^{42} was added prior to the IgM^{wt}.

It has been more convenient to use end-point dilution titers to compare the cytolytic activity of the mutant and normal IgMs. Thus, we have used agglutination and lysis titers on

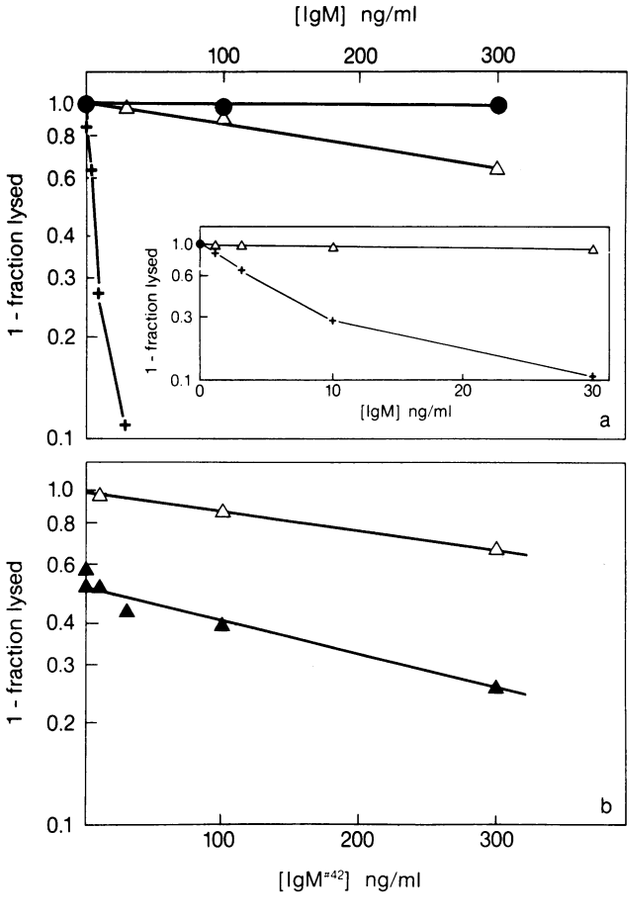


FIG. 2. Cytolytic activity of mutant and normal IgMs. (*a*) TNP-SRBC were preincubated at 37°C for 10 min with culture supernatants containing the indicated concentrations of IgM^{42} (transformant T/42A-1.1; △) and IgM^{wt} (transformant T/Sp6-1.4; +). Under these conditions, >90% of the IgM was bound to the TNP-SRBC. Complement was then added and the incubation was continued for 10 min. The fraction of cells that were lysed was assessed by the release of hemoglobin, as measured by the increased absorbance at 541 nm. No lysis was seen from the culture supernatant of the recipient cells igm-10 (●), which was assayed at dilutions comparable to those used for the transformants. The fraction of unlysed cells—i.e., 1 − fraction lysed—is plotted as a function of IgM concentration (0–300 ng/ml) and is plotted in the *Inset* on an expanded scale (0–30 ng/ml). (*b*) To test for possible inhibition of lysis by IgM^{42}, TNP-SRBC were preincubated for 10 min with culture supernatants (transformant T/42A-1.1) containing the indicated amount of IgM^{42} in the presence (▲) and absence (△) of 20 ng of IgM^{wt} (transformant T/Sp6-1.4). Complement was then added and the released hemoglobin was measured, as in *a*.

TNP-SRBC as a measure of the binding and cytolytic activity of the IgMs (Table 1). These results indicate that IgM^{42} has normal specific activity for agglutination. By contrast, IgM^{42} is less effective than IgM^{wt} by a factor of 30 at initiating hemolysis. Because the wild-type and mutant 42 IgMs have the same specific activity in the agglutination assay, the ratio of lysis to agglutination titers also measures whether the IgM is mutant or normal—that is, the ratio of hemolysis to hemagglutination titers indicates that the IgM encoded by the C_μ DNA from mutant 42 has ≈4% (≈3^{-3}) the hemolytic activity of normal IgM.

Mapping and Nucleotide Sequence of Mutation 42. The gene transfer results implied that the C_μ DNA from mutant 42 encodes an abnormal μ chain. To map the mutation we constructed recombinant μ-chain genes bearing DNA segments in part from the normal gene and in part from the mutant gene. For each construction we then transferred the recombinant genes and tested whether the resulting IgM was

Table 1. Comparative analysis of IgM derived from the wild-type and mutant 42 μ-chain genes

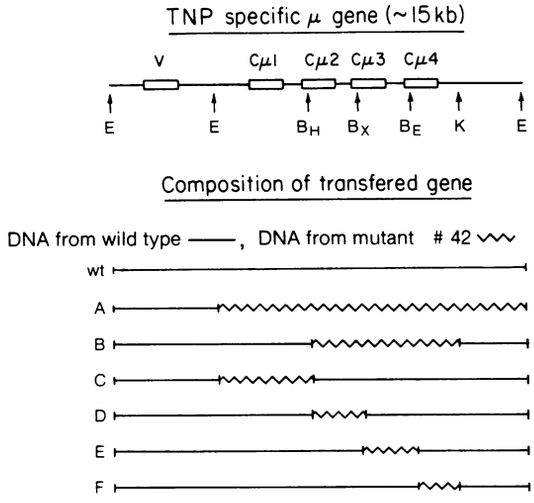
Transformant	IgM, μg/ml	Titer on TNP-SRBC, activity per ml		Specific activity, activity per μg		Ratio*
		Binding	Lysis	Binding	Lysis	
T/Sp6-1.4	13	3^6	3^5	56	19	3^{-1}
T/42A-1.1	5	3^5	3^1	49	0.6	3^{-4}

The IgM from transformants expressing the normal μ gene (T/Sp6-1.4) or the μ gene derived from C_μ DNA segment of mutant 42 (T/42A-1.1) was tested for TNP-specific binding and cytolytic activity as follows. The supernatants of T/Sp6-1.4 and T/42A-1.1 cultures grown to about 10^6 cells per ml were serially diluted 1:3. The IgM concentration in culture supernatants was determined by ELISA. Binding to TNP-SRBC was measured as agglutination titer—i.e., the highest dilution at which agglutination of TNP-SRBC occurred. The cytolytic titer of the IgM on TNP-SRBC represents the highest dilution at which a 2-μl aliquot caused lysis of TNP-SRBC suspended in agarose in the presence of complement (5% guinea pig serum) (5). The precision in the measurement of IgM concentration is about 1.5-fold; the precision in the lysis and agglutination titers is one dilution step (3-fold). Specific activity is calculated by dividing the agglutination or lysis titers by the IgM concentration.
*Lysis/binding ratio.

mutant or normal by measuring the ratio of hemolysis to hemagglutination titers (Fig. 3). These results show that the mutation lies on the 555-base-pair (bp) segment bounded by the indicated *Bst*XI and *Bst*EII sites.

The wild-type and 42 C_μ DNA subfragments bounded by the *Bam*HI and *Kpn* I sites (Fig. 3) were inserted into phage M13mp18 and the nucleotide sequence of the above mentioned 555-bp segments was determined by using the dideoxy method (Fig. 4). The segment from mutant 42 differs from the wild type at one position, resulting in a single amino acid substitution in the third C domain: codon AGT → AAT, so that the serine at amino acid position 406 is replaced by asparagine. We have used site-directed mutagenesis to prove that the Ser-406 → Asn change is sufficient to impair IgM-mediated cytolysis. The 22-nucleotide oligomer GGCACCTTCAACGCTAAGGGTG was synthesized and used to substitute the alternative Asn codon, AAC in the wild-type C_μ DNA at the site corresponding to position 406. As described in *Materials and Methods*, the oligonucleotide was hybridized to the M13-C_μ phage bearing the normal C_μ *Bam*HI–*Kpn* I segment and used to prime DNA synthesis. The *Bam*HI–*Kpn* I segment from phage bearing the mutation was then substituted for the normal segment in the pR-Sp6 vector to produce reconstructed mutant μ genes, denoted 42R. This vector was then transferred to igm-10, and the IgM of transformants (IgM^{42R}) was tested for its cytolytic activity (Table 2). These results indicate that IgM^{42} and IgM^{42R} have ≈4% of the cytolytic activity of normal IgM and are apparently identical.

Further Comparisons of Mutant and Normal IgMs. Another abnormality of the μ chain made by the original mutant 42 cell line was its slower than normal mobility in $NaDodSO_4$/PAGE (4). This feature can be seen for μ^{42} made by the transformants and is, moreover, apparent for the μ encoded by the reconstructed (42R) mutant gene (Fig. 5). As reported for the original mutant 42, the mutant and normal μ chains comigrate when made in the presence of tunicamycin, a compound that inhibits glycosylation (Fig. 5). The results indicate that the same mutation affects cytolytic activity and μ-chain mobility and suggest that this mutation causes the μ chain to be abnormally glycosylated. We have also analyzed the mobility of the mutant and wild-type IgMs without reducing disulfide bonds (Fig. 6). Although IgM^{42} is predominantly (≈70%) pentameric, a larger fraction of the mutant IgM migrates in $NadodSO_4$/PAGE as monomers than is the



TNP specific		Ratio
binding	lysis	lysis / binding
3^7	3^5	3^{-2}
3^6	3^2	3^{-4}
3^3	$<3^0$	$<3^{-3}$
3^5	3^4	3^{-1}
3^5	3^4	3^{-1}
3^6	3^2	3^{-4}
3^4	3^3	3^{-1}

FIG. 3. Mapping of the mutation in C_μ DNA from mutant 42. C_μ genes were recombined *in vitro* by cutting and religating segments into the pSV2-neo transfer vector, using the indicated restriction enzyme sites. The recombinant genes were transferred to the igm-10 cell line and transformants were selected by growth in G418. The TNP-specific binding and cytolytic activity of the IgMs from typical transformants were tested as described in the legend to Table 1. The results for transformants producing the most IgM are reported here. Restriction enzyme sites are indicated: E, *Eco*RI; B_H, *Bam*HI; B_X, *Bst*XI; B_E, *Bst*EII; K, *Kpn* I. kb, Kilobases.

case for wild-type IgM. The analysis of mixtures of IgM^{wt} and IgM^{42} (Fig. 2*b*) indicates that the monomers do not inhibit hemolysis.

DISCUSSION

Our analysis of the mutant IgM^{42} indicates that a single amino acid substitution, Ser-406 → Asn, in the third C domain of the μ heavy chain reduces the capacity of the mutant IgM to trigger complement-mediated lysis by a factor of 25–100.

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                    Asn (#42)
                       A
SerHisProAsnGlyThrPheSerAlaLysGlyValAlaSerValCysValGluAspTrp
AGCCATCCCAATGGCACCTTCAGTGCTAAGGGTGTGGCTAGTGTTTGTGTGGAAGACTGG
        10        20        30        40        50        60
    BstXI

AsnAsnArgLysGluPheValCysThrValThrHisArgAspLeuProSerProGlnLys
AATAACAGGAAGGAATTTGTGTGTACTGTGACTCACAGGGATCTGCCTTCACCACAGAAG
        70        80        90       100       110       120

           .....Cμ3)
LysPheIleSerLysProAsn
AAATTCATCTCAAAACCCAATGGTAGGTATCCCCCCTTCCCTTCCCCTCCAATTGCAGGA
       130       140       150       160       170       180

 CCCTTCCCGTACCTCATAGGGAGGGCAGGTCCTCTTCCACCCTATCCTCACTACTGTCTT
       190       200       210       220       230       240

       (Cμ4.....
       GluValHisLysHisProProAlaValTyrLeuLeuProProAlaArgGluGln
CATTTACAGAGGTGCACAAACATCCACCTGCTGTGTACCTGCTGCCACCAGCTCGTGAGC
       250       260       270       280       290       300

  LeuAsnLeuArgGluSerAlaThrValThrCysLeuValLysGlyPheSerProAlaAsp
AACTGAACCTGAGGGAGTCAGCCACAGTCACCTGCTTGGTGAAGGGCTTCTCTCCTGCAG
       310       320       330       340       350       360

  IleSerValGlnTrpLeuGlnArgGlyGlnLeuLeuProGlnGluLysTyrValThrSer
ACATCAGTGTGCAGTGGCTTCAGAGAGGGCAACTCTTGCCCCAAGAGAAGTATGTGACCA
       370       380       390       400       410       420

  AlaProMetProGluProGlyAlaProGlyPheTyrPheThrHisSerIleLeuThrVal
GTGCCCCGATGCCAGAGCCTGGGGCCCCAGGCTTCTACTTTACCCACAGCATCCTGACTG
       430       440       450       460       470       480

  ThrGluGluGluTrpAsnSerGlyGluThrTyrThrCysValValGlyHisGluAlaLeu
TGACAGAGGAGGAATGGAACTCCGGAGAGACCTATACCTGTGTTGTAGGCCACGAGGCCC
       490       500       510       520       530       540

  ProHisLeuValThrGlu
TGCCACACCTGGTGACCGAG
       550       560
          BstEII
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FIG. 4. Nucleotide sequence change in the C_μ gene of mutant 42. The nucleotide sequence of the normal and mutant μ gene between the *Bst*XI and *Bst*EII sites (Fig. 3) was determined. For this purpose, the antisense strands of the *Bam*HI–*Kpn* I segment of the wild-type and mutant 42 μ genes were inserted into phage M13mp18. Oligomers (15-mers) corresponding to nucleotide positions −38, 179, and 354 were synthesized and used as primers for sequencing. The nucleotide and corresponding amino acid sequences for the wild-type μ gene segment are shown; the single nucleotide difference between the wild-type and mutant sequences is indicated.

These results thus suggest that the structure of the $C_\mu3$ domain is important for complement activation, although it is, of course, possible that the Ser-406 → Asn substitution affects the structure of a region of IgM outside the $C_\mu3$ domain.

Various parts of the IgM molecule have been implicated by other workers in complement activation. Studies on μ-chain fragments suggest that the C1 component is activated by binding to a site in the $C_\mu4$ domain (16, 17). Siegel and Cathou argued that complement activation could be influenced by the second C domain of the μ heavy chain (18). Leptin and Melchers found a monoclonal antibody that binds to $C_\mu3$ blocks complement-dependent lysis, whereas antibodies against the other three C domains do not (19), suggesting that a site in $C_\mu3$ is important for complement activation. Analysis of the interactions of IgG and complement also leads to the expectation that the $C_\mu3$ domain is involved in C1 activation—that is, the second C ($C_\gamma2$) domain of the γ heavy chain is thought to be analogous to the $C_\mu3$ domain (20), and studies using fragments of IgG molecules have suggested a role for the $C_\gamma2$ domain in complement binding and activation (21, 22).

Possible Effects of Mutation 42 on the Glycosylation of the μ Chain. The μ chain of mutant 42 appears to be more highly glycosylated than the normal μ chain—that is, when the μ chains are unglycosylated (synthesized in the presence of tunicamycin), the wild-type and mutant chains comigrate; however, the glycosylated mutant μ chain migrates in two bands, both slower than the glycosylated normal μ chain (Fig. 5 and ref. 5). Glycosylation occurs at asparagine residues in the sequence Asn-Xaa-Ser/Thr (23–25). The

Table 2. Comparative analysis of IgM derived from the mutant 42 and 42R μ-chain genes

	Titer on TNP-SRBC, activity per ml		
Transformant	Binding	Lysis	Ratio*
T/Sp6-1.4	3^6	3^4	3^{-2}
T/42E-1.1	3^6	3^1	3^{-5}
T/42R-8.2	3^6	3^1	3^{-5}
T/42R-9.2	3^7	3^1	3^{-6}

The reconstructed mutation was introduced into a functional μ gene and this μ gene was transferred into igm-10 cells. The culture supernatant of two transformants (T/42R-8.2 and T/42R-9.2) was tested as described in the legend to Table 1. Culture supernatants from transformants (T/Sp6-1.4 and T/42E-1.1) expressing the normal μ gene and the mutant μ gene (structure E in Fig. 3), respectively, were included for comparison.
*Lysis/binding ratio.

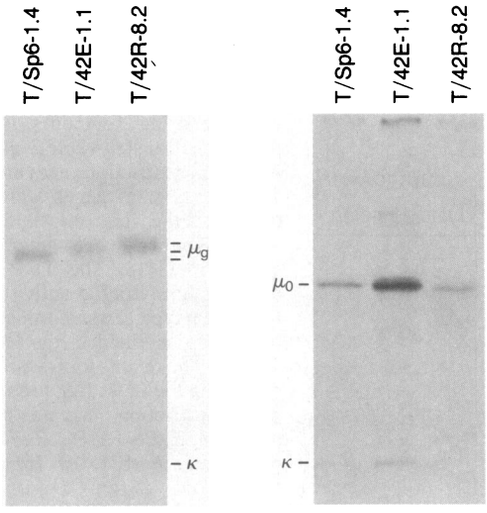

FIG. 5. Analysis by NaDodSO$_4$/PAGE of mutant and wild-type μ chains. The transformants T/Sp6-1.4, T/42E-1.1 and T/42R-8.2 express normal, mutant (structure E in Fig. 2), and reconstructed mutant μ genes, respectively. The left three lanes analyze secreted material biosynthetically labeled in the absence of tunicamycin. The right three lanes analyze total (extra- and intracellular) Ig labeled in the presence of tunicamycin. The material was allowed to react with rabbit anti-μ serum. For this analysis, precipitates were treated with 2-mercaptoethanol to reduce disulfide bonds and subjected to NaDodSO$_4$/PAGE (9). μ_g and μ_0 indicate the positions of the glycosylated and unglycosylated μ chains, respectively.

change Ser-406 → Asn generates the sequence Asn-Ala-Lys, so that this change is not expected to create a new site for glycosylation. It is, however, interesting that the mutation creates the sequence Thr-Phe-Asn (where asparagine is at position 406), which, in common with the canonical glycosylation sequence Asn-Xaa-Thr, brings threonine and asparagine into close proximity. We are not aware of information that would *a priori* preclude the possibility of glycosylation at the Asn-406 in the mutant μ chain. However, a more likely possibility is that the amino acid substitution influences the glycosylation at another asparagine in $C_\mu3$, or elsewhere. For example, glycosylation in $C_\mu3$ normally occurs at Asn-402 and Asn-364 (26), and the Ser-406 → Asn change might alter the pattern of glycosylation of these nearby oligosaccharides. Another possibility is that the Asn-Phe-Thr sequence at positions 347–349, which is not normally glycosylated (26), has become a substrate for glycosylation in the mutant 42 μ chain.

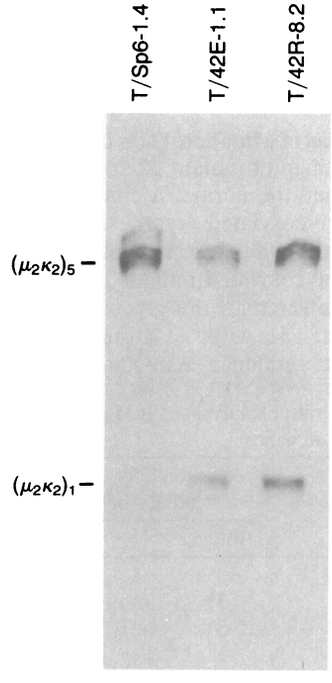

FIG. 6. Analysis by NaDodSO$_4$/PAGE of mutant and wild-type IgM. The anti-μ precipitated material described for Fig. 5, left three lanes, was subjected to NaDodSO$_4$/PAGE without reducing disulfide bonds (10). $(\mu_2\kappa_2)_5$ and $(\mu_2\kappa_2)_1$ indicate the positions of IgM pentamers and monomers, respectively.

IgG is glycosylated only at Asn-297 in the $C_\gamma2$ domain, and several studies indicate that deglycosylated IgGs of various types and species are less able to activate complement than normal IgG (27–30). Taken together, these results raise the possibility that the Ser-406 → Asn change might affect the interaction of IgM and complement by altering the glycosylation of the μ chain. If so, this mutant would offer an unusual opportunity to analyze how the amino acid sequence of the IgM protein influences the specificity of glycosylation and how, in turn, the oligosaccharide structure can influence protein function.

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