The Functional Architecture of an Acetylcholine Receptor-mimicking Antibody*

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Mo2-3 is a monoclonal antibody that partially mimics the nicotinic acetylcholine receptor (AChR). Its three-dimensional structure has been previously predicted by molecular modeling, suggesting that 29 complementarity determining region (CDR) residues and 2 framework residues are exposed to solvent. To identify the antibody residues that bind to the antigen, i.e. snake toxin that binds specifically to AChR, we (i) produced the scFv form of Mo2-3 fused to alkaline phosphatase, in the periplasmic space of Escherichia coli; (ii) submitted approximately 75% of exposed residues of the fused scFv to individual or combined mutations, and (iii) identified the residues whose mutations affect scFv binding to the toxin, using a sensitive enzyme-linked immunosorbent assay. 11 critical residues were identified, including 8 heavy chain residues, 2 framework residues, and 1 light chain residue. They cover a surface of approximately 800 Å2, with a subset of most critical residues (VHD31, VIY92, and VHGI01) and several aromatic residues. This functional architecture not only constitutes a plausible complementary binding surface for the snake toxin but also offers a structural basis to ultimately understand the capacity of the antibody to partially mimic AChR.

Understanding of the molecular events that are associated with activation and inactivation of channels by specific ligands requires an elucidation of the complementary interacting sites (1, 2). In this respect, crystallographic analyses of ion channel-ligand complexes would be most informative; however, due to their membranous character, ion channels are not readily amenable to crystallization. Therefore, alternative approaches are required to investigate the sites by which a ligand and an ion channel interact. One of them, which has not been much considered as yet, consists of exploiting the capacity of some antibody to mimic, at least to some extent, a receptor or an ion channel. Previous studies have revealed that some monoclonal antibodies raised against receptor-specific ligands share structural and/or functional properties with receptors (3–6). Evidently, such receptor-mimicking antibodies constitute interesting templates to approach the structure of a ligand binding site on its receptor. The goal of this study was, therefore, to elucidate the functional architecture of the paratope of Mo2-3, an antibody that shares a number of properties with the nicotinic acetylcholine receptor (AChR), a ligand-gated ion channel (6).

These properties include (i) the recognition of all short-chain curaremimetic toxins from snakes; (ii) elicitation of anti-AChR antibodies; (iii) a number of complementarity determining regions (CDR) residues that are identical to those found between positions 100/128 of the α-subunit of AChR, and (iv) binding to a snake toxin epitope that involves 10 residues among which 8 are also present in the determinant by which the same toxin binds to AChR (6).

Residues of an antibody paratope that interact with an antigen are usually identified by crystallographic analyses of the antibody-antigen complex (7, 8). However, despite our extensive efforts, no crystal of Mo2-3 alone or complexed to its antigen has ever been obtained. Therefore, to identify the functional structure of the antibody, we decided to combine modeling studies and mutational analyses. In this strategy, two steps are required. First, a predictive model of the antibody has to be established. Using the amino acid sequence of Mo2-3 deduced from its cloned cDNA, four predictive models of its combining antigen site including CDRs were previously selected (9). All models suggested that the putative combining site of Mo2-3 possesses 31 solvent exposed residues that presumably include most functional elements of the antibody combining site. The second step is the major objective of this study. It corresponds to an experimental identification of the functional residues of Mo2-3.

The goal of this paper is 4-fold. First, using a convenient bacterial expression system, we produced, in the periplasmic space of Escherichia coli, a functionally active scFv fragment of Mo2-3 inserted in the N-terminal region of the reporter bacterial alkaline phosphatase, as described previously for various antigens or antibodies (10–12). Second, we developed a sensitive ELISA that allowed us to determine the binding affinity of Mo2-3-toxin complex. Third, we introduced 43 individual mutations within the combining site of the chimeric form of scFvMo2-3 and using the ELISA, we identified the residues whose mutations affect the stability of the antibody-toxin complex. Fourth, we examined the compatibility of the proposed structural models of the antibody (9) with the results of our mutational analysis and analyzed the architecture of the identified functional paratope in the frame of its capacity to mimic some properties of AChR.

EXPERIMENTAL PROCEDURES

Construction of the scFv Fragment—The scFv fragment was constructed from the plasmid pLIP2/Fab, which contains the DNA fragments encoding VHCH1 and VLCL domains of Mo2-3 (11), using the PCR methodology (13) and following the procedures described previ-
ously (14, 15). A 14- amino acids linker-coding sequence (Lk), derived from Pantaloni et al. (16) was introduced between the C terminus and the N terminus of VL and VH domains, respectively. Primers VLFOR (5′-GAAAGGAGAGAGATCCGCAATATGTTAGTACCGCCAG-3′), VLVR (5′-GAAAGGAGAGAGATCCGCAATATGTTAGTACCGCCAG-3′), VLVA (5′-GAAAGGAGAGAGATCCGCAATATGTTAGTACCGCCAG-3′), and VLVB (5′-GAAAGGAGAGAGATCCGCAATATGTTAGTACCGCCAG-3′) were finally used. Radioimmunoassays (RIAs) were performed notech) for 2 h. 3,3

**Site-directed Mutagenesis—**Numbering of the amino acids forming the scFv of Moz-3 is that of Tenette et al. (9). The following nucleotide probes were used for mutagenesis: VLNSA1 (5′-CAAGATGCTGTTAGTACCTTGAACAGAAATA-3′), VLNSB2 (5′-CAAGATGCTGTTAGTACCTTGAACAGAAATA-3′), VLNSC3 (5′-CAAGATGCTGTTAGTACCTTGAACAGAAATA-3′), VLNSD4 (5′-CAAGATGCTGTTAGTACCTTGAACAGAAATA-3′), VLNSE5 (5′-CAAGATGCTGTTAGTACCTTGAACAGAAATA-3′), and VLNSF6 (5′-CAAGATGCTGTTAGTACCTTGAACAGAAATA-3′). The probes were used to express scFv-PhoA (alkaline phosphatase) hybrid proteins within the periplasmic space (11, 12).

**Production and Characterization of Recombinant scFvM<sub>2-3</sub>-PhoA Hybrid**

Our laboratory has previously developed a system to produce hybridizing recombinant colorimetric proteins in <i>E. coli</i>. They are bi-functional hybrids with a single chain protein that is introduced between residues +6 and +7 of the mature form of PhoA. Monomeric proteins that have been successfully associated to PhoA include a snake toxin (10), rat prolactin (19), human proinsulin (20), and an scFv derived from an anti-human IgG antibody (12). Another scFv was also successfully fused to PhoA by others, using a similar approach (21). Using an appropriate protocol, the hybrid can also accommodate the presence of the more complex structure of a Fab per PhoA subunit (11). In both cases, the gene encoding the scFv of PhoA preceded the gene encoding the mature amino acid sequence of PhoA. Clearly, the presence of the first six amino acids of PhoA favors expression of hybrids into the periplasm of <i>E. coli</i>, where their cysteine residues are oxidized into disulfide bonds and their native-like structure is formed (10–12, 20).

Moz-3, a toxin-specific monoclonal antibody (22), and its mutants were produced as colorimetric scFv fragments. In practice, the gene encoding the Moz-3 VL fragment was linked to the gene encoding its VH fragment by a DNA encoding a hybrid with the first six amino acids of PhoA favors expression of hybrids into the periplasm of <i>E. coli</i>, where their cysteine residues are oxidized into disulfide bonds and their native-like structure is formed (10–12, 20).

**Expression and Characterization of Wild-type and Mutants scFv-PhoA Hybrid Proteins—**Expression procedure, periplasmic extractions, and PhoA activity measurements were performed as described previously (12). Western blot experiments were achieved as follows. 10 μl of crude periplasmic extract was run on a 12.5% denaturing and reducing polyacrylamide gel. Proteins were then transferred on a nitrocellulose membrane (Optitran, Schleicher & Schuell). After saturation in a 2% milk-PBS solution for 10 min, revelation was made first using an anti-Moz-3 monoclonal antibody diluted to 1/10,000 in PBS-TP (16) for 2 h and second, after washing, a goat anti-mouse IgG coupled with peroxidase diluted to the 1/2000 in PBS (GAM-P0, Immotech) for 2 h. 3,3’-Diaminobenzidine tetrahydrochloride tablets (SIGMA) were finally used. Radioimmunoassays (RIAs) were performed using 3H-toxin α and crude periplasmic extracts concentrated 10-fold by ultrafiltration through a YM30 membrane (Amicon). Toxin-antibody complexes formed (using 0.1 ml of previously concentrated solution and 1.24 pmol of tritiated toxin) after 2 h of incubation at room temperature were precipitated using a 12.5% PEG 6000 solution (11, 17, 18). Enzyme immunoassays (EIAs), i.e. competition ELISA, were achieved as follows. Briefly, ELISAs were performed using 100 μl of crude periplasmic extracts containing 1.5 μg/ml scFv-PhoA proteins, as determined by measuring the PhoA activity (12), and microtiter plates coated overnight with 10 μl of a 10 μg/ml solution of purified toxin α in 0.05 M Tris-HCl, pH 7.4, buffer. Nonspecific binding was assessed by incubating the periplasmic extract with an excess of toxin α in solution (5.10<sup>-10</sup> mM). Revelation was made either using the PhoA substrate p-nitrophenyl phosphate (11) or using the following multiple-step revelation procedure. After washing, VIAP diluted to 1/10000 in 0.1 M Tris-HCl, pH 7.4, and 0.1% BSA was incubated at room temperature for 2 h. After a second washing, the W3110 (5′-TCCGGTAAATCCTCTGACAGGACCAAGGACCCAG-3′) and crude periplasmic extracts concentrated 10-fold by ultrafiltration were used. The same buffer was incubated at room temperature for 0.5 h. Microtiter plates were read with a Titertek Multiaskan MCC/340 spectrophotometer at 410 nm.
inter-chain disulfides in parent antibodies stabilizes the VH-VL interface (25).

Incubation of crude periplasmic extracts on microtitration plates coated with toxin α revealed the presence of PhoA activity. The corresponding colorimetric signal decreased in a dose-dependent manner in the presence of increasing amounts of toxin α, with an IC50 of 2.10⁻⁸ M (Fig. 3). Therefore, the periplasmic extract contains a bifunctional component that both recognizes specifically the coated toxin and generates a colorimetric signal. Since the bacterial PhoA is active as a periplasmic extract contains a bifunctional component that we anticipate that this component is the hybrid (scFvMα2-3)2-PhoA. In agreement with this view, we obtained the same result with the (FabMα2-3)2-PhoA conjugate, which was previously characterized (11) (Fig. 3). We have not quantitatively determined the activity of the PhoA moiety; however, since our previous constructions displayed high colorimetric activity (11, 12), we suspect that it is the same here. Therefore, our data strongly suggest that the periplasmic space of the bacteria contained the expected scFv form of Mα2-3. We also noted that neither inversion of the order of the variable domains (VH was followed by the 14-amino acids linker and VL) nor an increase of the length of the linker peptide from 14 residues (GSTSGSKPGSGEGSTKG),² as suggested in other instances (27), modified the specificity and affinity of the recombinant hybrid (data not shown). Therefore, the N terminus residues from VL or VH domains of Mα2-3 as well as the linker length play little role in the functional site of the antibody, in agreement with previous data obtained with other scFvs.

On the ELISAs Made With scFvMα2-3-PhoA Hybrids

The covalent association of PhoA activity with scFvMα2-3 offers a convenient means to rapidly detect a specific binding of the hybrid to a coated antigen, using a conventional ELISA. However, in such an assay, it is often unclear as to whether the colorimetric assay indeed reflects the actual affinity of the antigen for the antibody. To shed light on this issue, we compared binding data resulting from both ELISA and a sensitive RIA made with a soluble antigen (18). We used four (scFvMα2-3)2-PhoA mutants having a wide range of different affinities. The first two mutants were substituted in the CDR1L. For one of them, Asn-31 was changed into Ala and for the other an additional Ser was inserted between Ser-94 and Asn-31. These mutants were named, respectively, VLNS31A and VLS30+S. The other two mutants were substituted in the CDR3L. They had an additional proline after position Ser-94 and was named VLS94+P. The original sequence (VLYSS(92–94)) of the other mutant was changed into VLHRF(92–94)+P. The four mutants were produced and then analyzed by SDS-PAGE and Western blot experiments. Fig. 1B shows that their electrophoretic profiles were similar to that of the wild-type hybrid, the similarity of band intensities suggesting equivalent levels of production in all cases. Tritiated toxin bound specifically to each mutant and in a saturable manner, as indicated by an RIA performed in solution (18). Two typical binding curves and their derived Scatchard plots are shown in Fig. 4. The affinities of the four mutants had Kd values ranging from 28 nM to 500 nM (Fig. 5A). In parallel experiments, we monitored the colorimetric signal at 410 nm of the same mutants, using an ELISA made with coated toxin α. A simple glance at Fig. 5A is sufficient to reveal that the signal intensities determined by ELISA nicely parallel the Kd values derived from RIA. The mutant VLHRF(92–94)+P has a slightly higher affinity, as compared with the wild-type antibody, whereas VL(S30+S) has the weakest affinity in both cases. Fig. 5B illustrates the linear relationship of the competitive inhibition curves of the four scFvMα2-3-PhoA mutants with their IC50 calculated from competition curves give in both cases a value of 2.10⁻⁸ M.

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relationship between the colorimetric intensities and \( K_d \) values. Therefore, the ELISA allowed us to estimate \( K_d \) values using small amounts of hybrids and without the use of a radioactive tracer.

**On the Strategy of the Mutational Analysis**

In the absence of crystallographic data, the structure of Mo2-3 has been previously predicted by modeling analysis (9). Thus, 31 residues of a putative combining site were proposed to be exposed to solvent. As indicated by various x-ray diffraction studies of antibody-antigen complexes (28, 29), these exposed residues should include most functional residues, and therefore, they were investigated in priority. The amino acids predicted to be surface exposed are indicated in Fig. 6. Mutations at these positions are anticipated to not dramatically alter the structure of the antibody fragment, and as a result, a subsequent variation in binding affinity is likely to reflect the functionality of mutated residues. Some studies even suggested that an affinity change greater than 10-fold might indicate that the mutated residue is energetically important for the stabilization of the antibody-antigen complex (28). Thus, and although one must keep in mind that this value may vary from one complex to another, we have considered, at least in the first step of our analysis, that a mutated residue is functionally important.

For example, to clarify the role of the numerous tyrosines that surround the CDR3H, 7 of them were mutated into both Ser and Phe. Also, the role of CDR3H and CDR3L was investigated by multiple and random mutagenesis. Finally, the length of CDR1L and CDR3L was respectively modified by inserting one additional Ser or Pro residue. As a whole, 43 mutants were constructed to probe, individually or in combination, the role of 23 of 31 solvent exposed positions (29 from CDRs and 2 from framework).

**Functional Mapping of the Paratope of Mo2-3**

All mutants were produced as (scFv\(\text{Mo2-3}\))2-PhoA hybrids, and the correctness of their amino acid sequence was inferred from analysis of their nucleotide sequence. For all mutants, the level of production was approximately comparable with that of wild-type scFv-PhoA Mo2-3. The effect of mutations on stability of antibody-toxin complexes was investigated by ELISA, and the \( K_d \) values were then derived from the linear relationship shown in Fig. 5B. The results are compiled in Figs. 7 and 8.

**On the Functional Role of Heavy Chain Residues—Diffraction studies of a number of antibody/antigen complexes have shown that the heavy chain and especially its CDR3H plays a predominant interacting role with antigen (30–32). Our data obtained with scFv-Mo2-3 agree with this view since simultaneous mutations of the 5 residues MGATA (100–104) into STTY or of the four residues MGAT (100–103) into STTY completely abolished the capacity of the antibody to bind to the toxin. Therefore, 1 or more residues of the MGAT sequence may be functionally important (7) and also because VLY49 and VLY67 belong to the curious “tyrosine-ring,” which was suspected to include functionally important residues (9). Several different substitutions were introduced at various positions. For example, to clarify the role of the numerous tyrosines that surround the CDR3H, 7 of them were mutated into both Ser and Phe. Also, the role of CDR3H and CDR3L was investigated by multiple and random mutagenesis. Finally, the length of CDR1L and CDR3L was respectively modified by inserting one additional Ser or Pro residue. As a whole, 43 mutants were constructed to probe, individually or in combination, the role of 23 of 31 solvent exposed positions (29 from CDRs and 2 from framework).
substantial affinity decreases, some of them being quite large. Since each of these four residues is functionally important, it is not surprising that their concomitant mutations destroyed the binding capacity of the antibody. Mutation of the adjacent A104 into serine had virtually no effect on the complex stability. Therefore, though more mutants may be needed, we suspect that A104 plays a weak, if any, functional role. One may note that the mutation A102G caused little affinity change, whereas mutation A102S and especially A102V caused much larger effects. This type of situation is not uncommon. In effect, though a residue may establish clear contacts with residues of the binding partner, it may be more sensitive to some mutations than to others, as illustrated with Asp-101 of lysozyme whose mutations caused either none or large effects on the stability of a lysozyme-anti-lysozyme antibody complex (33). It is possible that introduction of an apparently inefficient mutation generates compensations in terms of local interactions and/or ratios of the kinetic constants of association to dissociation of the antibody-antigen complex.

The CDR1H and CDR2H have been predicted to contain respectively 3 (D31, Y32, Y33) and 6 (Y52, A54, S55, N57, T58, and K59) exposed residues. The three residues of the CDR1H and four of the six residues of the CDR2H have been individually mutated (Figs. 7 and 8). At least one type of mutation at positions 31, 32, 52, or 54 caused a substantial affinity change, suggesting the functional importance of these residues. The effect of mutations at position 31 deserves some comments. Mutation D31A caused a large affinity change, suggesting the functional importance of a negative charge at this position. However, mutation of D31 by the negatively charged glutamic acid also caused a large affinity change, virtually identical to that observed upon mutation by the neutral asparagine. We anticipate, therefore, that the negative charge of D31 establishes precise contacts with toxin residues, perhaps with an
exposed positively charged amino acid. One may note that mutations at the adjacent residue Y32 into phenylalanine or serine caused marked affinity decreases, indicating the critical importance of its hydroxyl group and perhaps of its aromatic side chain. Mutations at Y33, S55, and N57 had little effect on the binding affinity, suggesting that these residues do not belong to the functional paratope; however, one should keep in mind that positions 55 and 57 have undergone only one mutation.

In summary, the functional contribution of the heavy chain is associated with the involvement of at least D31, Y32, Y52, A54, M100, G101, A102, and T103. That so many residues of the heavy chain are functionally important does not mean that the isolated heavy chain can bind to the antigen. Unpublished data revealed that when produced alone, the Fd domain (VH-CH1) of Mα2-3 has no binding activity. Not unexpectedly, the presence of the light chain is required for the heavy chain to exert its functionality.

On the Functional Role of Light Chain Residues—Though the light chain tend to contribute less than the heavy chain to the paratope function, the CDR3L often includes functional residues (7). The CDR3L of Mα2-3 includes 8 residues (QQD-YSSYT), among which YSS at positions 92–94 were predicted to be solvent-exposed (9). The surface covered by these residues is close to 250 Å², a value that is smaller than that observed in other CDR3L of similar length, including those from HyHEL5 (430 Å²) and 17e8 (320 Å²) (34, 35). To probe the individual role of the only exposed and central tyrosine (Y92), we mutated it into either serine or phenylalanine. No affinity change was observed. To further explore the possible role of this loop, we then introduced an additional proline between Y95 and T96, and surprisingly, this modification, which makes the loop belong to another canonical group (36), caused an affinity decrease lower than 2-fold. In view of this lack of incidence, we decided not only to simultaneously mutate the 3 exposed residues YSS into HRF but also to maintain the additional proline. Despite such considerable changes, no alteration of the affinity was observed. A similar result was obtained upon mutations of YSSY into ARKQW. Therefore, the exposed residues of the CDR3L do not play a functional role in the paratope of Mα2-3.

The CDR2L contains 7 residues (YASSRYT) from positions 50 to 56. All but A51 are predicted to be exposed to solvent, like in addition, the adjacent framework-specific Y49 (FR2L) and Y67 (FR3L). At least one mutation at Y49, Y50, or Y67 caused a substantial affinity decrease (Figs. 7 and 8), suggesting that these residues play some functional role. Mutations at R54 caused an affinity decrease comprised between 3–10-fold, which could reflect its vicinity with the functional paratope, as observed in other instances (6). Mutations at S52, S53, and Y55 caused affinity changes lower than 3-fold, suggesting that they are excluded from the paratope. At present, only T56 has not been mutated individually because of unexpected difficulties in producing the corresponding mutants.

The CDR1L is characterized by both a close-to-canonical sequence and the presence of two individualized patches of residues exposed to solvent (9). These are K24, S26, and Q27...
and S28, S30 and N31. Among these, K24, S26, Q27, and S28 are at the periphery of the paratope, being located beyond the functionally unimportant CDR3L. In contrast, S30 and N31 occupy a more central position, being sandwiched between the critical residues Y50L and Y67L (Fig. 6A). N31 was therefore mutated into alanine; however, only a 3-fold affinity decrease was observed, suggesting that this residue is not functionally important. To further explore the potential role of this region, we inserted an additional serine between S30 and N31. Though an insertion is known to often cause local structural perturbations, we only observed a 14-fold affinity decrease associated with the insertion of the additional serine. This result further confirms that this region of the paratope is unlikely to be functionally critical; however, the affinity decrease that is associated with the serine insertion will have to be explored more deeply.

**Analysis of the Paratopic Region of Mo2-3**

We identified 12 residues as being outside the functional paratope. These excluded residues are colored in green and yellow in Fig. 9. These are VH(Y33, S55, N57, and A104) and VL(N31, S52, S53, R54, Y55, Y92, S93, and S94). By contrast, 11 other residues VH(D31, Y32, Y52, A54, M100, G101, A102, T103), VLY50, Y49FR2L, and Y67FR3L seem to be functionally important. They are nicely sandwiched between the excluded residues, a finding that further emphasizes the nice delineation of the paratope. The functionally important residues of the paratope are shown in Fig. 9. Residues in orange and red indicate that at least one type of mutation caused an affinity decrease comprised, respectively, between 10–100-fold and higher than 100-fold. Only mutations at three positions caused affinity decreases by more than 2 orders of magnitude, irrespective of the type of mutations that was introduced. These most critical residues, all located on the heavy chain, are VH(D31, Y32, and Y52). They may constitute a sort of energetic core of the functional paratope, as it occurs in other protein-protein complexes whose stabilization is driven by a small subset of energetically important residues (37–42).

The three-dimensional models of Mo2-3 (9) suggested that eight exposed residues are tyrosine residues, *i.e.* VH(Y32, Y33, and Y52) and VL(Y50, Y55, Y92, Y49, and Y67), forming a large aromatic ring. Five of them (VHY52, VHY32, VLY49, VLY50, and VLY67) are functionally important, covering a little more than half of the aromatic ring. As deduced from various mutations, their hydroxyl group and/or aromatic ring may be functionally important. This is not surprising since tyrosine residues are reported to often establish a diversity of interactions (43, 44), including van der Waals contacts and hydrogen bonds (45–48), and aromatic-aromatic interactions (49).

Therefore, a functional paratope of Mo2-3 has emerged from our mutational analysis. Interestingly enough, it possesses several features that agree with those of paratopes identified by x-ray diffraction studies. These include (i) eleven functional
residues that cover a homogeneous area of approximately 800 Å² (7, 34, 53), (ii) a subset of most critical functional residues (29, 37, 51), (iii) several residues of the heavy chain hypervariable loops (4, 50), (iv) a small number of framework residues (34, 52), and (v) several aromatic side chains (31, 44, 54). Moreover, the paratope shares some expected complementary characteristics with the toxin epitope (55) (see Fig. 10). Thus, the two interacting surfaces possess 10 and 11 functional residues (in red and orange) that cover similar surfaces of 700–800 Å². Also, the paratope covers a long shaped surface that is compatible with the shape of the epitope that essentially covers the central loop of the toxin. In addition, both the epitope and paratope include a core of hydrophobic and/or weakly polar residues, Trp-29, Ile-36, and Ile-50 for the epitope and Met-100, Gly-101, and Ala-102 for the paratope, surrounded by more polar residues. Finally, the epitope and paratope possess a comparable number (7 and 8, respectively) of critical residues (in red). Evidently, a clearer comparison will be possible when interacting pairs of residues are identified, a work that is now in progress. Therefore, by probing approximately 75% of residues predicted to be solvent-exposed, the model depicting three-dimensional structure of Mo2-3 (9) has now received an experimentally based validation.

**On the AChR-mimicking Architecture of Mo2-3**

As already mentioned, Mo2-3 shares some functional and structural properties with the nicotinic AChR (3). In particular, it was observed that the amino acid sequences of some of its CDRs can be aligned with the region 100–128 of the α-subunit of AChR from mouse and Torpedo (6). In principle, one may expect that some of these aligned paratope residues can exert a functional property. As shown in Fig. 11, residues Y50, A51, T56, and V58 of CDR2L can be aligned, respectively, with F100, A101, T106, and V108 of CDR2H and residues 115–128 of the receptor. Six aligned residues are exposed to solvent. These are Y52, A54, S55, N57, T56, and V58. The remaining functional residues of the CDR3H, i.e. MGA (100–102) display little analogies, with the corresponding residues of AChRs. Evidently, one may also expect that residues of the striking LLDY sequence play a functional role. These residues, not being solvent-exposed, were not investigated in priority; however, preliminary results indicated that mutation of L106 into tyrosine totally abolishes the binding capacity of the antibody. Since L106 is predicted to be buried, one may wonder whether its replacement by an aromatic residue may have destabilized the paratope structure? If this is the case, the leucine could play a similar structural role both in the antibody and receptor to maintain the structural organization of their toxin binding determinants (6). On the other hand, L106 might play a direct functional role, which would imply that this buried residue becomes accessible upon binding to the antigen. Such a scenario is compatible with various structural studies, which show that interacting zones of antigens and/or antibodies undergo substantial structural change upon complex formation (56).

Four residues of the CDR2H (I48, W50, P53, A54) can be aligned with identical residues in the AChR subunit. As a consequence, a correspondence is observed between VHT58 and S126 of the receptor (Fig. 11) and between residues 47–60 of CDR2H and residues 115–128 of the receptor. Six aligned residues are exposed to solvent. These are Y52, A54, S55, N57, T58, and K59, and of these, Y52 and A54 are functionally important. However, only the latter residue is strictly identical in both the CDR and receptor. Furthermore, VHA54 and A122α are both preceded by a proline residue. Possibly, a pair of similar residues has been selected in both cases, one of them (proline) playing a structural role and the other (an alanine) exerting a functional role. We have not investigated whether the corresponding I48/T116α, W50/W118α, and T58/S126α do play any functional or structural role.

In summary, of the CDR residues that were aligned with the α subunit of AChR residues, only 3 (VLY50, VLT56, VHA54) play a functional role in the paratope. Such a situation is not surprising since receptor mimicry by antibodies is not sequential but essentially topographical (52, 57). Moreover, if Mo2-3 shares a number of similarities with AChR, it also differs from it by various aspects including its inability to recognize all AChR ligands like the long chain toxins that are so analogous to the short chain toxins that the antibody does specifically recognize (6). Hence, the paratope might also reflect these differences. A detailed structural analysis of the antibody-toxin complex as well as an identification of the functionally important
AchR residues are now required to better understand the functional mimicry between the antibody and AchR, in particular regarding the regions to which the curaremimetic toxins bind.

CONCLUSION

This study reports on the delineation of the functional architecture of Mo2-3, a monoclonal antibody that partially mimics AchR. The interacting residues in the antibody paratope and antigen epitope now remain to be identified. This may be possible, using an appropriate mutational strategy of both the antibody and antigen (58, 59). In this respect, we may wonder whether the important negatively charged amino acid (VHDD1) of the paratope is in proximity to any of the 3 positively charged residues of the epitope (6). Preliminary data have already indicated that this is indeed the case (60).

REFERENCES

1. Changeux, J. P. (1990) Trends Pharmacol. Sci. 11, 408–412
2. Staple, P., Kolmakova-Partensky, L., and Miller, C. (1994) Biochemistry 33, 443–450
3. Davis, S. J., Schofield, G. A., Somoza, C., Buck, D. W., Healey, D. G., Rieber, E. P., Reiter, C., and Williams, A. F. (1992) Nature 358, 76–79
4. Doring, E., Sterger, R., Gruetz, G., Von Baehr, R., and Scheinder-Mergener, J. (1994) Mol. Immunol. 31, 1059–1067
5. Ducancel, F., Merienne, K., Fromen-Romano, C., Tremo, O., Pillet, L., Drevet, P., Zinn-Justin, S., Boulain, J-C., and Menez, A. (1996) J. Biol. Chem. 271, 31345–31353
6. Davies, D. R., and Padlan, E. A. (1990) Annu. Rev. Biochem. 59, 439–473
7. Padlan, E. A. (1996) Proteins Struct. Funct. Genet. 26, 9–31
8. Gillet, D., Ducancel, F., Pradel, E., Leomot, M., Mene, A., and Boulain, J. C. (1992) Prot. Eng. 5, 273–287
9. Ducancel, F., Gillet, D., Carrier, A., Lajeunese, E., Mene, A., and Boulain, J. C. (1993) Bio/Technology 11, 603–605
10. Carrier, A., Ducancel, F., Settiawan, N., Bantisc, C., Lemaire, B., Leomot, M., Drevet, P., Mene, A., and Boulain, J. C. (1995) J. Immunol. Methods 181, 177–186
11. Saik, R. K., Scharf, S., Falloona, M., Bullin, K. B., Horn, G. T., Erlich, H. A., and Arneheim, N. (1985) Science 230, 1350–1354
12. Skerra, A., and Pluckthun, A. (1988) Science 240, 1038–1040
13. Bird, R. E., Hardman, K. D., Jacobson, J. W., Johnson, S., Kaufman, B. M., Rines, J. F., and Hardman, K. D. (1991) J. Biol. Chem. 265, 593–604
14. Skerra, A., and Pluckthun, A. (1988) Annu. Rev. Biochem. 57, 7–12
15. Bird, R. E., Hardman, K. D., Jacobson, J. W., Johnson, S., Kaufman, B. M., Rines, J. F., and Hardman, K. D. (1991) J. Biol. Chem. 265, 593–604
16. Pantaliano, M. W., Bird, R. E., Johnson, L. S., Avel, D. D., Sudd, S. W., Wood, G. E., and Webster, R. G., and Colman, P. M. (1994) Biochemistry 33, 6828–6835
17. Finzel, B. C., and Davies, D. R. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8075–8079
18. Zhou, G. W., Guo, J., Huang, W., Scanlan, T. S., and Fletcher, R. J. (1994) Science 265, 1059–1064
19. Chothia, C., and Lesk, A. M. (1987) J. Mol. Biol. 196, 901–917
20. Novotny, P., Brucher, E. R., and Saul, P. A. (1989) Biochemistry 28, 4735–4749
21. Janin, J., and Chothia, C. (1990) J. Mol. Biol. 265, 16027–16030
22. de Vos, A. M., Ulls, M., and Kassikoff, A. A. (1992) Science 255, 306–312
23. Cole, J. C., and Arnheim, N. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3958–3962
24. Herb, S., Silvertown, E. W., Padlan, E. A., Cohen, G. H., Smith-Gill, S. J., Finzel, B. C., and Davies, D. R. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7807–7809
25. Dell’Aqua, V., Goldblum, E. R., Eisenstein, E., and Mariuzza, R. A. (1996) Biochemistry 35, 9676–9676
26. Boulain, J. C. (1996) J. Mol. Biol. 265, 1059–1064
27. Tenette, C., Ducancel, F., and Smith, J. C. (1996) Proteins Struct. Funct. Genet. 26, 9–31
28. Prasad, L., Sharma, S., Van den Selaar, M., Quail, J. W., Lee, J. S., Waygood, E. B., Wilson, K. S., Dauter, Z., and Delhate, L. J. T. (1993) J. Biol. Chem. 268, 10705–10708
29. Woche, G. W., Guo, J., Huang, W., Scanlan, T. S., and Fletcher, R. J. (1994) Science 265, 1059–1064
30. Novotny, P., Brucher, E. R., and Saul, P. A. (1989) Biochemistry 28, 4735–4749
31. Herron, J. N., Ho, L. M., Mox, M. S., and Edmunson, A. B. (1989) Proteins Struct. Funct. Genet. 5, 271–280
32. Rines, J. F., Schulze-Gahmen, U., and Wilson, I. A. (1992) Science 255, 959–965
33. Spring, J., Sha, Y., Prasad, L., and Delhaere, L. T. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 539–543
34. Burky, S. R., and Petsko, G. A. (1988) Adv. Protein Chem. 32, 125–159
35. Rieckmann, L., and Davies, J. (1995) J. Biol. Chem. 270, 141–152
36. Garcia, K. C., Ronco, P. M., Verruck, P. J., Bringer, A. T., and Amzel, L. M. (1992) Science 257, 502–507
37. Fields, B. A., Goldsmith, F. A., Yern, X., Poljak, R. J., and Mariuzza, R. A. (1995) Nature 374, 739–742
38. Braden, B. C., and Poljak, R. J. (1995) FASEB J. 9, 9–15
39. Davies, D. R., and Cohen, G. H. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 7–12
40. Tremo, O., Lemaire, C., Drevet, P., Pinkasfeld, S., Ducancel, F., Boulain, J-C., and Menez, A. (1995) J. Biol. Chem. 270, 9226–9239
41. Davies, D. R., and Chacko, S. (1995) Biochemistry 34, 421–427
42. Malby, R. L., Tulp, W. H., Harvel, V. R., McKinn-Breschkin, J. L., Laver, W. G., Webster, R. G., and Colman, P. M. (1994) Structure (Lond.) 2, 733–746
43. Schreiber, G., and Fersht, A. R. (1995) J. Mol. Biol. 248, 478–486
44. Hidalgo, P., and Mackinnon, R. (1995) Science 268, 307–310
45. Janon, K. (1996) In: Ingénierie Génétique des Anticorps: Mime du Site par Lequel Un Recepteur Lie ses Effecteurs. Ph.D. Thesis, Université de Paris XI, Paris, France
