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Mouse Immunoglobulin Kappa Chain MPC 11: Extra Amino-Terminal Residues

Abstract. *The kappa chain from the immunoglobulin of myeloma tumor MPC 11 has 12 extra residues at its amino terminus, the first six of which are identical to the residues at positions 1 to 6 of typical mouse kappa chains and at positions 13 to 18 of MPC 11 itself. Two of the peptide bonds within this extra 12-residue segment are cleaved under very mild conditions.*

I have discovered two unusual features of the kappa-type light chain from the mouse myeloma tumor line MPC 11. (i) When its first 75 amino acids are aligned with other mouse kappa chains, MPC 11 is seen to contain an extra 12 amino acids at its amino terminus. (ii) Two of the peptide bonds within these 12 extra amino acids undergo facile hydrolysis under very mild conditions which do not ordinarily promote peptide bond cleavage.

A clone of cells (clone 45–6) in continuous culture derived from MPC 11 (*1*) was supplied by M. D. Scharff. These cells were injected intraperitoneally into BALB/c mice, which formed 3 to 12 ml of peritoneal fluid a few weeks later. The MPC 11 myeloma protein was isolated from the supernatant of this fluid by precipitation three times with an equal volume of 3.5*M* ammonium sulfate at 0°C. Light chains were separated from heavy chains by chromatography on Sephadex G-200 in 5*M* guanidinium chloride after cleaving all disulfide bonds in 7*M* guanidinium chloride. This cleavage was effected either by reduction with 10 m*M* dithiothreitol and alkylation with 22 m*M* iodoacetamide (*2*) or by mixed disulfide formation (*3*) with 5 m*M* dithiothreitol and 0.25*M* diethanol disulfide or diethylamine disulfide (cystamine).

A partial amino acid sequence for positions 1 to 51 of the MPC 11 light chain was determined by the methods of Smithies *et al.* (*4*) on an Edman-Begg sequenator (*5*) (Illitron division of Illinois Tool Works). The positions of the half-cystine residues were determined with [^{14}C]carboxamidomethyl-labeled light chain by counting a portion of each step from the sequenator.

In order to extend this sequence, [^{14}C]carboxamidomethyl light chain (20 mg/ml) was cleaved at its methionine residues (*6*) by CNBr (70 mg/ml) in 70 percent formic acid at room temperature for 1 hour, which was sufficient time to destroy all the methionine residues. Amino acid analysis had indicated that the light chain contains four methionine residues. Three of these had already been located at positions 4, 16, and 23 in the partial sequence of the undigested light chain. The fourth was presumed to correspond to a constant-region methionine located 39 positions from the end of mouse kappa chains (*7*). Consequently, I expected five CNBr fragments of very different sizes: a very large one (CNBr-I) extending from position 24 to the constant-region methionine; a 39-residue fragment (CNBr-II) extending from the residue following this methionine to the end of the chain; and three small peptides of 4, 7, and 12 amino acids. I tried to separate these fragments by chromatography on Sephadex G-100 in 10 percent formic acid. This procedure resolved the CNBr digest into three peaks. The first peak contained 50 percent of the radioactivity; since it was excluded from the gel, it presumably consisted of large aggregates. The second peak contained 37 percent of the radioactivity; the sequence data reported below indicate that it was an aggregate containing both CNBr-I and CNBr-II (CNBr-I/II). The third peak, containing 13 percent of the radioactivity, was not characterized.

CNBr-I/II was sequenced for 52 steps by the same methods used for the undigested light chain (as described above). Two sequences were present. One of these corresponded to the 39-residue constant-region fragment CNBr-II, which was expected to have a sequence identical to that at the corresponding positions of other mouse kappa chains [for example, positions 176 to 214 of the kappa chain of tumor MOPC 21 (*7*)]. The residues from this fragment decreased in yield as the sequenator degradation progressed, and could not be detected at all beyond step 24. Presumably this decrease in yield was due to partial loss of the short residual peptide during extraction of excess reagents at each step of the degradation. The other sequence obtained from CNBr-I/II continued for all 52 steps and corresponded to the expected fragment CNBr-I. The first 28 residues of this sequence were entirely consistent with the partial sequence that had already been determined for positions 24 to 51 of the undigested light chain.

Taken together, the data from CNBr-I/II and undigested light chain sufficed to determine the sequence of the first 75 amino acid residues of the MPC 11 light chain. In Fig. 1, this sequence is compared to the mouse kappa chain MOPC 21 (*7*) and to other sequences that will be discussed later. Residues 13 to 75 of MPC 11 align very well with residues 1 to 63 of MOPC 21. By contrast, if residues 1 to 75 of MPC 11 are aligned with residues 1 to 75 of MOPC 21 (or of any other mouse or human kappa chain) no homology is evident except at residues 1 to 6, which are similar or identical to the corresponding residues of typical mouse kappa chains and to residues 13 to 18 of MPC 11 itself (Fig. 1, underlined residues). MPC 11 therefore has 12 extra residues at its amino terminus.

These extra residues call to mind the extra residues that are found at the amino terminus of the precursor polypeptides coded by messenger RNA's (mRNA's) of mouse kappa chains. This precursor has been found by four groups of investigators using mRNA's for tumors MOPC 41 (*8*), MOPC 21 (*9*), and MOPC 321 (*10*). In the case of MOPC 41, the precursor migrated in sodium dodecyl sulfate gel electrophoresis as if it were about 20 residues longer than mature light chain. By the same criterion, the precursor for MOPC 21 seemed to be about 14 residues longer than mature light chain; it was also shown to have an altered NH_2-terminal tryptic peptide and to contain a chain-

initiating methionine. Schechter (*10*) determined the positions of the leucine residues among the first 60 residues of the MOPC 321 precursor: they were found at positions 6, 7, 8, 11, 12, 13, 24, 31, and 35. Among its first 40 residues, mature MOPC 321 kappa chain contains leucine residues at positions 4, 11, and 15 (*11*); as shown in Fig. 1, these align perfectly with the leucine residues at positions 24, 31, and 35 of the precursor. The MOPC 321 precursor therefore undoubtedly contains 20 extra NH_2-terminal residues. The number, 20, is identical to the estimated number of extra residues in the MOPC 41 precursor; the difference between it and the apparent number of extra residues in the MOPC 21 precursor, 14, is probably no greater than the experimental uncertainty in the latter.

MOPC 321 is very similar in sequence to MOPC 70, and the genes for these two proteins therefore probably diverged very recently in evolutionary time (*12*); the divergence between MOPC 70 and MOPC 41, on the other hand, is the oldest (or one of the oldest) among the known mouse and human kappa chains (*13*). The extra precursor amino acids are found in both evolutionary branches (one leading to MOPC 41, the other to MOPC 321 and MOPC 70) emanating from the earliest known kappa chain divergence. They are consequently very likely to be of ancient origin and to be shared by all human and mouse kappa chains.

The extra NH_2-terminal residues in MPC 11 are probably not the extra precursor amino acids themselves. First, not one of the leucine residues at positions 6, 7, and 8 of the MOPC 321 precursor coincides with a leucine in MPC 11 (see Fig. 1). Second, considering the probably ancient origin of the extra precursor amino acids, it is difficult to believe that those corresponding to residues 1 to 6 of MPC 11 would be so similar to residues 1 to 6 of mature kappa chains.

It is much more likely that the MPC 11 gene was produced by a recent breakage and reunion of two kappa variable region genes. One of these genes (the left-most) could have given rise to residues 1 to 6, the other (right-most) gene to the residues from position 13 to the end of the variable region. This hypothesis accounts for the identity of residues 1 to 6 and 13 to 18, but leaves the ancestry of residues 7 to 12 to be explained. According to the reasoning of Smithies *et al.* (*14*), this sequence of six amino acid residues might have one of three ancestries, depending on the exact configuration of the broken strands of DNA that were joined to form the MPC 11 gene. They might derive (i) from codons 7 to 12 of the left-most gene, (ii) from the codons for the last six of the extra precursor residues coded by the right-most gene, or (iii) from either or both of these with much scrambling or mutation of the sequence.

The first ancestry is countered (but not ruled out altogether) by the absence of any significant homology of residues 7 to 12 of MPC 11 to residues 7 to 12 of other mouse kappa chains (*15*). According to the second ancestry, residues 7 to 12 of MPC 11 should be homologous to the last six of the extra NH_2-terminal residues in kappa chain precursors. This ancestry is consistent with the absence of a leucine residue in positions 15 to 20 of the MOPC 321 precursor, since there is no leucine in positions 7 to 12 of MPC 11; but proof or disproof of this ancestry will probably not be possible until a more complete precursor sequence is determined. There is no evidence either for or against the third ancestry.

Several preparations of MPC 11 myeloma protein which had been subjected to carboxymethylcellulose chromatography at *p*H 5.5 showed variable amounts of an altered light chain which migrated faster toward the cathode in starch-gel electrophoresis than did the light chain from freshly isolated MPC 11 (*16*). A short Edman degradation revealed that this altered light chain was a mixture of two sequences, one lacking the NH_2-terminal four residues,

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                                                                      5                   10                  15                  20
MPC 11                                          Asp Ile Val Met Thr Gln Phe Ala Gly Val Asp Gly Asp Ile Val Met Thr Gln Ser His
                                                                                                                      5
MOPC 21                                                                                                 Asn Ile Val Met Thr Gln Ser Pro
                                      5                   10                  15                  20                  25
MOPC 321 precursor    •   •   •   •   •   Leu Leu Leu •   •   Leu Leu Leu •   •   •   •   •   •   •   •   •   •   Leu •   •   •   •
                                                                                                                      5
MOPC 321                                                                                              Asp Ile Val Leu Thr Gln Ser Pro

                                      25                  30                  35                  40                  45
MPC 11                Lys Phe Met Ser Thr Ser Val Gly Asx Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asx Val Ser Thr Thr Val Ala Trp Tyr
                          10                  15                  20                  25                  30                  35
MOPC 21               Lys Ser Met Ser Met Ser Val Gly Glu Arg Val Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Val Thr Tyr Val Ser Trp Tyr
                          30                  35                  40                  45                  50                  55
MOPC 321 precursor    •   •   Leu •   •   •   Leu •   •   •   •   •   •   •   •   •   •   •   •   •   •   •   •   •   •   •   •   •
                          10                  15                  20                  25                  30                  35
MOPC 321              Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser Val Asn Thr Tyr Gly Asn Ser Phe

                          50                  55                  60                  65                  70                  75
MPC 11                Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Asx Arg Phe Thr
                                  40                  45                  50                  55                  60
MOPC 21               Gln Gln Lys Pro Glu Gln Ser Pro Lys Leu Leu Ile Tyr Gly Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr
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Fig. 1. Comparison of the amino acid sequences of MPC 11 (this report), MOPC 21 (*7*), the MOPC 321 precursor (*10*), and MOPC 321 (*11*). The amino acids repeated at positions 1 to 6 and 13 to 18 of MPC 11 are underlined. Residue 1 of MPC 11 was not determined by sequence analysis to be Asp rather than Asn, but this is assumed on the basis of the electrophoretic behavior of the light chain (*16*). At those positions indicated by a dot (•) in the MOPC 321 precursor sequence, Leu was not present, but it was not determined which of the other amino acid residues *was* present. The abbreviations for the amino acid residues are: Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, half-cystine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine; Gln, glutamine; Asn, asparagine; Asx, either asparagine or aspartic acid.

the other the NH_2-terminal seven residues. An altered light chain electrophoretically identical to this adventitious one could be produced at will by incubating the whole myeloma protein at *p*H 4.8 (0.15*M* NaCl, 0.05*M* acetic acid, *p*H adjusted with NaOH) for 1 to 2 hours at 37°C.

One other instance of an association between facile hydrolysis and unusual chain length has been reported for the human myeloma protein Sac (*4, 14, 17*). The light chain of this protein has lost 65 internal amino acid residues within its variable region (*14*). Because the missing residues are internal, this loss is undoubtedly due to a deletion at the DNA level. This shortened light chain undergoes hydrolysis at the first peptide bond with loss of some of its NH_2-terminal residue (*4*). Moreover, the heavy chain in the same protein lacks almost its entire variable region, including the amino terminus (*17*). While it is possible that the loss in the heavy chain is due to deletion at the DNA level, such an explanation requires the coincidence of two rare deletions in the same cell line. It is simpler to attribute the "deletion" in the Sac heavy chain to peptide-bond hydrolysis facilitated in some way by the unusual length of the Sac light chain, in a manner analogous to the loss of one amino acid residue from the Sac light chain itself, or of four or seven residues from the unusually long MPC 11 light chain, as discussed in this report.

Laskov and Scharff (*1*) found a small difference between tryptic peptide chromatograms of MPC 11 light chains produced by a cloned cell line and by the original tumor line. Different degrees of conversion of the two preparations of light chain to the altered form described here would provide a simple explanation of this difference.

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Serum Dopamine-β-Hydroxylase Activity: Sibling-Sibling Correlation

Abstract. *Dopamine-β-hydroxylase activity is released into the blood with catecholamines from the adrenal medulla and sympathetic nerves. This enzyme activity has been measured in the blood of 317 normal children and 227 normal adults. A significant sibling-sibling correlation of serum dopamine-β-hydroxylase values was found in the 94 sibling pairs tested. Frequency distributions of serum enzyme values in both children and adults suggest the existence of two populations with regard to serum activity of this enzyme.*

Dopamine-β-hydroxylase (DBH) is the enzyme that catalyzes the conversion of 3,4-dihydroxyphenylethylamine (dopamine) to the neurotransmitter norepinephrine (*1*). The enzyme is localized to the catecholamine-containing vesicular structures in sympathetic nerve terminals and in the adrenal medulla (*2*). In response to stimulation of sympathetic nerves and the adrenal gland, DBH is released with catecholamines (*3*). A serum DBH activity described in both man and experimental animals is biochemically and immunochemically identical to the activity found in sympathetic nerves and in the adrenal medulla (*4*). This enzyme activity is increased in the blood of experimental animals and man under stress (*5*), and is decreased in the rat after partial chemical sympathectomy with the drug 6-hydroxydopamine (*6*). It has been suggested that serum DBH activity might be a useful measure of the level of function of the sympathetic nervous system (*4*). In many clinical studies, abnormal levels of circulating DBH activity have been described in patients with various diseases, including familial dysautonomia, neuroblastoma, torsion dystonia, and Down's syndrome (*7*). A wide range of values in control human subjects has been reported in all studies performed. Little is known of the factors that regulate serum or tissue DBH activity in man. We wish to describe the results of a study of serum DBH activity in large populations of normal children and adults, the results of which demonstrate a significant correlation of DBH activity between siblings and suggest that there may be at least two populations in humans with regard to serum levels of this catecholamine biosynthetic enzyme.

Blood samples from 317 children age 6 through 12 years were obtained as part of a survey of lipoprotein values in children attending the Rochester, Minnesota, schools. Blood was obtained at school in the early morning after an overnight fast. Blood samples from 227 adults were obtained from normal blood donors receiving no medications.

Activity of DBH was determined by a sensitive radiochemical enzymatic assay (*4, 7, 8*). The only alteration in the assay procedure as previously described was that 1*M* acetate buffer, *p*H 4.9, was substituted for 1*M* tris(hydroxymethyl)-aminomethane hydrochloride buffer, *p*H 6. This change resulted in a final incubation *p*H of 5.2, the optimal *p*H for the determination of DBH activity in human serum in this assay system. One unit of enzyme activity represents the produc-