Genetic Mechanism Accounting for Precise Immunoglobulin Domain Deletion in a Variant of MPC 11 Myeloma Cells

Abstract. *A variant of the MPC 11 cell line, M 311, produces a short immunoglobulin heavy chain. When compared with the parental γ_{2b} heavy chain, M 311 was found to have a carboxyl terminal deletion comprising the C_H3 domain. The COOH-terminal cyanogen bromide (CNBr) cleavage fragment of M 311 is identical to a corresponding segment of a parental heavy chain CNBr fragment, with the exception of a substitution of asparagine for lysine at the COOH-terminal residue. This observation enabled prediction of both the parental DNA sequence in this region and the genetic mechanism which generated the variant, a frameshift followed by premature termination. This hypothesis is supported by studies of the DNA sequence of the MPC 11 γ_{2b} constant region gene.*

A number of variants in immunoglobulin production have been isolated from the mouse myeloma cell line MPC 11 ($IgG_{2b,\kappa}$). Some variants have lost the ability to synthesize immunoglobulin heavy chains (*1*); others make altered heavy chains. Among the latter are those that synthesize short heavy chains (*2*) and others that make heavy chains containing serological and structural markers distinctive of the IgG_{2a} subclass (*3*). In order to decipher the genetic mechanisms by which these variants have arisen, we have undertaken structural analysis of the parental MPC 11 heavy chain (*4*). Such studies have provided a helpful comparison to the sequence of the DNA that codes for the γ_{2b} heavy chain constant region (*5*, 6) and have enabled us to analyze in detail two variant heavy chains having characteristics of a second subclass—IgG_{2a}. We have shown that one variant has an Fc fragment which is entirely γ_{2a}-like (*4*), while a second variant makes a γ_{2b}-γ_{2a} hybrid chain with the crossover point located in the C_H2 domain between residues N-308 and N-332 (counting from the amino terminal) (*7*).

We have studied the protein synthesized by the M 311 cell line, which was derived from the MPC 11 cell line after its exposure to melphalan, a phenylalanine mustard used to treat human multiple myeloma (*2*). The M 311 cells synthesize a short heavy chain of 40,000 daltons in comparison to the parental heavy chain of 55,000 daltons. Other distinctive features of the variant molecule include loss of both Fc and γ_{2b} subclass-specific serological markers and an aberrant assembly pathway leading to the secretion of both the complete immunoglobulin (H_2L_2) and the half immunoglobulin (HL) molecules (*2*, *8*). In addition, 30 percent of the secreted H_2L_2 and HL remain unglycosylated (*8*).

One way to account for a decreased molecular size in M 311 is by a COOH-terminal deletion. Therefore, we focused our investigation on the COOH-terminal portion of the molecule and have used our studies of the parental MPC 11 immunoglobulin as a guide. When MPC 11 H_2L_2 is cleaved with CNBr, it gives rise to two major fragments: pool I, a doublet of ~100,000 daltons, comprising almost all of the N-terminal half of the molecule; and pool II, a smaller fragment of ~29,000 daltons, comprising the COOH-terminal half of the heavy chain (*4*). Both H_2L_2 and HL from M 311 were subjected to cleavage by CNBr and two major fragments (pools I′ and II′) were separated by gel filtration on a Sephadex G-75 column equilibrated in 0.05*M* formic acid. The larger fragment from M 311 H_2L_2 (pool I′) was similar in size to pool I of the parent. Figure 1A shows the comparison by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of pool II from MPC 11 with the smaller fragment (pool II′) from M 311 H_2L_2 and HL. The smaller fragments (pool II′) from M 311 H_2L_2 and HL were identical in size to each other but considerably smaller than the corresponding fragment from MPC 11, indicating that there was a deletion in this segment. Parental pool II contains three CNBr-cleavage fragments that can be separated by gel filtration after total reduction and alkylation (*4*). The fragments were named by their order of elution and were aligned (*4*) as follows: NH_2-II.2-II.1-II.3-COOH (see Fig. 2). When the smaller fragment (pool II′) from M 311 was treated under similar conditions, the elution profile was considerably altered (Fig. 1B); M 311 lacked the COOH-terminal parental fragment, II.3; and instead had a new fragment, termed II.C, which was identified in this procedure solely by its content of radioalkylated cysteine.

Three pools were obtained (Fig. 1B) and subjected to amino acid analysis and automated sequential degradation. The first pool (not named) was an aggregate arising from incomplete reduction. The second pool contained a fragment that had an amino acid composition and an NH_2-terminal sequence identical to that of II.2 from MPC 11 (*4*). The third pool contained a fragment, II.C, which had no homoserine (Fig. 2B), thus indicating it to be the COOH-terminal fragment. Automated sequential degradation of II.C (Fig. 2B) showed that its sequence was identical to the NH_2-terminal segment of parental fragment II.1 for 23 steps. At the 24th step, M 311 contained an asparagine residue instead of the parental lysine residue and the fragment terminated there. The amino acid composition of II.C was consistent with the sequence. Additional confirmation of the

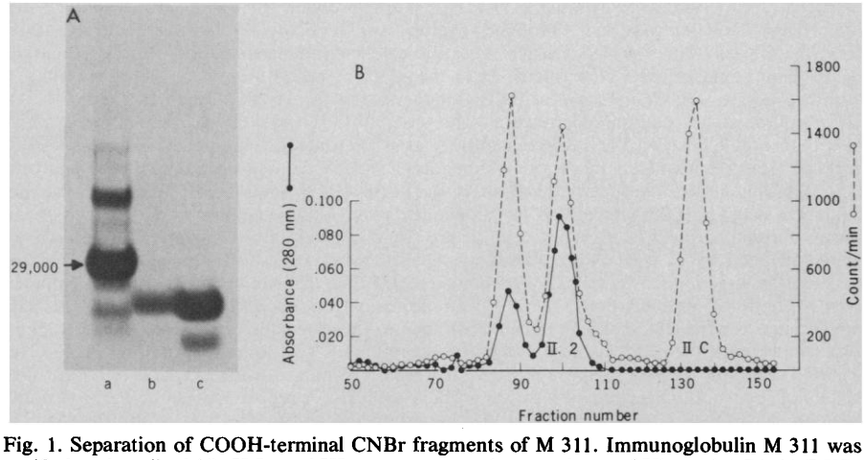

Fig. 1. Separation of COOH-terminal CNBr fragments of M 311. Immunoglobulin M 311 was purified as described (*4*). H_2L_2 and HL were separated on a column of Ultrogel ACA 44 (LKB, Washington, D.C.), equilibrated in 0.15*M* NH_4HCO_3. Both M 311 H_2L_2 and HL were submitted to CNBr cleavage in 70 percent formic acid (*4*). The cleavage products, pools I′ and II′, were separated on a Sephadex G-75 column, equilibrated in 0.05*M* formic acid. (A) Comparison by 5 percent SDS-PAGE of (a) pool II of MPC 11 (29,000 daltons); (b) pool II′ from M 311 H_2L_2; and (c) pool II′ from M 311 HL. (B) Separation of CNBr-cleavage fragments from pool II′ of M 311 HL after total reduction and alkylation with tritiated iodoacetic acid on a column of Sephadex G-75, equilibrated in 8*M* urea, 0.1*M* formic acid. Fraction volume was 5 ml; 0.05 ml of every other tube was taken for liquid scintillation counting.

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COOH-terminal sequence was achieved by treating II.C with carboxypeptidase A (Fig. 2B).

From the genetic code, we predicted a DNA sequence for the II.C region that might account for the substitution of asparagine for lysine as well as premature termination of the chain (Fig. 2B). By postulating a deletion of two nucleotides affecting lysine at N-340, the lysine codon is altered so that the next obligatory nucleotide triplet becomes AAU (A, adenine; U, uracil) which codes for asparagine. The codon following that for asparagine would then be UAA, a nonsense triplet which directs premature termination.

Tucker *et al.* (*5*, *6*) report the DNA sequence of the MPC 11 γ_{2b} constant region gene. Our predicted DNA sequence matches the actual DNA sequence for this segment. In the parental DNA, the codon for lysine 342 is split by an intervening sequence and is read as sense only when three adenine nucleotides, positions 1222, 1223, and 1332, have been made contiguous by RNA processing (*6*). The nucleotide at 1223 would represent the third base in the termination codon UAA in M 311, generated as described above.

Thus, we propose that, in M 311, the C_H3 domain of the heavy chain is precisely deleted by a mechanism that involves a short sequence of adenine-rich nucleotides immediately preceding the intervening sequence separating the C_H2 and C_H3 domains of the γ_{2b} gene. This phenomenon would appear to be independent of RNA splicing. In the gene coding for bacteriophage T4 lysozyme, Streisinger, Okada, and colleagues previously observed that sketches of identical nucleotides, particularly an adenine-rich region like that found in the MPC 11 DNA sequence, are especially susceptible to frameshift mutations (*9*). How melphalan might have facilitated the induction of such a frameshift in M 311 remains unclear. The proof of this mechanism for the generation of M 311 rests on the determination of its DNA sequence.

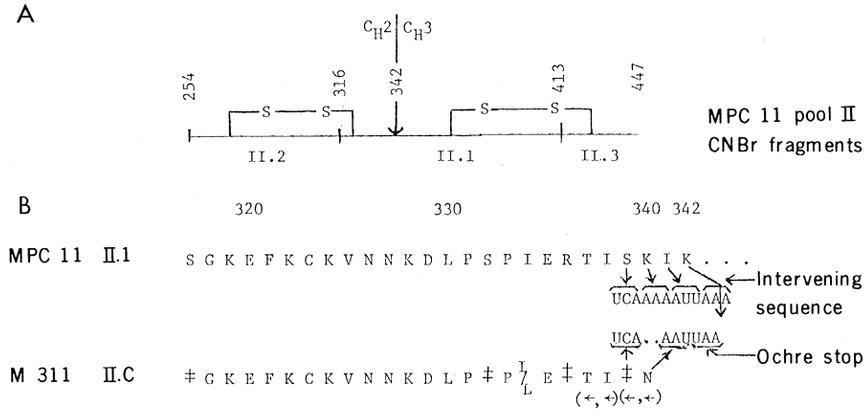

Fig. 2. (A) The CNBr fragments (*4*) from MPC 11 pool II. Lysine 342 has been confirmed as the C_H2-C_H3 boundary by Tucker *et al.* (*5*, *6*). The codon for lysine 342 is split by the intervening sequence and only brought together after splicing of heterogeneous RNA to form mature messenger RNA. Vertical lines indicate methionine residues. Roman numerals below the line name the CNBr fragments in the order in which they were eluted from a gel filtration system. (B) Comparison of CNBr fragments II.1 from MPC 11 and II.C from M 311 HL by automated sequential degradation. The parental II.1 sequence was taken from (*4*), except that residue position 332 has been identified as serine and residue position 336 was taken as arginine from the sequence of the complementary DNA (*5*, *6*). Automated sequence analysis (*19*) of fragment II.C was carried out on a Beckman 890 C sequencer [dimethylallylamine double-cleavage program (10/29/74)]. The amino acid phenylthiohydantoins were identified either by gas chromatography (Beckman GC-65) (*20*) or by thin-layer chromatography (*21*). Residues were identified by at least two methods or separate analyses. The following yields for one degradation were found: Step 2, Gly, 665 nmole; step 5, Phe, 557 nmole; step 9, Val, 413 nmole; step 14, Leu, 426 nmole; step 15, Pro, 271 nmole; step 17, Pro, 275 nmole; step 18, Leu, 426 nmole; step 22, Ile, 282 nmole. The symbol ‡ marks those residues that were not identified. The arrows mark the residues seen with carboxypeptidase. A treatment, carried out at 25°C in 0.05*M* ammonium acetate, *p*H 8, with 50 μg of carboxypeptidase A for 2, 5, and 18 hours. The results of digestion were analyzed by amino acid analysis. A representative amino acid composition of M 311 II.C is (residues): *S*-carboxymethylcysteine (C), 1.3; Asp (D), 3.1; Thr (T), 0.74; Ser (S), 1.7; Glu (E), 1.9; Pro (P), 1.9; Gly (G), 1.3; Ala (A), 0.7; Val (V), 1.3; Ile (I), 2.0; Leu (L), 1.4; Tyr (Y), 0.2; Phe (F), 0.94; Lys (K), 4.3; Arg (R), 1.3. In other analyses, serine was found to be 2.7, 2.2, and 2.8 residues, and little or no alanine was detected. The predicted nucleotide sequence presented for amino acids N-339 to N-342 corresponds to nucleotides 1213 to 1223 and 1334 of the γ_{2b} DNA sequence (*5*, *6*). The abbreviations for the amino acid residues are Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, cysteine; Glu, glutamic acid; Gly, glycine; Ile, isoleucine; Leu, leucine; Lys, lysine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine. Asparagine (N) and glutamine (Q) are deamidated by hydrolysis with HCl and are detected by amino acid analysis as aspartic acid and glutamic acid, respectively.

It seems likely that mutations that lead to DNA deletion or to improper splicing may account for some variant immunoglobulins. Some possible examples include the IF2 mutant derived from MOPC 21 (*10*), which lacks the complete C_H1 domain (*11*), several heavy chain disease proteins (*12*), a short mouse IgA heavy chain with a COOH-terminal deletion (*13*), a human IgA heavy chain with a COOH-terminal deletion (*14*), a group of three human myeloma proteins with identical hinge region deletions (*15*), and a variant light chain (*16*). However, our studies have shown that mechanisms other than DNA deletion and aberrant splicing could account for complete domain deletions occurring at exon-intron boundaries. Whether a specific mutation at or near such a processing point will affect successful splicing remains to be determined. A comparison of the primary transcription products and their intermediates previously obtained for the parental MPC 11 cell line (*17*) with those of M 311 may be informative.

Other large deletions or rearrangements at the protein level can take place that are independent of domain boundaries and are, therefore, not generated by disorders in splicing. For example, the IF1 mutant of MOPC 21 occurs as a result of a frameshift of the triplet coding for residue N-358, some 26 residues away from the C_H2-C_H3 boundary (*10*, *18*). Our studies of a γ_{2b}-γ_{2a} hybrid chain synthesized by a variant of MPC 11 place the crossover in the carboxyl terminal segment of the C_H2 domain some 8 to 32 amino acid residues away from the end of the domain (*7*).

The deletion we have defined in M 311 may not be the only alteration in its structure in comparison to the parent. The fact that the heavy chain is synthesized and secreted in multiple forms suggests that other parts of the molecule might either be affected by the deletion of the C_H3 domain or contain additional independent mutations.

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Benzo(*a*)pyrene-7,8-dihydrodiol 9,10-Oxide Adenosine and Deoxyadenosine Adducts: Structure and Stereochemistry

Abstract. *The structure and absolute stereoconfigurations of four adenosine adducts with (±)-7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene (BPDE) and their deoxyadenosine analogs have been determined. They result from both* cis *and* trans *addition of the* N^6 *amino group of adenine to the 10 position of both enantiomers of BDPE. This was determined from studies of the nuclear magnetic resonance spectra, mass spectra, and circular dichroism spectra, as well as from their* pK_a *values and chemical reactivities.*

Considerable evidence has accumulated that the ultimate oncogenic metabolite of the ubiquitous environmental carcinogen benzo(*a*)pyrene (BP) is a 7,8-dihydrodiol 9,10-oxide (BPDE) (*1*). In previous studies from this laboratory (*2*) we demonstrated that the predominant RNA and DNA adduct present in human cells exposed to BP is formed by covalent linkage of the amino group at the 2 position of guanine (N^2) to the 10 position of a specific isomer: (+)-7β,8α dihydroxy-9α, 10α-epoxy7,8,-9,10-tetrahydrobenzo(*a*)pyrene (7*R*-BPDE). This adduct has also been detected in rodent cell cultures (*3*, *4*) and in mouse skin (*5*), and its complete stereochemistry has also been elucidated (*6*). Lesser amounts of N^2-substituted deoxyguanosine adducts derived from (±)-7β,8α-dihydroxy-9β,10β-expoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene have also been detected in vivo (*7*), and there is indirect evidence for the reaction of BPDE at the 7 position of guanine (*8*) and with phosphates of the DNA backbone (*5*).

In vitro studies with BPDE have demonstrated that under particular conditions there can also be significant reaction with adenine and to a lesser extent cytosine residues in synthetic homopolymers, RNA, and DNA (*9*, *10*). Small amounts of the DNA adducts have been tentatively identified in certain cell culture systems exposed to BP (*4*). There is also a report suggesting that the conformation of the DNA at sites of BPDE modification of adenine residues differs from that at sites of guanine modification (*11*). Because at present it is not known which of these types of nucleic acid modification are the most critical with respect to the carcinogenic process, we have elucidated the complete chemical structure and stereochemistry of certain BPDE-adenine adducts.

The quantities of deoxyadenosine derivatives that could be prepared were limited by the poor reactivity of deoxyadenosine monophosphate (dAMP), by the high cost of polydeoxyadenylate [poly(dA)], and by the difficulty experienced in hydrolyzing BPDE-modified poly(dA). Experiments were therefore directed toward elucidating the structures of the adenosine derivatives formed by modifying polyadenylate [poly(A)] with BPDE and then comparing these products with those formed with poly(dA) or dAMP. Poly(A) was reacted with (±)-BPDE and hydrolyzed to mononucleosides, and the modified residues were separated by LH-20 column chromatography and high-pressure liquid chromatography (HPLC). As was previously described (*10*), we obtained four major derivatives, which were shown to be BPDE-adenosine adducts by virtue of their pyrene-like ultraviolet absorption spectra and their circular dichroism (CD) spectra. The CD spectra of these four compounds (A-BPDE, **1** to **4**) were essentially identical in both shape and magnitude, except that the pairs **1** and **2** were opposite in sign from pairs **3** and **4** (Fig. 1). This result indicates that the spacial relationship between the pyrene and adenine chromophores is the same and thus the position of attachment of the two moieties is the

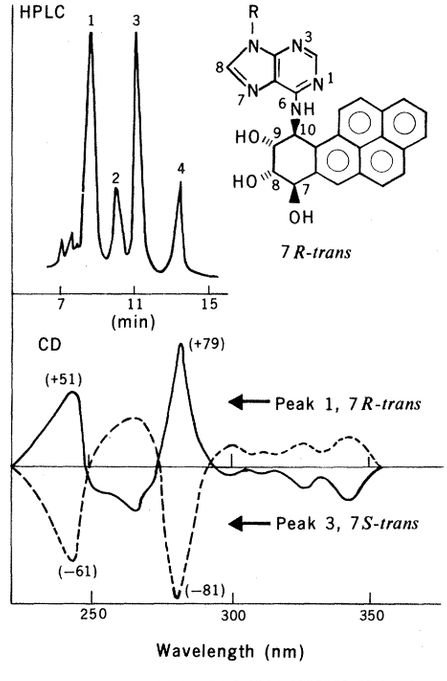

Fig. 1. High-pressure liquid chromatography profiles were obtained on a Du Pont 830 instrument with a Rainin C-18 column, 5-μm particle size, 250 by 2.1 mm (A-BPDE derivatives) or a Whatman ODS-2 column (dA-BPDE derivatives) operated with water-methanol mixtures at 50°C; the eluant was monitored at 280 nm. Preparative runs were performed on Rainin columns (250 by 6.4 mm). The CD spectra were measured in mixtures of water and methanol (1:1) on a JASCO J-40 instrument (*10*, *13*). All the A-BPDE and dA-BPDE derivatives, designated to **1–4** and **1′–4′**, respectively, by their HPLC elution order, had similar ultraviolet spectra which were dominated by the pyrene chromophore and were similar to that seen previously for the guanosine derivative (*2*). All the CD spectra were similar to each other except in sign. The molar ellipticities (Δε) at 244 and 279 nm, the absolute stereo configurations of the 7-hydroxyl group on addition of the adenine moities to the original BPDE were as follows: A-BPDE peak 1, +51, +79, 7*R-trans*; peak 2, +35, +60, 7*S-cis*; peak 3, −61, −81, 7*S-trans*; peak 4, −49, −81, 7*R-cis*; dA-BPDE peak 1′, +50, +68, 7*R-trans*; peak 2′, −49, −66, 7*S-trans*; peak 3′, +40, +61, 7*S-cis*; peak 4′, −36, −67, 7*R-cis*.

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