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MECHANISMS OF DNA SEQUENCE SELECTIVE ALKYLATION OF GUANINE-N7 POSITIONS BY NITROGEN MUSTARDS

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We have previously reported that nitrogen mustards react preferentially at certain guanines in DNA and that the preferred sites may depend on the structure of the nitrogen mustard (Mattes et al. NUCLEIC ACIDS RES 14:2971, 1986). We have now carried out quantitative determinations of relative rates which suggest structural hypotheses of the origin of the reaction selectivities.

Restriction fragments of pBR322 or SV40 DNA, 32P-labeled at a 3' or 5' end, were reacted with nitrogen mustards for 1 hr at 20°C. Guanine-N7 alkylations were converted to strand breaks by heating in 1M piperidine and the resulting fragments were resolved by gel electrophoresis (Maxam-Gilbert method). Band areas were determined by computer analysis of digitized densitometer scans. Corrections were applied for proximal cuts relative to the label site and for band overlap as a function of position on the gel. Baseline corrections were taken at sites at least 2 bases removed from the nearest G.

Several mustards gave reaction intensity patterns that were similar to each other. These included HN2 (bis(2-chloroethyl)methylamine), phosphoramide mustard (PM), L-phenylalanine mustard (L-PAM), spirohydantoin mustard and chlorambucil. The reaction intensities for these mustards at low ionic strength varied from a range of 10-fold to a range of 20-fold. Dimethylsulfate gave a different pattern with an intensity range of only 5-fold. The correlation coefficients for the reactions of a given mustard relative to HN2 were between 0.81 and 0.93.

The reaction intensities of the above mustards correlated with the negative molecular electrostatic potential (MEP) induced at the G-N7 position by the nearest neighbor base pairs, as calculated by Pullman and Pullman (QUART REV BIOPHYS 14:289, 1981). The correlation coefficients were between 0.63 and 0.90.

When 2mM Mg was included in the reaction solvent, the degree of selectivity and the dependence on MEP were reduced by 40-50% for HN2 and L-PAM; 10mM Na produced smaller reductions (25-30%). PM showed no such reductions. This can be understood from the cationic nature of the reactive intermediates of HN2 and L-PAM, as opposed to the zwitterion in the case of PM.

We had previously reported that uracil mustard (UM) and quinacrine mustard (QM) produce distinctive reaction patterns, differing from those of the mustards discussed above. We have now characterized these differences in terms of base sequence and find that they can be accounted for on the basis of plausible structural hypotheses.

UM reacts strongly at G's in the sequence 5'-YGC-3', which constitutes weak sites in the case of the other mustards (table 1). When these sites are excluded from consideration, the reaction intensity pattern of UM correlates well with that of the other mustards. Moreover, the reaction intensities of UM at these sites are less affected by Mg than are other sites. Molecular modelling suggests that, as the reactive aziridinium group of UM approaches G-N7, the UM-O4 can interact with the NH_2 of the 3'-cytosine and counter its positive effect on MEP (fig. 1). The presence of pyrimidines on both sides of the G, according to Calladine's rules (Dickerson, JMB 166:419, 1983), would force the G to slip in the direction of its sugar. This would improve the geometry of the postulated interaction.

QM reacts at very low concentrations and exhibits an unusually wide range from weakest to strongest sites, in accord with an initial intercalative binding of the quinacrine group prior to alkylation. QM reacts most strongly with G's that are followed on the 3' side by a G or T, followed by a purine (table 2). This effect is not reduced by Mg or Na. This preference is plausible on the basis of intercalation of the quinacrine group between the 2nd and 3rd base pairs 3' to the guanine to be alkylated (fig. 2). (Although fig.2 suggests a correlation of reaction intensity with MEP, detailed examination of the data indicates that the intensities are independent of the identity of the base pair on the 3' side of the reacting G.)

These findings point to the possibility of design of DNA sequence specific alkylating agents.

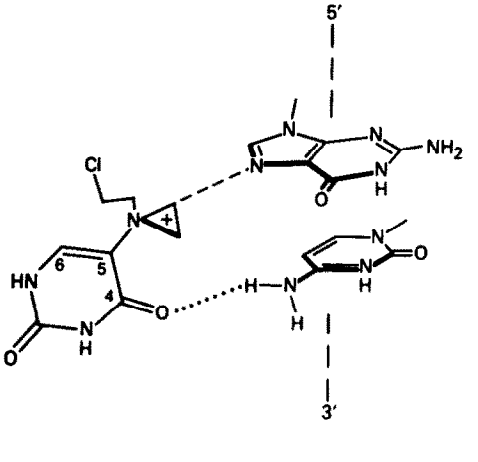
Table 1: Specific Enhancement of Reaction With Uracil Mustard.

$\bar{\sigma}$ = variance of deviations from the linear regression of LN(intensity) values for uracil mustard versus HN2, calculated for each experiment excluding G's that are followed by 3'C. The table lists the number of reaction sites of each type falling within the indicated limits of $\bar{\sigma}$. (R=purine; Y=pyrimidine; D=not C; N=any base.)

Reaction Sites (G)	<+$\bar{\sigma}$	>+$\bar{\sigma}$ <+2$\bar{\sigma}$	>+2$\bar{\sigma}$ <+3$\bar{\sigma}$	>+3$\bar{\sigma}$	Totals
5'-YGC-3'	2	3	5	29	39
5'-RGC-3'	9	6	7	1	23
5'-NGD-3'	106	24	2	0	132
Totals	117	33	14	30	194

Table 2: Quinacrine mustard reaction intensities at various sequence configurations in pBR322 DNA (experiments 022 and 031). The reaction is at the underlined G; the 2 bases following in the 3' direction are indicated (R=purine, Y=pyrimidine, n=number of sites). Delta-MEP is the calculated effect of the nearest neighbor base pairs to the reacting G on the molecular electrostatic potential as calculated by Pullman and Pullman, 1981.

	expt. 022			expt. 031		
	n	LN(intensity)	delta-MEP	n	LN(intensity)	delta-MEP
GGR	11	1.99 ± 0.28	-14.9 ± 4.2	6	1.80 ± 0.33	-13.9 ± 4.0
GGY	15	0.55 ± 0.35	-12.8 ± 3.5	4	0.75 ± 0.33	-14.2 ± 2.9
GTR	6	1.65 ± 0.44	-7.4 ± 4.5	7	1.37 ± 0.42	-4.0 ± 2.9
GTY	2	0.34 ± 0.41	-7.2 ± 3.4	6	0.46 ± 0.17	-5.6 ± 3.7
GAR	1	0.51	-12.9	5	-0.57 ± 0.62	-8.9 ± 3.3
GAY	4	-0.39 ± 1.01	-8.0 ± 2.8	6	-0.67 ± 0.23	-6.1 ± 0.0
GCR	10	-0.90 ± 0.65	-4.1 ± 3.3	2	-0.11 ± 0.16	1.4 ± 1.4
GCY	10	-0.67 ± 0.83	-1.6 ± 4.3	5	-0.87 ± 0.48	0.3 ± 3.7



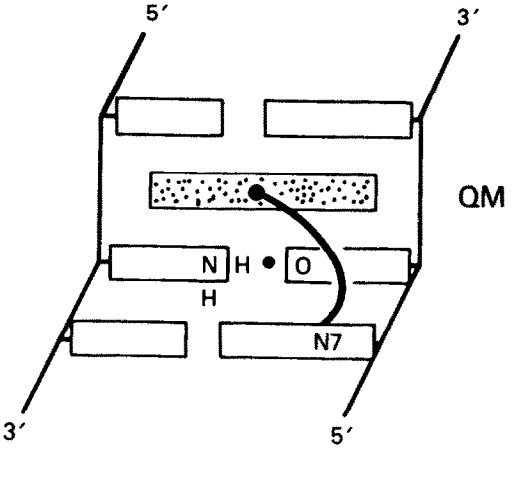


Figure 1: Possible configuration of uracil mustard (aziridinium form) at the initiation of the reaction with guanine-N7, showing the proposed interaction of the uracil-O4 atom with the N-H of a 3'-cytosine. The attacking aziridinium carbon is modelled at a van der Waals distance from the guanine-N7 atom and in the plane of the guanine ring.

Figure 2: Schematic view into the major groove, showing a quinacrine mustard (QM) molecule intercalated between the first and second base pair 3' to a guanine-N7 reaction site. The hydrocarbon side chain which tethers the mustard group to the quinacrine ring system is shown curving over an intervening thymine residue.