Tetrahedron Letters No. 29, pp 2481 - 2484, 1975. Pergamon Press. Printed in Great Britain.

STEREOCHEMISTRY OF THE REACTIONS OF BIOPOLYMERS IV.

Explanation of stereospecific and asymmetric reactivity of DNA in cross-linking alkylation

L. Ötvös and I. Elekes

Central Research Institute for Chemistry of the Hungarian Academy of Sciences
Budapest, Hungary

(Received in UK 23 May 1975; accepted for publication 5 June 1975)

In a previous paper[1] the steric inhibitory effects in the cross-linking alkylation of DNA was discussed. It was also suggested that the configuration of the carbon atom bearing a given substituent of the alkylating agents has also a definite role in the reactivity. First direct arguments for the asymmetric cross-linking alkylation of DNA were given by Verly, Brakier and Feit[2], as well as us[3] using dianhydro derivatives of tetritols and hexitols. No explanation has been given, however, for the stereospecificity. The purpose of this paper is to show that the stereospecificity observed in the alkylation with meso-, L- and D-diepoxybutane stereoisomers is due to the asymmetric right-handed helix structure of the DNA and the difference in the reactivity can be correlated to the absolute configuration of the reagents. The amount of the interstrand cross-links was determined by measuring the renaturability of alkylated DNA compared with the renaturability of the nucleic acid containing no cross-linkage[4]. Table I. shows renaturability data obtained in the alkylation of chicken blood DNA /500 μg/ml/ with diepoxybutane isomers /0,03 M/, in the first three hours of the reaction. The accuracy of the measurements is ± 1-3 %.

Table I.

Reaction time min. 37°	Renaturability of DNA /%/ alkylated with DL- diepoxybutane	Renaturability of DNA /%/ alkylated with meso diepoxybutane	Stereospecificity /%/ $\left(1-\frac{meso}{DL}\right)\times 100$	Renaturability of DNA /%/ alkylated with L- diepoxybutane	Renaturability of DNA /%/ alkylated with D- diepoxybutane	Stereospecificity /%/ $\left(1-\frac{D}{L}\right)\times 100$
10	19	0	-	35	4	89
20	25	2	-	43	7	84
30	28	2	-	46	10	78
40	30	4	92	51	13	75
60	38	4	89	60	20	67
90	45	4	89	67	29	57
120	52	5	90	71	34	52
180	58	6	90	73	40	45
	Mean value		90	Extrapol. to t = 0 min		95

From the data of Verly[2], total stereospecificity can be calculated in the case of the meso compound related to both L- and D-isomers, since no formation of interstrand cross--link was observed in the alkylation of DNA with meso-diepoxybutane.

As Table I. shows, the stereospecificity calculated from the experimental data is not quantitative but of a high degree.

The different reactivity of optically active and meso compounds can be explained with the different steric position of the hydroxyl substituents of cross-linkage formed in the reaction. The cross-links resulted primarily by the alkylation of N^7-atoms of guanine bases in the opposite strands of DNA contain two hydroxyl groups /Fig.1/.

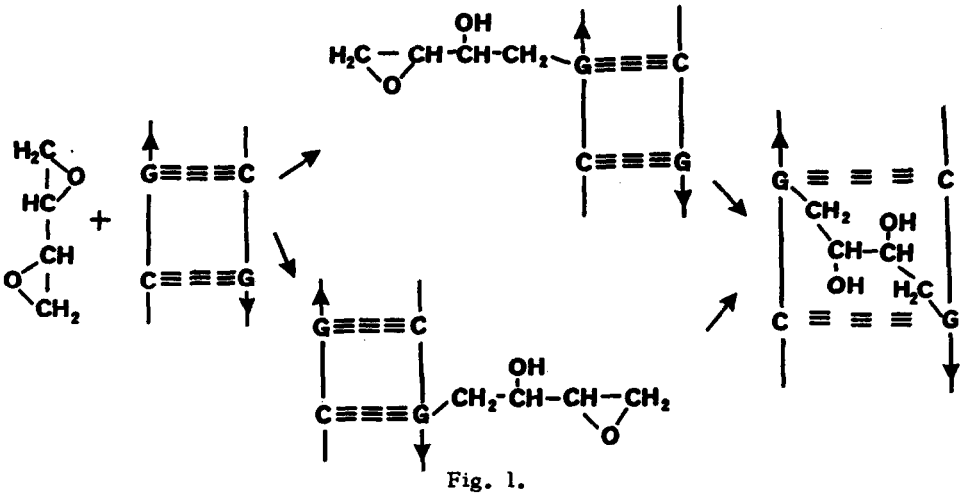


Fig. 1.

The first step of the reaction /monoalkylation/ with both DL- and meso-diepoxybutane takes place without any difficulty. However, in the case of the meso compound, the second step,that is,the nucleophilic attack of the N^7-atom of guanine on the opposite strand is inhibited strongly. The inhibition is resulted from the internal pressure between DNA and the hydroxyl group formed in the monoalkylation and oriented toward the inside of the nucleic acid /introverted or "τ" substituent[1]/. Such a steric hindrance does not come about in the cross-linking alkylation with optically active isomers, because the hydroxyl group formed in the monoalkylation is oriented opposite the inside of DNA /extraverted or "μ" substituent/.

The renaturability data of DNA alkylated with D- and L-diepoxybutanes are summarized in Table I. The different reactivity of the D- and L-isomers shows that the DNA has an asymmetric reactivity in the cross-linking alkylation.

Somewhat lower stereospecificity was found by Verly et al.[2] in the first period of the reaction, using different DNA and reaction medium. The results are very similar after a reaction time of six hours. However, this period can not be compared owing to the differences in the depurination of DNA treated with L- and D-diepoxybutane and other secondary

processes. The asymmetric reactivity of DNA is interpreted in terms of the asymmetric molecular structure of nucleic acid /right-handed double helix/ for neither the reaction centre in the macromolecule /N^7-atom of guanine/ nor its vicinity shows any chirality.

Fig. 2 shows such neighbouring base pairs between which interstrand cross-link may come about. The distance between the N^7-atoms of G_1 and G_2 guanines is remarkably smaller than that of G_3 and G_4 owing to the helix structure of DNA. /Fig.3 and Fig.4./ Formation of an interstrand crosslink containing four carbon atoms will cause distorsion of the native DNA molecule even in case of linkage between G_1 and G_2 while cross-linkage between G_3 and G_4 is entirely excluded. $B_1 \equiv B_1'$ and $B_2 \equiv B_2'$ may be arbitrary base pairs. /A = T, T = A, G ≡ C, C ≡ G./ The steric position of these plays an important role in the asymmetric reactivity of DNA in the reaction discussed.

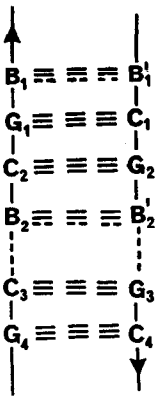


Fig.2

In Fig.3 the plane of the base pair C_1-cytosine and G_1-guanine, shown schematically from top view, is in a position turned by 36° to the right with respect to the plane of the G_2-C_2 base pair.

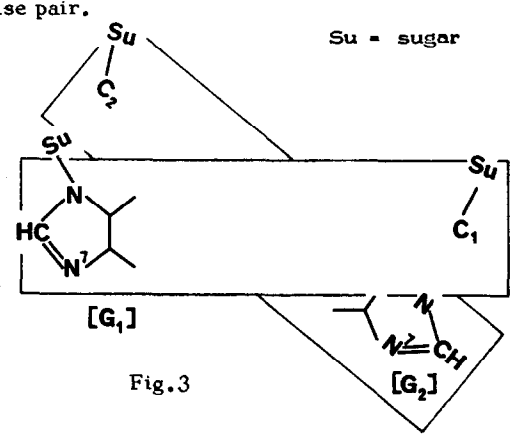


Fig.3

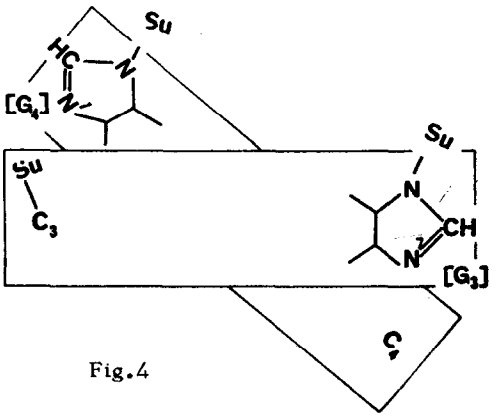


Fig.4

Supposing that the first step of alkylation takes place on the N^7 atom of guanine G_1, L- and D-diepoxybutanes give the monoalkylated products shown in Figs.5 and 6, respectively. Stereospecificity is explained in terms of the different reactivities of these two intermediates.

Because of the right-handed helix, the plane of base pair G_2-C_2 is twisted by 36° to the right to that of the B_2-B_2', i.e., the B_2-B_2' plane is turned by 36° to the left as compared to G_2-C_2. In the Fig.6 the H_a atom is oriented towards B_2'. The orientation of H_b is opposite this base /Fig.5/.

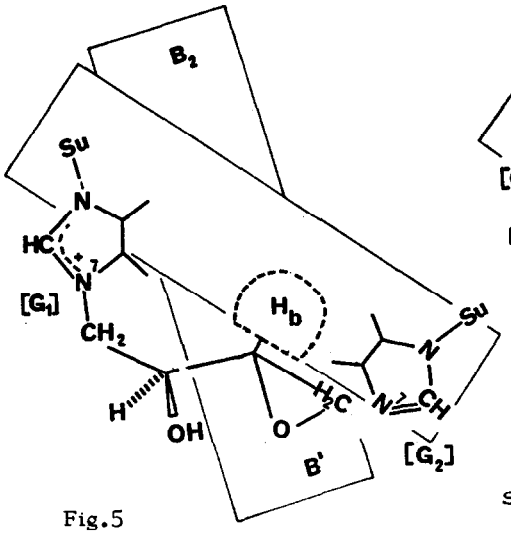
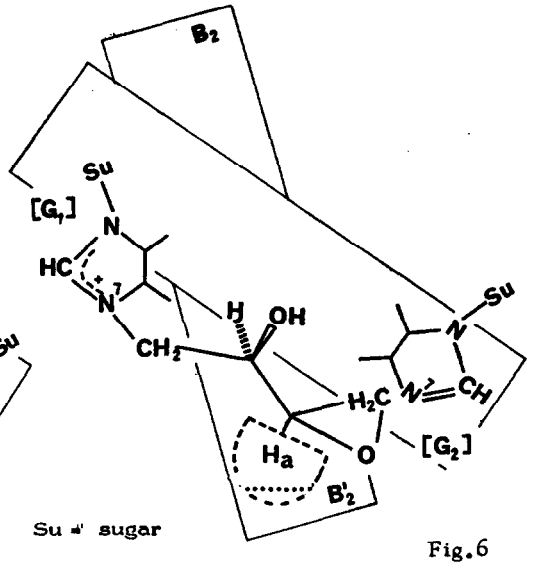


Fig.5



Su = sugar

Fig.6

The strain arising between the B_2' base and the H_a atom of the monoepoxy derivative formed from D-diepoxybutane hinders the approach of the epoxide methylene group to the N^7-atom of G_2 /Fig.6/, necessary to establish the cross-linkage. Since there is no strain between the B_2' base and the H_b atom of the monoepoxy intermediate, resulting from L--diepoxybutane /Fig.5/, cross-linking occurs here more readily than in the case of the D-isomer.

The situation is quite analogous if the first step of crosslinking alkylation takes place on the N^7-atom of G_2. For steric reasons, in this case the B_1 base hinders the approach of the reactive site of the monoepoxy derivative, resulting from the D-isomer, to the N^7-atom of G_1.

The asymmetric reactivity of DNA is the first example in reactions of biopolymers where the selectivity can be correlated with the secondary structure /right-handed helix/ of the native macromolecule.

The diepoxybutanes were prepared in our laboratory from D- and L-tartaric acids with a slight modification described by Feit[5].

REFERENCES

1./ Submitted for publication to Tetrahedron Letters.
2./ W.G.Verly, L.Brakier and P.W.Feit; Biochim.Biophys.Acta, 228, 400 /1971/
3./ L.Ötvös, I.Elekes, F.Kraicsovits and L.Institoris; Magy.Kém.Folyóirat, 77,646/1971/
L.Ötvös, I.Elekes; VII th International Symp.on the Natural Product, Riga, 1970. Abstract of Papers p. 209.
4./ P.D.Lawley and P.Brookes; J.Mol.Biol., 25, 143 /1967/
5./ P.W.Feit; Chem.Ber., 93, 116 /1960/
P.W.Feit; J.Med.Chem., 7, 14 /1964/