THE STRUCTURE OF DNA-DERIVED THYMINE DIMER

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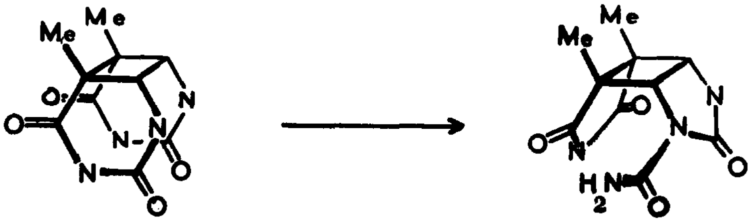
Thymine dimer obtained from ultraviolet-irradiated bacterial DNA is shown to be identical with thymine ice-dimer. This is known to have the structure, (I), expected from intrastrand dimerisation of adjacent thymine residues.

Ultraviolet irradiation of bacterial DNA leads on hydrolysis to a thymine photoproduct. This has been shown previously to be similar to the dimer obtained by irradiation of a frozen thymine solution both in its chromatographic behaviour (Wacker, 1963; Boyce and Howard-Flanders, 1964) and in its photoreversion to thymine in aqueous solution (Beukers, Ijlstra, and Berends, 1959).

The rearrangement of thymine ice-dimer (I) to a triazatri-cyclodecane derivative (II) is a reaction sterically denied the other three isomeric dimers (Blackburn and Davies, 1965). Accordingly, it can be used as a rigorous test of the identity of the dimers from the two sources.



cis-syn
(I)

(II)

FIG I

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Labelled DNA was isolated from cultures of a thymidine-requiring strain of Escherichia coli grown in a medium supplemented with thymidine-6-T(n).[+] After irradiation with ultraviolet light, filtered to remove short wavelength radiation, two thymine photoproducts were isolated chromatographically using the method of Boyce and Howard-Flanders.

The major product, which accounted for 2% of total thymine, was mixed with thymine ice-dimer as carrier, with which it was chromatographically identical. Repeated crystallisation of this mixture resulted in no significant change in its specific activity, (983 ± 37 cts/mg/min). On treatment with sodium hydroxide and bromine it gave the rearrangement product (II) with retention of 70% of the specific activity (690 ± 30 cts/mg/min).

The radioactivity of the rearrangement product (II) shows that the major DNA-derived thymine dimer is identical with the ice-dimer (I). This is the isomer predicted to result from linkage of adjacent thymine residues in the same DNA strand (Wulff and Fraenkel, 1961). While the loss of 30% of the specific activity could arise from the presence of a chromatographically indistinguishable photoproduct which, moreover, co-crystallised with thymine ice-dimer, the discrepancy is almost certainly due to counting difficulties with solid samples. Experiments using 2-^{14}C-thymine are in progress to resolve this question.

The minor photoproduct accounted for 0.6% of total thymine. It is possible that ultraviolet-induced interstrand cross-linking in DNA is caused by the formation of thymine dimers (Marmur and Grossman, 1961), which would account for the formation of this

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minor photoproduct. C.Nagata *et al.* (1965) have suggested that such a dimer would have either *cis-anti* or *trans-syn* stereochemistry of the cyclobutane ring, while Wulff and Fraenkel (1961) from their inspection of a model of DNA preferred the *cis-anti* and *trans-anti* structures.

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REFERENCES.

Beukers, R., Ijlstra, J., and Berends, W., Rec.Trav.Chim., 79, 101 (1959)

Blackburn, G.M., and Davies, R J H., Chem.Comm., 215 (1965).

Boyce, R.P., and Howard-Flanders, P., Proc.Natl.Acad.Sci.U.S., 51, 293 (1964).

Marmur, J., and Grossman, L., Proc.Natl.Acad.Sci.U.S., 47, 778 (1961).

Nagata, C., Imamura, A., Tagashira, Y., Kodama, M., and Fukuda, N., J.Theoret.Biol., 9, 357 (1965).

Wacker, A., Progress in Nucleic Acid Research, Vol.I, p.362, Academic Press, New York, 1963.

Wulff, D.L., and Fraenkel, G., Biochim.Biophys.Acta, 51, 332 (1961).