Reactions of 'K'-Region Epoxides of Polycyclic Hydrocarbons with Nucleic Acids and Polyribonucleotides

By P. L. GROVER and P. SIMS (*Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London SW3 6JB, U.K.*)

'K'-region epoxides have been detected as microsomal metabolites of the polycyclic hydrocarbons phenanthrene and benz[a]anthracene (Grover *et al.*, 1971b), of pyrene and benzo[a]pyrene (Grover *et al.*, 1972) and of 7,12-dimethylbenz[a]anthracene (Keysell *et al.*, 1972). Synthetic 'K'-region epoxides have been found to possess biological activity as mutagens (Huberman *et al.*, 1971; Cookson *et al.*, 1971), to cause malignant transformation of rodent cells *in vitro* (Grover *et al.*, 1971c) and to be alkylating agents that react with the nucleic acids of cells in culture (Grover *et al.*, 1971a). In view of current interest in the role that 'K'-region epoxides may play in hydrocarbon carcinogenesis *in vivo*, we are examining in more detail the reactions of these compounds with nucleic acids.

In the present investigations, ³H-labelled 'K'-region epoxides (0.04 μmol) were incubated with nucleic acid (4.0 μmol of P) at 37°C for 2 h in neutral aqueous solution, and the water-soluble products were passed through a Sephadex G-25 column. The extents of reaction (mmol/mol of P) with DNA, RNA and apurinic acid respectively were: for phenanthrene 9,10-oxide, 0.015, <0.007 and <0.007; for benz[a]anthracene 5,6-oxide, 0.68, 0.51 and 0.021; for 7-methylbenz[a]anthracene 5,6-oxide, 1.63, 0.96 and 0.051; for dibenz[a,h]anthracene 5,6-oxide, 0.33, 0.42 and 0.020.

In similar experiments with synthetic polyribonucleotides, the 'K'-region epoxides did not appear to react with poly(U) or poly(C), reacted with poly(A) (0.02–0.57 mmol/mol of P) but reacted more extensively with poly(G) (0.29–1.86 mmol/mol of P). These results suggest that it is probably the purines in nucleic acids that are alkylated by polycyclic hydrocarbon epoxides; a study of the products formed is in progress.

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Cookson, M. J., Sims, P. & Grover, P. L. (1971) *Nature New Biol. (London)* **234**, 186–187

Grover, P. L., Forrester, J. A. & Sims, P. (1971a) *Biochem. Pharmacol.* **20**, 1297–1302

Grover, P. L., Hewer, A. & Sims, P. (1971b) *FEBS Lett.* **18**, 76–80

Grover, P. L., Sims, P., Huberman, E., Marquardt, H., Kuroki, T. & Heidelberger, C. (1971c) *Proc. Nat. Acad. Sci. U.S.* **68**, 1098–1101

Grover, P. L., Hewer, A. & Sims, P. (1972) *Biochem. Pharmacol.* in the press

Huberman, E., Aspiras, L., Heidelberger, C., Grover, P. L. & Sims, P. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 3195–3199

Keysell, G. R., Booth, J., Sims, P., Grover, P. L. & Hewer, A. (1972) *Biochem. J.* **129**, 41 p

The Formation of an Epoxide in the Microsomal Metabolism of 7,12-Dimethylbenz[a]anthracene

By G. R. KEYSELL, J. BOOTH, P. SIMS, P. L. GROVER and A. HEWER (*Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London SW3 6JB, U.K.*)

The metabolism of 7,12-dimethylbenz[a]anthracene by rat liver microsomal fraction yields dihydrodiols and phenols (Sims, 1970), which probably arise from epoxide precursors: with phenanthrene and benz[a]anthracene (Sims *et al.*, 1971) and with pyrene and benzo[a]pyrene (Grover *et al.*, 1972) 'K'-region epoxides are formed. 'K'-region epoxides react with DNA, RNA and protein both chemically and in cells in culture (Grover *et al.*, 1971a), and possess biological activities (Grover *et al.*, 1971c) not shown either by the parent hydrocarbons or by the dihydrodiols and phenols into which they are converted. 7,12-Dimethylbenz[a]anthracene is a potent carcinogen that presumably also requires metabolic activation, and the possibility that this involves the formation of a 'K'-region epoxide was investigated.

[³H]7,12-Dimethylbenz[a]anthracene was incubated with rat liver microsomal fraction, an NADPH-generating system and an epoxide hydrazine inhibitor, and the metabolites were chromatographed on alumina (Grover *et al.*, 1971b). A radioactive metabolite eluted with benzene–light petroleum (b.p. 60–80°C) (2:1, v/v) was (a) incubated with the 'epoxide hydrazine' of rat liver microsomal fraction to yield a product that was chromatographically identical with *trans*-5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[a]anthracene, (b) treated with HCl to yield a phenol that was identical with 5-hydroxy-7,12-dimethylbenz[a]anthracene, (c) allowed to react with GSH in aqueous acetone at pH 8.0 to form a water-soluble product that had the chromatographic characteristics of an acid-labile GSH conjugate, and (d) incubated with poly(G) in a neutral aqueous solution and the products passed through Sephadex G-25 to give a radioactive peak coincident with that of the u.v.-absorbance peak at 260 nm.

The conversion of the metabolite into a dihydrodiol

and a phenol and its reaction with GSH and poly(G) shows that the metabolite is an epoxide. The formation of products with the chromatographic characteristics of *trans*-5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[a]anthracene and 5-hydroxy-7,12-dimethylbenz[a]anthracene justifies its identification as the 'K'-region epoxide 7,12-dimethylbenz[a]anthracene 5,6-oxide. The 'K'-region dihydrodiol is formed in small amounts in the metabolism of 7,12-dimethylbenz[a]anthracene, although the major product is the non-'K'-region dihydrodiol 8,9-dihydro-8,9-dihydroxy-7,12-dimethylbenz[a]anthracene (Boyland & Sims, 1967). 7,12-Dimethylbenz[a]anthracene 8,9-oxide was not detected in these experiments, probably because it decomposed on the alumina column.

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- Boyland, E. & Sims, P. (1967) *Biochem. J.* **104**, 394-403
 Grover, P. L., Forrester, J. A. & Sims, P. (1971a) *Biochem. Pharmacol.* **20**, 1297-1302
 Grover, P. L., Hewer, A. & Sims, P. (1971b) *FEBS Lett.* **18**, 76-80
 Grover, P. L., Sims, P., Huberman, E., Marquardt, H., Kuroki, T. & Heidelberger, C. (1971c) *Proc. Nat. Acad. Sci. U.S.A.* **68**, 1098-1101
 Grover, P. L., Hewer, A. & Sims, P. (1972) *Biochem. Pharmacol.* in the press
 Sims, P. (1970) *Biochem. Pharmacol.* **19**, 795-818
 Sims, P., Hewer, A. & Grover, P. L. (1971) *Biochem. J.* **125**, 28 P

Tertiary-Structure Transitions of Supercoiled Deoxyribonucleic Acid

By DOUGLAS J. JOLLY and AILSA M. CAMPBELL
 (Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, U.K.)

The solution structure of supercoiled DNA with respect to its superhelix density, σ (Bauer & Vinograd, 1968), is still a subject of controversy. Recently the complete form of the plot of sedimentation coefficient against σ from -0.085 to 0 has been established (Upholt *et al.*, 1971). This suggested there are at least three different structures of superhelical DNA in different superhelix density regions. However, a previous attempt to correlate sedimentation coefficient with solution structure (Gray, 1967) had limited success, and Upholt *et al.* (1971) resorted to electron microscopy. They proposed that from $\sigma = -0.035$ to -0.018 supercoiled DNA had a rigid straight interwound structure, and from -0.018 to -0.005 a spherically coiled form. However, light-scattering investigations on bacteriophage ϕ X174 RFI DNA at

$\sigma = -0.025$ has ruled out the straight interwound structure and suggested a Y-shape structure as the most likely (Campbell & Jolly, 1972; Jolly & Campbell, 1972). It was therefore decided to follow the structure transition with changing σ by light-scattering.

The superhelix density was altered by temperature change. Increasing temperature unwinds the DNA duplex, thus decreasing the absolute value of σ , by a fairly accurately known amount per °C (Upholt *et al.*, 1971; Wang, 1969a,b).

In all experiments the molecular weight was $3.21 \times 10^6 \pm 0.1 \times 10^6$. Between 14.9°C and 53.4°C, i.e. $\sigma = -0.0265$ to $\sigma = -0.0207$, no great structural changes were apparent from either the root-mean-square radius values or the reciprocal of the scattering factor at scattering angle θ , $P(\theta)^{-1}$, against $\sin^2(\theta/2)$. The root-mean-square radius varied from 85.4 nm at 14.9°C through 95.2 nm at 45°C to 83.2 nm at 53.4°C. However, at 74.5°C and $\sigma = -0.0175$ the root-mean-square radius decreased to 41.5 nm and the $P(\theta)^{-1}$ curve altered considerably, evidence of some structural change. None of the previously suggested models, the Y-shape, the straight interwound model or the toroid, seems appropriate here for what is apparently a very compact molecule. It could be a very short fat interwound cylinder, or more probably a form of very compact toroid with a very large 'small' radius.

As the root-mean-square radius falls rapidly, the sedimentation coefficient starts to fall also; this is the opposite of what would be expected and implies that the molecule has become more 'free-draining' (Tanford, 1961).

The findings also show that the results obtained with formaldehyde temperature titrations of supercoiled DNA (Follet & Crawford, 1967; Rhoades & Thomas, 1968) are artifacts, since these indicate structure transition at lower temperatures.

- Bauer, W. & Vinograd, J. (1968) *J. Mol. Biol.* **33**, 141-171
 Campbell, A. M. & Jolly, D. J. (1972) *Biochem. J.* **127**, 39 P
 Follet, E. A. C. & Crawford, L. V. (1967) *J. Mol. Biol.* **28**, 455-459
 Gray, H. B., Jr. (1967) *Biopolymers* **5**, 1009-1019
 Jolly, D. J. & Campbell, A. M. (1972) *Biochem. J.* **128**, 569-578
 Rhoades, M. & Thomas, C. A. (1968) *J. Mol. Biol.* **37**, 41-61
 Tanford, C. (1961) *Physical Chemistry of Macromolecules*, p. 343, John Wiley and Sons, New York
 Upholt, W. B., Gray, H. B., Jr. & Vinograd, J. (1971) *J. Mol. Biol.* **62**, 21-38
 Wang, J. C. (1969a) *J. Mol. Biol.* **43**, 25-39
 Wang, J. C. (1969b) *J. Mol. Biol.* **43**, 263-272