Uncoiling of Bacteriophage- ϕ X174 Replicative Form Deoxyribonucleic Acid by Ethidium, Daunomycin and Actinomycin

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A specific prediction of the intercalation hypothesis is that binding of intercalating drug molecules to DNA should be associated with local uncoiling of the double helix (Lerman, 1964; Fuller & Waring, 1964). Evidence that such uncoiling occurs when ethidium bromide interacts with DNA has been obtained by showing that the supercoils of closed circular duplex DNAs can be progressively removed and reintroduced by increasing amounts of the drug (Crawford & Waring, 1967a,b). These studies showed that equivalence between drug-induced uncoiling and initial supercoiling turns occurs at an r value of about 0.037 (r being the number of drug molecules bound per nucleotide) for DNA from three animal viruses. Other workers have shown similar equivalence ratios using ethidium and supercoiled mitochondrial DNA (Ruttenberg, Smit, Borst & Van Bruggen, 1968), bovine papilloma virus DNA (Bujard, 1968) and SV-40 virus DNA (Bauer & Vinograd, 1968). With the intracellular replicative form of bacteriophage ϕ X174 DNA (ϕ XRF) equivalence occurs at about $r = 0.04$ with ethidium. Thus, if each insertion of an ethidium molecule causes a 12° uncoiling (Fuller & Waring, 1964), ϕ XRF contains about 12 full turns of supercoiling under our conditions. The effects of daunomycin and of actinomycin on ϕ XRF are qualitatively similar to those of ethidium, including the generation of left-handed supercoils at high ratios of drug binding. There is evidence that daunomycin binds to DNA by intercalation (Ward, Reich & Goldberg, 1965; Kersten, Kersten & Szybalski, 1966), but hitherto there has been no reason to believe that actinomycin might intercalate, and indeed a well-defined 'outside binding' model has been described (Hamilton, Fuller & Reich, 1963). Equivalence between the supercoiling turns of ϕ XRF and uncoiling by actinomycin binding occurs at $r = 0.043$, which is very similar to the ratio found with ethidium, showing that the angle of uncoiling caused by binding of an actinomycin molecule is similar to that caused by binding of ethidium, or very slightly less (perhaps 11°). This observation cannot be taken as proof that actinomycin binds to DNA by intercalation: it would be consistent with such a hypothesis, but might also be explained in other ways—for example, by interaction between the peptide rings and groupings in the narrow groove of DNA.

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Enzymic Fragmentation of a Human Myeloma γ -Macroglobulin

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γ -Macroglobulin (19s IgM) (M_w 900 000) most probably consists of five 7s sub-units (M_w 180 000) each containing two light κ - or λ -chains (M_w 20 000) and two heavy μ -chains (M_w 70 000) linked by disulphide bridges (Miller & Metzger, 1965a,b; Lamm & Small, 1966).

To obtain more structural information, a human myeloma IgM was fragmented with enzymes and the products were separated by gel filtration and Pevicon-block electrophoresis. Fragments were examined on the analytical ultracentrifuge and their antigenic determinants investigated by immunoelectrophoresis and immunodiffusion. Tryptic peptide 'maps' were prepared after reduction with dithiothreitol in 6M-guanidine-HCl.

Treatment with trypsin gave two main fragments. One had $S_{20,w}$ 6.4s, M_w 140 000, κ determinants and some of the specific determinants of IgM. It appeared to be similar to, but slightly bigger than, the $F(ab')_2$ fragment reported by Miller & Metzger (1966). The other fragment had $S_{20,w}$ 3.6s, M_w 46 000 and only κ determinants. It was probably identical with the Fab_u fragment reported by Miller & Metzger (1966). Peptide 'maps' of the two fragments showed all the κ -chain peptides and some of the μ -chain peptides. The 6.4s fragment gave more μ -chain peptides than the 3.6s fragment.

Treatment of IgM with chymotrypsin gave similar products to those obtained with trypsin except that there was a 6.1s fragment that gave fewer μ -chain peptides than the tryptic 6.4s fragment.

Treatment with papain in the presence of cysteine gave two 3.5s fragments similar to those reported by Onoue, Kishimoto & Yamamura (1967). One of

these fragments was similar to the 3-6s tryptic fragment. The other fragment (pap-Fc) had only specific IgM determinants that were partially deficient. None of these determinants were common to those on the 6-4s tryptic fragment. A peptide 'map' showed that only μ -chain peptides were present.

Treatment of IgM with pepsin gave very similar fragments to the tryptic ones. Small amounts of a fragment (pep-Fc) having only specific IgM determinants were also obtained. This fragment had more determinants than the pap-Fc fragment and some of these were common to those on the 6-4s tryptic fragment.

These results are consistent with a sub-unit structure for IgM; each sub-unit having a Fab portion containing a light chain and part of the heavy chain, and a Fc portion containing the rest of the heavy chain.

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The Labelling of Brain Phosphatidylethanolamine and Ethanolamine Plasmalogen from Cytidine Diphosphate Ethanolamine *in vitro*

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The formation of choline plasmalogen from 2-acyl 1-alk-1'-enyl glycerol (plasmalogenic diglyceride) and CDP-choline has been demonstrated by Kiyasu & Kennedy (1960) in the presence of a liver particulate fraction and very recently by Poulos, Hughes & Cumings (1968) with a particulate fraction from cardiac muscle. Both McMurray (1964a) and Ansell & Metcalfe (1966) have shown that labelling of both phosphatidylethanolamine and ethanolamine plasmalogen of brain from CDP-ethanolamine occurs when homogenates are incubated in the absence of an exogenous lipid acceptor.

When brain tissue (10mg.) from 20-day-old rats was incubated in 60mm-tris-HCl buffer, pH 8.0, for 1 hr. at 39° with 0.8mm-CMP-[³²P]phosphorylethanolamine (CMP-³²PE), the incorporation of [³²P]phosphorylethanolamine into the ethanolamine plasmalogen was stimulated sevenfold by the addition of 8mm-acyl alkenyl glycerol. This was a much greater stimulation than that found by McMurray (1964b). The activity of this enzyme, ethanolamine phosphotransferase (EC 2.7.8.1) to-

wards acyl alkenyl glycerol and diacyl glycerol in brain subcellular fractions was then determined. On the basis of both fresh brain weight and protein content, the activity was highest in the microsomal fraction, but significant activity was also found in a fraction consisting mainly of small myelin and synaptosomes, and also in the supernatant; less than 5% of the total activity was present in mitochondria. The distribution of activity towards diacyl and acyl alkenyl glycerol was very similar in all fractions examined. Subsequent experiments with the microsomal fraction showed that the K_m for acyl alkenyl glycerol was 1.7mm and that for diacyl glycerol 1.5mm, in the presence of saturating concentrations of CMP-³²PE.

For determining the activity of the enzyme in brain microsomes from animals of different ages this fraction (0.5-2mg. of protein) was incubated at 39° for 30min. in a volume of 1ml. containing: 60-80mm-tris-HCl, pH 8.0, 1.0-1.2mm-CMP-³²PE, 10mm-MnCl₂, and 0.03% Tween 20. In the absence of exogenous lipid substrate, the formation of ethanolamine plasmalogen was less than 1 μ mole/mg. of protein/hr. and that of phosphatidylethanolamine approx. 3 μ mole/mg. of protein/hr. At a saturating concentration of acyl alkenyl glycerol (10mm), the formation of ethanolamine plasmalogen was 70 μ mole/mg. of protein/hr., and at a saturating concentration of diacyl glycerol (15mm), the formation of phosphatidylethanolamine was 260 μ mole/mg. of protein/hr. Maximal activity towards substrates was between 16-30 days *post partum*. The variation in transferase activity towards acyl alkenyl glycerol during development was very similar to that observed towards diacyl glycerol. At 3 days *post partum*, ethanolamine phosphotransferase activities towards diacyl and acyl alkenyl glycerol were 58 and 56% respectively of the peak values at 16 days. The corresponding figures for the adult animals were 69 and 62% respectively of the peak values.

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