No peptides containing *N*-terminal arginine, lysine, cystine or cysteine were included.

This work is sponsored by the Agricultural Research Service of the U.S. Department of Agriculture under the Authority of Public Law 480.

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The Hydrolysis of Unimolecular Films of ^{32}P -Labelled Lecithin, Phosphatidylethanolamine and Phosphatidylinositol with Phospholipase A (*Naja naja* Venom)

By R. M. C. DAWSON

*Biochemistry Department, Agricultural Research Council
 Institute of Animal Physiology, Babraham, Cambridge*

(Received 20 December 1965)

Previous studies on the hydrolysis of unimolecular films of lecithin by venom phospholipase A (EC 3.1.1.4) used changes in the surface potential to measure the extent of the breakdown (Hughes, 1935*a*; Colacicco & Rapport, 1965). It was assumed that the lysolecithin formed remained in the film and that the replacement of lecithin by lysolecithin led to a decrease in the surface potential corresponding to the known differences between the surface potentials of films of pure lecithin and lysolecithin (Hughes, 1935*b*).

The present experiments, with a surface radioactivity technique (Bangham & Dawson, 1960), show that when unimolecular films of three ^{32}P -labelled phospholipids are treated with cobra (*Naja naja*) venom a considerable proportion of the lyso-compound formed actually leaves the film. This loss allows the physicochemical and biochemical determinants of the reaction to be investigated more directly.

The apparatus consisted of a cylindrical glass dish (9 cm. diam., 4.5 cm. deep) with a central glass-plate barrier (1 cm. deep) fused across the diameter of the open end and which divided the surface of the aqueous contents into two semi-

circular areas. The vessel was nearly filled with 0.9% NaCl solution (275 ml.) in 0.73 M tris-HCl buffer, pH 7.2, and a solution of the radioactive phospholipid (20 μg . of P/ml.) in CHCl_3 -ether (1:4, v/v) inserted on the surface of one semicircular area with a microsyringe and the solvent allowed to evaporate. The [^{32}P]lecithin and [^{32}P]phosphatidylethanolamine were isolated from ^{32}P -labelled *Saccharomyces cerevisiae* (Dawson & Bangham, 1959; Dawson, 1963). [^{32}P]Phosphatidylinositol was isolated from ^{32}P -labelled *Lipomyces lipofer* by using chromatography on formaldehyde-treated paper (Hörhammer, Wagner & Richter, 1959) for the final purification. The surface radioactivity was measured with a mica-window Geiger-Müller counter coupled to a rate-meter and pen recorder. The surface pressure was recorded with a Wilhelmy (1863) dipping plate and torsion balance. Reagents and venom were inserted into the other side of the vessel and the whole contents were stirred magnetically from the underside.

Fig. 1(*a*) shows a typical result of adding 1 mg. of venom (0.00036%) under a unimolecular film of [^{32}P]lecithin at a pressure of about 30 dynes/cm. ^{32}P was rapidly lost from the film, the surface

Fig. 1. Effect on the surface radioactivity of unimolecular films of ^{32}P -labelled phospholipids when cobra venom is added underneath. The ordinates represent the surface radioactivity in arbitrary units. The bulk phase consisted of 275 ml. of 0.145 M-NaCl in 0.73 mm-tris-HCl buffer, pH 7.2. ∇ , [^{32}P]Phospholipid film formed; $\circ$, 0.73 mm- CaCl_2 added; $\bullet$, 1 mg. of cobra venom added. (a) and (b) [^{32}P]Lecithin film at a surface pressure of 30 dynes/cm. (c) —, [^{32}P]Lecithin at a surface pressure of 42.8 dynes/cm. when venom was added; pressure was decreased to 21.2 dynes/cm. by sucking away part of the film. ----, [^{32}P]Lecithin film containing 15% (molar) dicetylphosphoric acid at a surface pressure of 44.2 dynes/cm. when venom was added. (d) [^{32}P]Phosphatidylethanolamine at a surface pressure of 38.1 dynes/cm.

radioactivity declining to a stable level which was some 15–20% of the original. The possibility was considered that this loss was due to the presence in the venom of a phospholipase B (Doery & Pearson, 1964) that was converting the lysolecithin formed into water-soluble glyceryl[^{32}P]phosphorylcholine. However, when the radioactive component was isolated after a film had been digested to completion it all behaved on solvent partition and thin-layer chromatography as lysolecithin. This suggests therefore that at the completion of the reaction the lysolecithin formed is in equilibrium,

with about 80–85% in the bulk aqueous phase and the remainder in the film.

The requirement for Ca^{2+} (0.73 mm) was absolute (Fig. 1b) whatever the surface pressure of the film. It could not be replaced by adding stearylamine to the film or Mg^{2+} to the bulk phase: indeed, the addition of Mg^{2+} antagonized the action of Ca^{2+} . EDTA (1.1 mm) completely inhibited the hydrolysis even when the reaction had started.

With films of lecithin at a surface pressure near the collapse (46 dynes/cm.) the loss of radioactivity on adding Ca^{2+} and venom was minimal (Fig. 1c). When the pressure was gradually lowered a rapid loss of radioactivity began at a pressure of approx. 29–33 dynes/cm. Below this pressure the initial rate of reaction (time for half substrate disappearance) appeared to be not appreciably affected by the pressure of the film. Addition of 15% dicetylphosphoric acid (but not stearylamine) to the lecithin caused a greatly increased loss of ^{32}P from high-pressure films in the presence of venom (Fig. 1c). However, as the pressure fell an accelerated rate of loss again became apparent at about 30 dynes/cm.

The behaviour of [^{32}P]phosphatidylethanolamine and [^{32}P]phosphatidylinositol films was similar to that of lecithin in that Ca^{2+} was obligatory for the enzymic hydrolysis. Whereas the loss of radioactivity from high-pressure phosphatidylinositol films was very slow, that from similar films of phosphatidylethanolamine was rapid even at high pressures (Fig. 1d).

If it is assumed that the slow loss of radioactivity from high-pressure lecithin films is due to a low rate of hydrolysis rather than an inability of the lysolecithin to leave the film, the present results suggest that yeast lecithin in a unimolecular film is not readily attacked by *Naja naja* venom unless the area/molecule is greater than about $75\text{\AA}^2$. The ability of dicetylphosphoric acid to enhance hydrolysis at film pressures above this may be due to the surface dilution caused by the repulsion of the anionic groups introduced into the film and the consequent increased spacing of the lecithin molecules (cf. Bangham & Dawson, 1960). However, it must be emphasized that a similar enhanced hydrolysis is not produced when cationic groups (stearylamine) are introduced into the lipid-water interface. Rather surprisingly, dicetylphosphoric acid does not stimulate the hydrolysis of lecithin particles by cobra venom and indeed acts as a potent inhibitor when the system has been activated with diethyl ether (Dawson, 1963). The hydrolysis of high-pressure films of phosphatidylethanolamine is probably due to these possessing a negative ζ potential owing to there being only partial ionization of the phospholipids' amino groups at the pH used (7.2). It was noted previously (Dawson, 1963) that

large phosphatidylethanolamine particles are hydrolysed without the necessity for the addition of diethylether. This latter solvent probably activates the hydrolysis of lecithin particles by causing a wider spacing of the phospholipid molecules orientated at the lipid-water interface as well as preventing the accumulation of the liberated fatty acids at this interface. The lack of hydrolysis of high-pressure phosphatidylinositol films is not consistent with this idea, but here other factors may be operative. Thus such films have a much higher affinity for ^{45}Ca than either lecithin or phosphatidylethanolamine (Dawson, 1965, and unpublished work), and this may have the effect of masking the stimulating effect of the interfacial anionic groupings.

I am indebted to Dr H. B. Stewart of the University of Western Ontario for a culture of *Lipomyces lipofer*.

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Biochem. J. (1966) **98**, 37c

Heat-Sensitive Deoxyribonuclease Activity in Cells Infected with Herpes Simplex Virus

By J. M. MORRISON and H. M. KEIR

Institute of Biochemistry, University of Glasgow

(Received 29 December 1965)

The activity of deoxyribonuclease (EC 3.1.4.5) from mammalian cells (BHK 21-C13) grown in tissue culture increases markedly after infection of the cells with herpes simplex virus (Keir & Gold, 1963). The increment could be ascribed to a stimulation or to a raised level of synthesis of the host-cell enzyme, induced by virus infection. It is also possible that the increment could arise from synthesis of new deoxyribonuclease, directed by the invading viral DNA, and this possibility is consistent with the observation that herpes-virus-specific RNA is transcribed from the herpes DNA after infection of the cells (Hay, Köteles, Keir & Subak-Sharpe, 1966). We now report briefly some observations which indicate that the increase of deoxyribonuclease activity found after infection is attributable to an enzyme(s) that differs in several respects from the corresponding enzyme(s) in the uninfected control cells.

BHK 21-C13 cells (derived from baby hamster kidney; MacPherson & Stoker, 1962) were grown as described by Russell *et al.* (1964) in 80 oz. bottles. The cell monolayers were infected with herpes

simplex virus (strain α) at a multiplicity of exposure 10. At 6-8 hr. later, the cell sheets were washed with phosphate-buffered saline (7.7 mM- Na_2HPO_4 -1.5 mM- KH_2PO_4 -0.14 M- NaCl) and removed from the glass with 0.5 mM-EDTA in phosphate-buffered saline. The cells were then washed twice with phosphate-buffered saline by centrifugation at 0° , and were stored at -75° until required. After the cells had been thawed, an initial enzyme extraction was carried out essentially as described by Keir & Shepherd (1965), to give a 105 000g supernatant fraction (S1) in 0.05 M-tris-HCl buffer (pH 8.0)-0.15 M-KCl-1 mM-EDTA-5 mM-2-mercaptoethanol. The 105 000g sediments were suspended in buffer of the same composition, homogenized vigorously for 1 min. in a Potter-Elvehjem homogenizer, and centrifuged at 105 000g to give a second soluble enzyme extract (S2). Further re-extraction of the 105 000g sediments did not yield significant amounts of enzyme activity.

Deoxyribonuclease was assayed by a standard procedure (Keir, 1962) that measured the release of acid-soluble DNA fragments from native or