Issued, March 1985

Should be yellow form

NATO Grants

for international collaboration in research

NATO Scientific Affairs Division, B-1110 Brussels, Belgium

GRANT CLOSURE

(Please submit in triplicate)

Reçu le - 8 NOV. 1989

The Project Co-ordinator should submit these final scientific and financial reports not later than two years after the last award. The Project Co-ordinator is responsible for reporting on the full amount of the latest award, even when it has been divided between the Principal Investigators. Invoices, receipts, tickets, etc. should not be enclosed but kept on file as stated in the conditions of award.

SCIENTIFIC REPORT

1. **Project title** PHOTOCHEMICAL STUDIES OF THE MECHANISM OF DNA PHOTOLYASE **Research Grant No.** RG86.0071 /

2. (a) **Project Co-ordinator** *(please give name, official address, telephone and telex numbers)*

Dr P.F.Heelis
Faculty of Science
The North East Wales Institute
Deeside,Clwyd,CH5 4BR,Great Britain

(b) **Other Principal Investigators** *(please give names only)*

Professor Aziz Sancar

3. **Publications resulting from the project**

No.	*(Please list the publications from the entire grant period and enclose one set of reprints and manuscripts accepted for publication)*	NATO support acknowledged YES	NO
1	Photochemical properties of E.Coli DNA photolyase. A flash photolysis study, P. F. Heelis and A. Sancar, Biochem., (1986), 25, 8163-8166.	✓	
2	Purification of the yeast PHR1 photolyase from E. Coli. Overproducing strain and characterisation of the intrinsic chromophores of the enzyme. G. B. Sancar, F. W. Smith and P. F. Heelis, J.Biol.Chem., (1987), 262, 15457-15465.	✓	
3	The active form of E. Coli DNA photolyase contains a fully reduced flavin and not a flavin radical, both in vivo and in vitro. G. Payne, P. F. Heelis, B. R. Bohrs and A. Sancar. Biochem., (1987), 26, 7121-7127.	✓	
4	Photochemical properties of E. Coli, DNA photolyase: Selective photodecomposition of the second chromophore,P. F. Heelis, G. Payne and A. Sancar. Biochem. 1987, 27, 4634-4640.	✓	
5	Doublet-Quartet intersystem crossing of flavin radical in DNA photolyase.T.Okamura,A.Sancar,P.F.Heelis,Y.Hirata and N.Mataga . J.Am.Chem.Soc.1989,111,5967.	✓	
6	Excited state properties of E.Coli DNA Photolyase in the picosecond to millisecond time scale.P.F. Heelis, T.Okamura, A.Sancar, Y. Hirata, N. Mataga and B.Thomas. (In Press)	✓	

(Continue on the last page, if necessary)

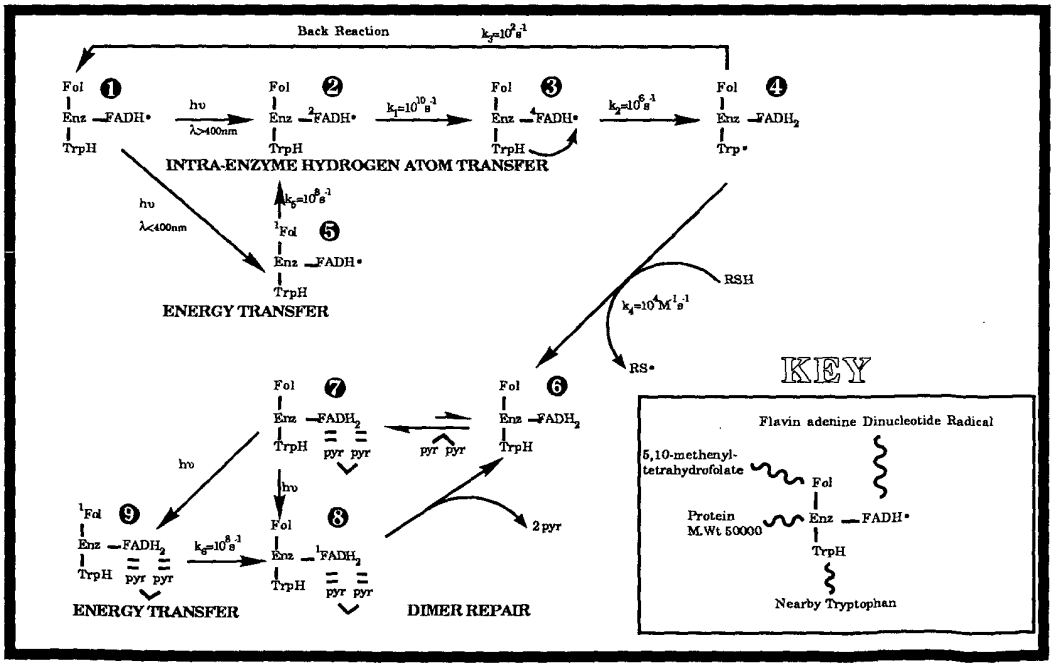
4. Abstract of the work accomplished and the results obtained

(Please refer to the publication numbers overleaf. A more detailed report should be submitted if the project did not result in any publication.)

Considerable progress has been made in understanding the mechanism of DNA repair. The primary process 'in vitro' has been shown to be photoreduction of the flavin radical ('❶' in figure 1,see papers 1 & 3). This proceeds via formation of the excited doublet state (❷) and subsequent intersystem crossing to give the quartet state (❸) as confirmed by picosecond flash photolysis (paper 5). Intra-enzyme hydrogen atom transfer from a tryptophan residue (❸ → ❹) is followed by either a back reaction (❹→ ❶) or electron transfer from reducing agents ❹ →❻ (paper 6).

The role of the second chromophore (now identified as a folate derivative (figure 2, page 6) has been shown to gather light (❶ →❺) in an analagous fashion to antennae pigments in photosynthesis and transfer energy to the flavin either in its radical (❺→❷) or reduced (❾ →❽) form (paper 4). The above results raised the question of whether the active form 'in vivo' or 'in vitro' is in fact the fully reduced enzyme. This was confirmed by a combination of e. s. r. and photochemical experiments on purified enzyme (paper 3). Comparative studies were carried out on the DNA photolyse enzyme from yeast. It was shown that although this enzyme is isolated in a fully reduced form, upon storage the flavin oxidises to its free radical form (as detected by e. p. r.) in an analogous manner to that of the E.coli enzyme. Subsequent irradiation results in radical reduction in the same way as outlined for the E.Coli enzyme (paper 2).

It has now been established that the main event in DNA repair occurs following excitation of the reduced flavin DNA complex (❽). Considerable information on the nature of the steps leading to DNA repair (❽→ ❻) have been obtained during picosecond flash photolysis carried out during the final trip of the award.The excited flavin ❽ has been observed for the first time and its quenching by pyrimidine dimers in DNA observed.While the nature of the quenching process is unknown ,this represents the first direct observation of the repair process.



THE PHOTOCHEMISTRY OF DNA PHOTOLYASE