Role of RNA cofactor of *PhrA* photolyase from *E. coli* in its binding with UV-irradiated DNA

Naresh C. Verma, Vidyasagar S. Hejmadi and Madhusoodan V. Hosur*

Radiation Biology Section, *Neutron Physics Division, Bhabha Atomic Research Centre, Bombay 400 085, India

RNA cofactor of *PhrA* photolyase has been digested with pancreatic RNase in the presence of either UV-irradiated or unirradiated DNA. Binding of photolyase with UV-irradiated DNA protects the RNA cofactor against digestion with RNase. The results are explained by building a theoretical model of a ten base-pair long DNA duplex with a thymine dimer in the middle. A qualitative model for the structure of the photolyase: UV-irradiated DNA complex has been proposed which is consistent with the functional properties of the enzyme.

THE *PhrA* photolyase (EC 4.1.99.3), a photoreactivating enzyme from *E. coli*, monomerizes *cis-syn* cyclobutane type pyrimidine dimers in UV-irradiated DNA, in the presence of visible light (350–600 nm)[1]. The photoreactivation involves two steps: first, the formation of a complex of photolyase with UV-irradiated DNA at the dimer site and second, the monomerization of the dimer on exposure to visible light and subsequent release of the photolyase from DNA. *PhrA* photolyase consists of an apoprotein of about 36,000 daltons and a non-covalently bound RNA (10–15 bases long) as the cofactor[2]. Although the role of RNA cofactor is not completely understood, its removal by digestion with pancreatic RNase[3], by adsorption on activated charcoal[2], or by prolonged dialysis[4] results in the loss of enzymatic activity. Furthermore, Hejmadi and Verma[5] have shown that in the absence of the RNA cofactor, the apoprotein of *PhrA* photolyase binds irreversibly and non-specifically with both UV-irradiated and unirradiated DNA. In this study we present evidence for the involvement of the RNA cofactor in the binding of *PhrA* photolyase with pyrimidine dimers bearing DNA. A model of a ten-base-pair long DNA duplex with a thymine dimer (T[]T) in the middle has been built to elucidate the nature of binding of the photolyase with UV-irradiated DNA.

Methods

Enzyme purification and assay

The enzyme was isolated and purified from an overproducing strain W3350(λcI857S7D→J) of *E. coli* by the method of Snapka and Sutherland[2]. The activity of *PhrA* photolyase was assayed by transformation assay using DNA with streptomycin resistance marker

(isolated from *H. influenzae*) as described earlier[5]. For *in vitro* photoreactivation, samples containing 350 ng DNA and 2 μg photolyase in 0.51 ml TED buffer (Tris-HCl, pH 7.2, 1 mM EDTA, 1 mM dithiothreitol) were exposed to 1000 W mercury vapour lamp for 60 min at 37°C.

RNase treatment

To digest the RNA cofactor, 10 μl RNase solution (1 mg/ml in TED buffer, heat-treated for 5 min at 80°C) was added in dark, to 100 μl photolyase solution (2 μg protein) either before or after mixing with 100 μl DNA solution (containing 350 ng either UV-irradiated or unirradiated DNA). The digestion was terminated after 15 min by adding 300 μl of RNase inhibitor (Sigma 7253).

Molecular model for UV-irradiated DNA and RNA cofactor

Molecular modelling was done on the VG3400 graphic terminal controlled by a PDP 11/34 (DEC, USA) computer, using the software package FRODO[6].

UV-irradiated DNA: We have assumed that the geometry of dimerized thymidylyl(3'–5')thymidylate (TpT) in crystal represents the geometry of the photodimer in UV-irradiated DNA[7]. Using the atomic coordinates of TpT we have generated the complementary strand ApA to obtain the dimerized duplex (TpT·ApA) as follows. The thymine base of a Watson–Crick T·A base pair in B-DNA geometry[8] was fitted by least squares method, in turn, to the thymine at the 5'- and 3'-ends of TpT dimer to obtain the trial coordinates of complementary adenines. However the N_9 atoms of adenines in these positions were too far apart (9.4 Å compared to 4.5 Å in B-DNA) to be spanned by the sugar-phosphate backbone. We could achieve backbone connectivity of these adenines by making the base-pair at the 3'-end of the dimer to be of the reverse Watson–Crick type. The ApA strand was then regularized for stereochemistry using the procedures described by Herman and McQueen[9]. This dimer duplex (TpT·ApA) was extended, in stages, in both directions by adding and regularizing B-DNA fragments of desired sequence. The model thus built was of the sequence [d(GCGTT[p]TTGAC)·d(GTCAAAACGC)]. The complete model was then subjected to two cycles of

idealization using restrained least squares refinement procedures of Hendrickson and Konnert[10] implemented in the programme NUCl SQ[11]. In this refinement the structure of the dimer T[]T was held fixed and the interstrand hydrogen bonding was maintained.

RNA cofactor: Although the RNA cofactor of photolyase is known to be 10–15 residues long[12], we believe that only a small non-helical portion interacts with UV-irradiated DNA and the rest of the RNA cofactor binds with the photolyase apoprotein. We have therefore built a model of only 4 residues long segment of the RNA cofactor. In the absence of any structural information about RNA cofactor, we have assumed a sequence GGAG for the 4 bases in the fragment of the RNA cofactor. However this assumption is not expected to seriously affect the results.

Results

To understand the role of the RNA cofactor, attempts were made to digest the RNA cofactor by treating the photolyase with pancreatic RNase under three different conditions. (i) Before binding with either UV-irradiated DNA or unirradiated DNA, (ii) After binding with UV-irradiated DNA, and (iii) After mixing with unirradiated DNA. The effect of RNase treatment on the activity of photolyase was studied by the transformation of *str* DNA in *H. influenzae* strain *uvr*-1, which is sensitive to streptomycin and also lacks the repair mechanisms for UV-damage in DNA. The results of transformation are given in Table 1 as per cent survival of *str* marker in different sample conditions taking the value for unirradiated and untreated DNA (control) as 100% (sample 1). All the samples except 1 and 2 were photoreactivated for 60 min at 37°C. Various additions were made in the same order as given in the table. The UV-irradiation of *str* DNA reduced the *str* survival to 10% (sample 2) which on photoreactivation with photolyase increased to 91% (sample 3). In sample 4, photolyase was first treated with RNase for 15 min in the dark. The digestion was terminated with RNase inhibitor before binding with UV-irradiated DNA and

Table 1. Effect of RNase treatment on *PhrA* photolyase in the absence and presence of either UV-irradiated or unirradiated *str* DNA as studied by transformation in *H. influenzae* uvr-1.

Sample description*	*str* survival (%)
N-DNA	100
UV-DNA	10
UV-DNA + *PhrA*-P	91
PhrA-P + RNase + RNaseI + UV-DNA	7
PhrA-P + UV-DNA + RNase + RNaseI	91
PhrA-P + N-DNA + RNase + RNaseI	7
RNase + RNaseI + N-DNA	>99

PhrA-P, *PhrA* photolyase; N-DNA, unirradiated DNA; UV-DNA, UV-irradiated DNA.

subsequent photoreactivation. In this case the survival was reduced to 7%, suggesting the loss of photoreactivating activity of the photolyase due to digestion of its RNA cofactor. However, when the photolyase was allowed to bind with UV-irradiated DNA before the RNase treatment, the survival of *str* marker was 91% (sample 5). Obviously the binding of photolyase with UV-irradiated DNA protects its RNA cofactor against digestion with RNase. This protection is completely lost when the UV-irradiated DNA is replaced with unirradiated DNA (sample 6). Sample 7 shows that RNase and RNase inhibitor alone do not affect the efficiency of transformation. These results suggest that the binding of photolyase with UV-irradiated DNA is such that the RNA cofactor gets sandwiched between the protein part of the photolyase and the pyrimidine dimer site of the UV-irradiated DNA thus becoming inaccessible to RNase.

The nature of interaction between RNA cofactor and the pyrimidine dimer site would depend on the deformations introduced in the structure of DNA by the presence of pyrimidine dimers. Husain *et al.*[13] have experimentally shown that the UV-irradiated DNA is bent by approximately 30° at the pyrimidine dimer site. A kink of 26° at the dimer site was also predicted by Pearlman *et al.*[14] in their energy refined theoretical model, which also predicts a slight unwinding of the helix at the dimer site (the helical twist being 23.6°). However, this model is based on the crystal structure of dimerised 1,3-dimethylthymine (DDMT) which has the cyclobutyl ring similar to the one in pyrimidine dimer, but lacks the connecting sugar-phosphate backbone[15]. Pearlman and coworkers had assumed B-DNA geometry for these phosphodiester bonds. However this assumption is not justified in view of the recent work by Cadet *et al.*[7] on the crystal structure of dimerized thymidylyl(3′ − 5′)thymidylate (TpT), which contains both the *cis–syn* cyclobutyl ring and the sugar-phosphate backbone. In this structure the relative orientation of the dimerized bases and the conformation of the phosphodiesters are radically different from that used by Pearlman *et al.* Therefore we have used the atomic co-ordinates of TpT in crystal and built a model of a ten base-pair long DNA duplex with a thymine dimer T[]T in the middle using the method described earlier (Figure 1). Our model also predicts a kink of 26° at the dimer site in agreement to Pearlman *et al.* However, in contrast to + 23.6° in their model, a helical twist of − 29° is predicted in our model. This negative helical twist, accompanied by the unusual phosphodiester conformation, manifests itself in a gross destacking of the purines on the complementary strand opposite to the pyrimidine dimer. These destacked purines may serve as a target for the photolyase to selectively bind at the dimer site through stacking interactions with its RNA cofactor. Such an interaction would demand that the region of
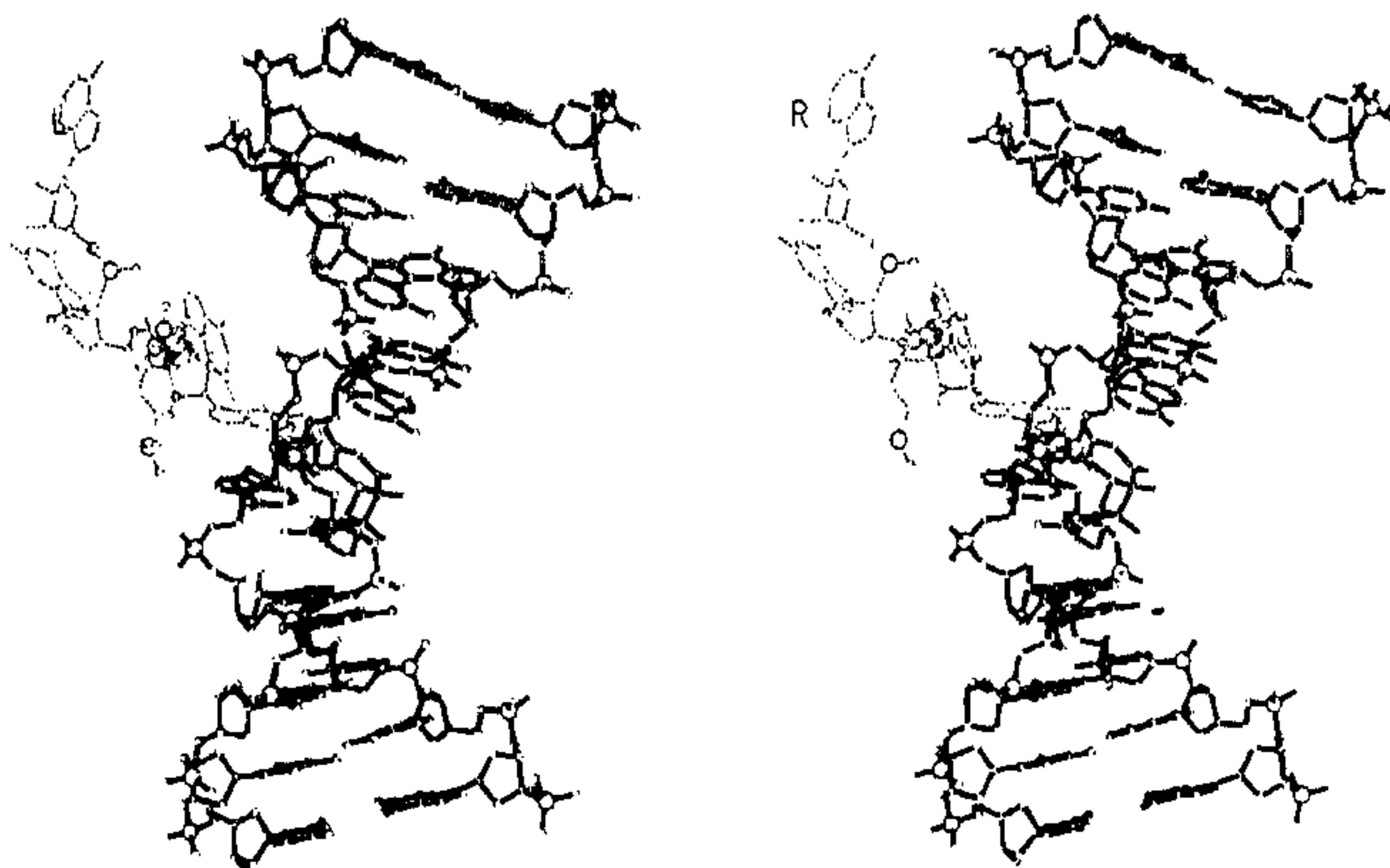
Figure 1. Stereo diagram of decamer double-helical DNA structure having a sequence [d(GCGT[TT]TGAC)·d(GTCAAAACGC)] with a thymine dimer T[]T in the middle (solid lines) and part of the RNA cofactor (dashed lines) four residues long with a sequence GGAG interacting with DNA at the dimer site. The adenine of RNA cofactor is shown stacked on the destacked adenine on the complementary strand of DNA duplex opposite to T[]T dimer.

the RNA cofactor which interacts with the dimer-site should also have destacked bases. Since RNA cofactor is known to have a partial double helical structure[12], one of the destacked purine bases from its single stranded region may be a good candidate to stack on the destacked target purines on the UV-irradiated DNA as shown in Figure 1. These ideas are schematically represented in Figure 2. A number of factors support the stacking interaction proposed above. For example, this mode of interaction is sequence non-specific and as such would explain the ability of photolyase to bind at the site of pyrimidine dimers of all types, viz., T[]T, T[]C, C[]T and C[]C. Appearance of a new absorption band at 350 nm and the hypochromacity observed at 260 nm on formation of the photolyase: UV-irradiated complex[16] can also be explained on the basis of such stacking interactions. It may be pointed out here that while making the model for the RNA cofactor we have assumed a sequence GGAG. According to our model the exact sequence of the RNA cofactor is not important so far as its binding through stacking interactions with UV-irradiated DNA is concerned. However, there may be other interactions (such as hydrogen bondings) in the photolyase: UV-irradiated DNA complex which may warrant specific sequence and geometry of the RNA cofactor. More experimental work is required to elucidate the exact structure of the complex.

Although qualitative, the model proposed above explains the functional properties of PhrA photolyase. It is obvious from our model that photolyase can bind only to UV-irradiated DNA and not to unirradiated DNA or free pyrimidine dimers. The model further
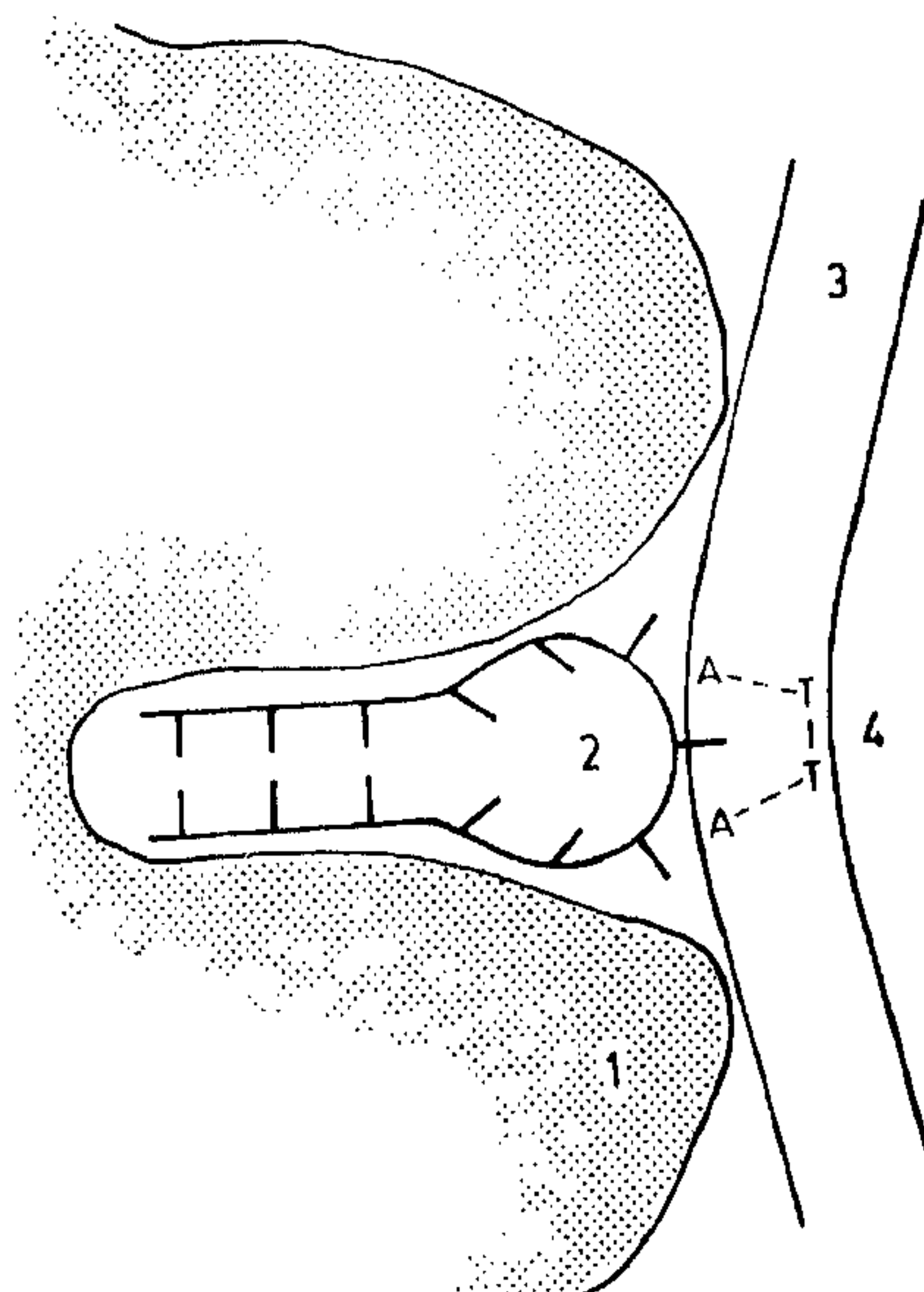

Figure 2. Schematic diagram of PhrA photolyase: UV-irradiated DNA complex. The UV-irradiated DNA (3) is bent at the T[]T dimer site (4) with its destacked adenines interacting with the RNA cofactor (2) The protein part of the PhrA photolyase is shown as shaded area (1).

predicts that any change in the structure of UV-irradiated DNA (such as breaking of phosphodiester or

N-glycosyl bonds at the dimer site) which affects the destacking of purines at the dimer site will also affect its binding with photolyase. In fact, Sutherland and coworkers[17] have shown that both the phosphodiester bonds internal to the dimer and the *N*-glycosyl bond joining the pyrimidine base to the deoxyribose must be intact for the *PhrA* photolyase to act. Breaking of either of the above mentioned bonds will remove the constraints imposed by the connectivity via sugar-phosphate backbone. As a consequence, the pyrimidine dimer will become flexible and adjust to a geometry similar to that of the dimerized dimethylthymidine. The structure of DNA under these conditions will be similar to that described by Pearlman *et al.*, which does not predict much destacking of the purines on the complementary strand. Therefore such a nicked DNA will not act as a substrate for the *PhrA* photolyase.

The model proposed above is mainly based on the stacking interactions between a base of RNA cofactor and the destacked target purines, which is essential for the recognition of the dimer site by the photolyase. However, other interactions between photolyase protein and DNA and hydrogen bonding between RNA cofactor and DNA backbone may exist and stabilize the photolyase: UV-irradiated DNA complex.

1. Sutherland, B. M., in *The Enzymes* (ed. Boyer, P. D.), Academic Press, New York, 1981, vol. 17, p. 481.
2. Snapka, R. M. and Sutherland, B. M., *Biochemistry*, 1980, 19, 4201.
3. Koka, P., *Biochemistry*, 1984, 23, 2914.
4. Snapka, R. M. and Fuselier, C. O., *Photochem. Photobiol.*, 1977, 25, 415.
5. Hejmadi, V. S. and Verma, N. C., *Indian J. Biochem. Biophys.*, 1987, 24, 189.
6. Jones, T. A., *J. Appl. Cryst.*, 1978, 11, 268.
7. Cadet, J., Voituriez, L., Hruska, F. F. and Grand, A., *Biopolymers*, 1985, 24, 897.
8. Arnott, S. and Hukins, D. W. I., *Biochem. Biophys. Res. Commun.*, 1972, 47, 1504.
9. Hermans, J. S. and McQueen, J. F., *Acta. Crystallogr.*, 1974, A30, 730.
10. Hendrickson, W. A. and Konnert, J. H., in *Biomolecular Structure, Conformation, Function and Evolution* (ed. Srinivasan, R.), Pergamon Press, Oxford and New York, 1981, vol. 1, p. 43.
11. Westhof, F., Dumas, P. and Moras, D., *J. Mol. Biol.*, 1985, 184, 119.
12. Cimino, G. and Sutherland, J. C., *Biochemistry*, 1982, 21, 3914.
13. Husain, I., Griffith, J. and Sancar, A., *Proc. Natl. Acad. Sci. USA*, 1988, 85, 2558.
14. Pearlman, D. A., Holbrook, S. R., Pirkle, D. and Kim, S. H., *Science*, 1985, 227, 1304.
15. Cameraman, N. and Cameraman, A., *J. Am. Chem. Soc.*, 1970, 92, 2523.
16. Wun, K. I., Gih, A. and Sutherland, J. C., *Biochemistry*, 1977, 16, 921.
17. Sutherland, B. M., Oliveira, O. M., Ciarrocchi, G., Brash, D. F., Haseltine, W. A., Lewis, R. I. and Hanawalt, P. C., *Biochemistry*, 1986, 25, 681.

Genetic variation in some wild populations of *Drosophila busckii*

Ravi Parkash, Jyoutsna and J. P. Yadav
Department of Biosciences, Maharshi Dayanand University, Rohtak 124 001, India

Isofemale lines derived from two natural population samples of *D. busckii* were analysed through horizontal starch gel electrophoresis for genetic variation in nine gene–enzyme systems. Five autosomal loci code for dimeric APH, ODH, MDH, ME and AO phenotypes; as well as allelic variants are represented by segregating single-band variants. Complex ACPH patterns are controlled by a duplicate locus while polymorphic esterase loci code for monomeric enzymes. However, α-GPDH and ADH are controlled by a single locus each and homozygous strains depict epigenetic two-banded phenotypes. Data on genetic structure at sixteen polymorphic loci in two populations of *D. busckii* are discussed here.

ELECTROPHORETIC techniques have revolutionized the status of empirical studies in population genetics, systematics and evolutionary biology[1,2]. Electrophoretic banding patterns can be transformed into allelic and/or non-allelic genetic variations which are used to describe the genetic structure of natural populations[3,4]. Data exist on the extent of genetic variability in local, regional and continental populations of *D. melanogaster* but there is little information on *D. busckii* which is the only known species of the subgenus Dorsilopha[1,5]. The present paper reports the patterns of genic variations occurring at loci coding for glucose-metabolizing enzymes and other enzyme systems in two populations of *D. busckii*.