netite–ilmenite–garnet–zircon suite. Magnetite and ilmenite are in equal proportions (32–34%), garnet, zircon and augite are less than 10% each, sillimanite and hornblende are about 2–5% each, chlorite, monazite and kyanite are about 1–2% and biotite, rutile and tourmaline are less than 1% each. An ilmenite–garnet–zircon–monazite suite characterizes VB beach placers. Ilmenite is most abundant (> 50%), followed by magnetite (13%), zircon (9%) and monazite (8%). Garnet is up to 9%, hypersthene is about 1% and hornblende, chlorite, biotite, kyanite, rutile and tourmaline less than 1% each. HM and VGU beach placers have higher amounts of magnetite, pyroxenes and amphiboles, indicating their derivation from the Deccan Traps. The Eastern Ghats provenance is the major source of ilmenite, rutile, monazite, zircon, sillimanite and garnet in VB. Higher percentages of magnetite, pyroxenes and amphiboles from HM and VGU[1,2] and ilmenite from VB[3] were also reported.

Magnetite and/or ilmenite constitute the bulk of black sand concentrates, other minerals are only an accessory. Magnetite being the major contributor to *K* merely indicates the magnetite abundance in sands and does not provide a measure of other minerals. On the other hand, magnetic susceptibility studies combined with radiometric studies reported earlier[11,12] provide criteria to estimate the relative abundance of magnetite, ilmenite and monazite in the beach placers of Andhra Pradesh. Magnetite is the most magnetic mineral in the beach sands along the Andhra Pradesh coast. Ilmenite, rutile, monazite, zircon, sillimanite and garnet contents are relatively high in the beaches north of Godavari River mouth, especially at Visakhapatnam–Bhimunipatnam and low in the beaches south of the Godavari River mouth as in Hamsaladivi–Manginipudi. The beach placer deposits are controlled by inputs from different provenances feeding the beaches. The Eastern Ghats provenance is the major source of minerals mentioned above, while the Deccan Traps have contributed higher amounts of magnetite, pyroxenes and amphiboles. Magnetic studies in conjunction with radiometric studies, would be an efficient tool in evaluating the relative abundances of magnetite, ilmenite, monazite and other associated economic minerals in the beach placers along the East Coast of India. The measurements are more rapid, easy and inexpensive than any other conventional exploration methods. These studies demonstrate that there is no mixing of mineral suites between the regions, since the river inputs and tidal-inlet currents act as a hydrodynamic barrier, trapping the longshore drift.

1. Krishnaiah Setty, B. and Dhana Raju, R., *J. Geol. Soc. India*, 1988, **31**, 491–494.
2. Dhanunjaya Rao, G., Krishnaiah Setty, B. and Rami Naidu, Ch. in *Exploration and Research for Atomic Minerals* (ed. Bhatnagar, R. K.), Nava Prakash Printing Press, Bombay, 1989, pp. 147–155.
3. Ramamohana Rao, T., Shanmukha Rao, Ch. and Sanyasi Rao, K., *J. Geol. Soc. India*, 1982, **23**, 284–289.
4. Currie, R. G. and Bornhold, B. D., *Mar. Geol.*, 1983, **51**, 115–127.
5. Narayana, A. C., Pandarinath, K., Karbassis, A. R. and Raghavan, B. R., *J. Geol. Soc. India*, 1991, **37**, 164–171.
6. Nagamalleswara Rao, B. and Kshirasagar, T. V. S. R., *Indian J. Mar. Sci.*, 1991, **20**, 292–293.
7. Folk, R. L. and Ward, W. D., *J. Sedment. Petrol.*, 1957, **57**, 3–27.
8. Radhakrishna Murty, C. and Likhite, S. D., *Bull. Nat. Geophys. Res. Inst.*, 1965, **3**, 1–8.
9. Ramachandran, C. J., *Geol. Soc. India*, 1990, **35**, 395–403.
10. Krishna Rao, J. S. R., *Sci. Cult.*, 1964, **30**, 552–553.
11. Nagamalleswara Rao, B. and Kshirasagar, T. V. S. R., *Indian J. Env. Prot.*, 1992, **12**, 646–648.
12. Nagamalleswara Rao, B., Some aspects of the Beach placer deposits, Central East Coast of India, Ph D thesis, Andhra University.

ACKNOWLEDGEMENTS. I thank Profs T. V. S. R. Kshirasagar and M. Subba Rao for a critical reading of the manuscript and Dr G. V. S. Poornachandra Rao of NGRI, Hyderabad for help in magnetic studies.

Received 28 August 1993; revised accepted 23 December 1993

Influence of oxidative and non-oxidative pathways of radiation damage on peroxidase activity in barley seeds

Ashu Bhan Tiku, S. P. Singh and P. C. Kesavan
School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

In comparison with nitrogen and nitrous oxide, oxygen-saturated medium greatly enhanced injury and peroxidase activity in eight-day-old seedlings raised from barley seeds exposed to ^{60}Co-γ-rays. The radiation damage in O_2-saturated medium not only increased peroxidase activity, but also induced two additional peroxidase bands, not present in any other situation, as detected by non-denaturing polyacrylamide gel electrophoresis (PAGE). These observations are briefly discussed in the light of available reports in the literature.

A wide range of cells and organisms respond to environmental stress, including oxidative stress, by forming stress proteins, some of which are similar to heat-shock proteins (hsps)[1–4]. When ionizing radiation is the causal agent of stress, the oxygen-dependent and oxygen-independent pathways account for the observed radiobiological effect[5,6]. It is generally believed that oxygen present *during* or after irradiation enhances radiobiological damage quantitatively, but does not produce any qualitative change. This accounts for the term oxygen enhancement ratio (OER)[7]. There is therefore a need to investigate whether oxic hydration merely enhances damage, or whether it also induces qualitative change(s). Use of seedling injury by radiation damage in barley seeds maintained in oxic or anoxic media, and/or chromosomal

aberrations in shoot-tip cells[8] have not revealed any qualitative differences. It was, however, reasoned that the study of degradative enzymes, which comprises several isoenzymes, might reveal subtle qualitative differences induced by ionizing radiation in the presence and absence of oxygen. Bagi *et al.*[9] studied such an enzyme, RNase, but their experimental design did not permit the resolution of the oxic and anoxic pathways of radiation damage. Singh and Kesavan[10] studied peroxidase activity of eight-day-old seedlings and found that post-irradiation in oxic medium not only increased peroxidase activity, but also induced two new molecular forms. The present study aims to verify if this is valid also for the kind of oxygen-dependent damage observed with the presence of oxygen during irradiation.

Seeds from a pure line, hull-less strain of barley (*Hordeum vulgare*) were used. Seeds were soaked in distilled water for 4 h at 25 ± 1°C and then transferred to fresh hydration medium saturated with oxygen, nitrogen or nitrous oxide for 2 h.

For preparation of hydration medium, double-distilled water was degassed by boiling for 20 min and allowed to cool to 10°C. Oxygen, nitrogen or nitrous oxide were then bubbled through for 20 min (for details see ref. 11). The oxygen contamination in nitrogen-and nitrous oxide-saturated water was 20–30 and 90–100 ppm respectively.

Vials containing presoaked seeds were exposed to 100 and 200 Gy of γ-rays (^{60}Co, 204 TBq, obtained from BARC, Bombay, India) at a dose-rate of 0.95 Gy/s as determined by Fe^{+2}/Fe^{+3} dosimetry. Vials were left for 2 h at 25°C. The seeds were plated in three replicate sets in petri dishes containing moist germination paper and kept in the dark at 25 ± 1°C, until the emergence of shoot tips. Thereafter they were illuminated continuously in a growth chamber. At 192 h after irradiation, seedling height was measured and seedling injury computed using the formula of Conger *et al.*[12].

Enzyme activity was assayed in 8-day-old seedlings. 3500 mg of seedlings was homogenized in 5 ml of 0.05 M Tris–HCl buffer (pH 7.2) in a pre-chilled mortar and pestle at 4°C. The homogenate was centrifuged at 20,000 *g* for 20 min at 4°C. The supernatant was used for further analysis. Total protein was estimated by the method of Bradford[13] using bovine serum albumin as standard. Peroxidase (EC 1.11.1.7) activity was determined by the procedure of Scandalios[14]. For total peroxide content Hochanadel's[15] method was used.

For non-denaturing PAGE, 500 mg of 8-day-old seedlings was homogenized in 5 ml buffer containing 20 mM Tris–HCl (pH 7.6), 3 mM magnesium acetate, 10 mM β mercaptoethanol and 0.5 mM phenylmethylsulphonyl fluoride. The homogenate was centrifuged at 20,000 *g* for 20 min at 4°C and the supernatant was used for electrophoretic studies. Peroxidase isozymes were analysed by the method of Laemmli[16]. Buffer containing 125 mM of Tris–HCl (pH 6.8), 15% glycerol and 0.01% bromophenol blue was added to extracts and resolved electrophoretically at 4°C in 16.0 × 14.0 cm gel (thickness 1.5 mm) using 10% acrylamide. Electrophoresis was carried out at 150 V at 4°C until the bromophenol blue migrated to the bottom of the gel. After electrophoresis, gel was washed with distilled water and then equilibrated with 1 : 4 (v/v) glacial acetic acid : water for 2 min. The gel was incubated in 15 mM benzidine solution for 3 min, followed by 20 mM hydrogen peroxide for 2 min. As soon as the bands appeared they were photographed.

There is no effect of O_2-, N_2- and N_2O-saturated water on seedling growth, peroxidase activity and total peroxide content of eight-day-old seedlings raised from *un*irradiated barley seeds (Table 1). However, the nature of the medium exerts a remarkable influence on the magnitude of effects in the irradiated seeds. Maximum damage to irradiated seeds is caused in O_2-saturated water; N_2- and N_2O-saturated water results in appreciably reduced damage (Table 1). Although seedling injury and peroxidase activity increase with radiation dose, the OER based on seedling injury remains constant (1.7 and 1.8 for 100 and 200 Gy respectively). It is also noted that as the seedling injury increases with dose, the peroxidase activity also increases but there is no correlation between seedling injury and peroxidase activity.

In this study, N_2O does not enhance radiation-induced seedling injury in comparison with that produced in N_2. There are several reports of N_2O-mediated increase in radiosensitivity[17–23]. The enhanced sensitivity has been ascribed to the conversion of hydrated electrons into hydroxyl radicals[24]. No radiosensitization by N_2O has, however, been observed in lymphocytes[25], Chinese hamster V 79 cells[26] and vegetative cells of bacteria[27, 28]. The sensitizing action of N_2O is influenced by factors such as cell concentration, dose-rate, and irradiation temperature[22]. Contrary to reports dealing with radiosensitization by N_2O, Singh and Kesavan[29] have shown that N_2O affords radioprotection to very dry seeds. This has been explained on the basis of restoration by N_2O of a more favourable balance between reducing and oxidising species[29]. In the present study, N_2O exerts neither a sensitizing nor protective effect (Table 1).

When metabolizing seeds are irradiated, the radiation chemistry is initiated as follows:

$$RH_2 \longrightarrow RH^{\cdot},\ H^{\cdot},\ OH^{\cdot},\ e^{-}_{aq}, \qquad (1)$$

where RH_2 is the targets molecule, e.g. DNA. Oxygen present in the medium reacts with these radicals as follows:

Table 1. Influence of nature of hydration medium on seedling growth, peroxidase activity and total peroxides in metabolizing barley seeds

Dose (Gy)	Treatment	8-day-old seedling height (cm ± SE)	% seedling injury	Total protein (mg/g seedling)	Peroxidase activity (units/mg protein)	Peroxidase activity (units/g seedling)	Total peroxides ($\times 10^{-5}$ M)
0	Oxygen water	15.85 ± 0.16	0	7.47	12.5	93.6	2.8
0	Nitrogen water	15.70 ± 0.16	0	8.57	10.3	88.5	3.0
0	Nitrous oxide water	15.32 ± 0.17	0	9.09	10.8	98.6	3.2
100	Oxygen water	9.00 ± 0.63	42	6.91	56.6	391.9	2.5
100	Nitrogen water	11.73 ± 0.46	25	8.21	23.3	191.2	3.2
100	Nitrous oxide water	11.75 ± 0.35	25	7.44	28.5	212.0	3.6
200	Oxygen water	4.59 ± 0.50	71	8.45	94.1	795.3	1.5
200	Nitrogen water	9.61 ± 0.47	39	11.14	18.9	210.2	3.5
200	Nitrous oxide water	9.88 ± 0.40	37	10.74	26.0	280.6	3.8

$$RH^{\bullet} + O_2 \longrightarrow RHO_2^{\bullet}, \quad (2)$$

$$H^{\bullet} + O_2 \longrightarrow HO_2^{\bullet}, \quad (3)$$

$$e_{aq}^{-} + O_2 \longrightarrow O_2^{-}, \quad (4)$$

$RHO_2^{\bullet}$ and $HO_2^{\bullet}$ may be irreversible reaction products resulting from 'O_2-fixation' of the damage. This may account for the substantial damage to irradiated seeds following hydration in oxygen-saturated water.

The results (Table 1) clearly show that with increase in seedling injury there is concomitant increase in peroxidase activity. Non-denaturing PAGE analysis of peroxidase isozymes shows that increase in peroxidase activity is manifested as enhanced intensity of peroxidase bands in treated compared to control samples. The intensity is maximal for seeds given oxygenated post-hydration (Figure 1). Such quantitative increase in peroxidase activity has been observed in sweet potato following irradiation[30]. It is noted (Table 1, Figure 1) that besides the quantitative increase, two additional bands also appear in the non-denaturing PAGE analysis of the sample extracted from seedling raised from seeds given oxic post-hydration. However, the increase in peroxidase activity in N_2 and N_2O is not linked with the induction of new peroxidase isozymes. This is further confirmed by the *absence* of two additional bands even when the experiment is repeated with equal peroxidase activity in all the lanes. Furthermore, in the case of dry barley seeds also, the dramatic potentiation of anoxic radiation damage by caffeine results in enhanced peroxidase activity but *without* the induction of new peroxidase bands[10]. Thus, the increase in peroxidase activity is *not* always accompanied with the induction of new isozymes, but it is the anoxic or the oxic condition which influences the production of new isozymes detectable in the form of additional peroxidase bands.

These data support the contention that the reduction products of oxygen ($RHO_2^{\bullet}$, $HO_2^{\bullet}$, O_2^{-}, H_2O_2) may trigger the expression of certain gene(s)[10,31]. The present studies suggest that, in spite of the lack of $OH^{\bullet}$ and e_{aq}^{-}, dry

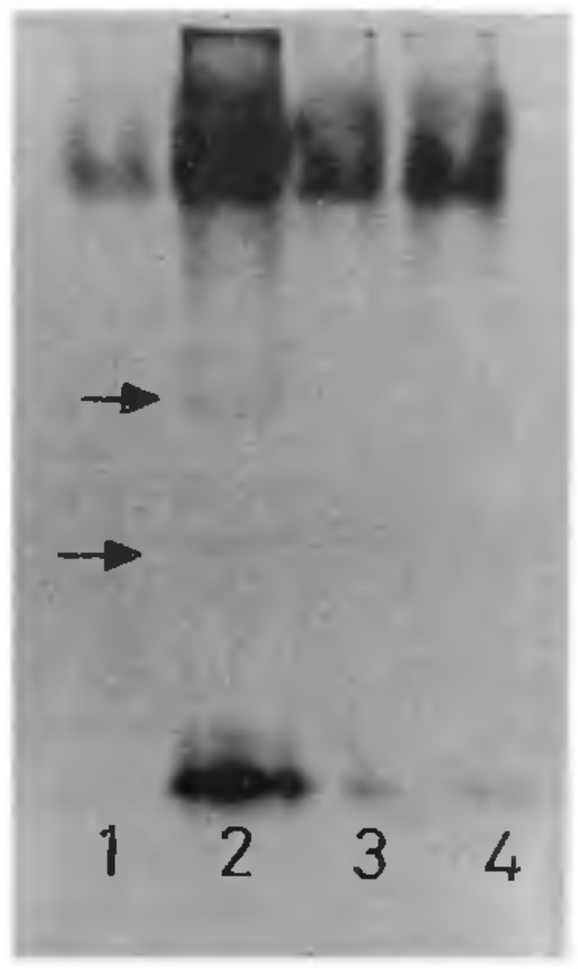

Figure 1. Activity gel of peroxidase Lane 1: unirradiated control; Lanes 2–4 irradiated (200 Gy) and hydrated in oxygen-, nitrogen- and nitrous oxide-saturated water respectively. Arrows indicate new isozyme bands. Sample load = 50 µg protein per lane.

seeds, like the metabolizing seeds, provide signals to express the peroxidase gene(s) and/or to influence post-transcriptional events.

1. Ashburn, M. and Bonner, J. J., *Cell*, 1979, **17**, 241–254.
2. Schlesinger, M. H., Ashburner, M. and Tissieres, A. (eds.), *Heat Shocks from Bacteria to Man*, Cold Spring Harbor Laboratory, 1982.
3. Tanguay, R. M., *Can. J. Biochem. Cell. Biol.*, 1983, **61**, 387–394.
4. Kapoor, M. and Lewis, J., *Can. J. Microbiol.*, 1987, **33**, 162–168.
5. Powers, E. L., *J. Cell. Comp. Physiol. Suppl.* 1, 1961, **58**, 13–25.
6. Kesavan, P. C., Trasi, S. and Ahmad, A., *Int. J. Radiat. Biol.*, 1973, **24**, 581–587.
7. Alper, T., *Cellular Radiobiology*, Syndich Cambridge-Press, 1979.
8. Kesavan, P. C. and Ahmad, A., *Mutat. Res.*, 1974, **23**, 337–346.
9. Bagi, G., Bornemisza-Pauspert, P. and Hidvegi, E. J., *Int. J. Radiat. Biol.*, 1988, **53**, 507–519.
10. Singh, S. P. and Kesavan, P. C., *Int. J. Radiat. Biol.*, 1991, **59**, 1227–1236.

11. Kesavan, P. C., Sharma, G. J. and Afzal, S. M. J., *Radiat. Res*, 1978, 75, 18–30
12. Conger, B V., Nilan, R. A. and Konzak, C. F., *Radiat. Bot.*, 1968, 8, 31–36
13. Bradford, M. M, *Anal. Biochem.*, 1976, 72, 248–254.
14. Scandalios, J. G., *Biochem. Genet*, 1969, 3, 37–79.
15. Hochanadel, C. J, *J. Phys. Chem.*, 1952, 56, 587–594.
16. Laemmli, U. K., *Nature*, 1970, 227, 680–685.
17. Powers, E. L. and Cross, M., *Int J. Radiat. Biol.*, 1970, 17, 501–514.
18 Ewing, D., Fielden, E. M and Roberts, P. B., *Radiat. Res.*, 1974, 58, 481–488.
19. Kesavan, P. C. and Powers, E. L., in Proceedings of the 8th International Congress of Radiation Research, Edinburgh (eds Fielden, E. M., Fowler, J. F., Hendry, J. H. and Scott, D) *Radiat Res.* 1, 1987, C30-4P.
20 Brustad, T. and Wold, E., *Radiat. Res.*, 1976, 66, 215–230.
21. Samuni, A. and Czapski, G., *Radiat. Res*, 1978, 76, 624–632.
22. Watanabe, H., Iizuka, H. and Takehisa, M., *Radiat. Res.*, 1982, 89, 325–333.
23. Roots, A., Chatterjee, A., Blakely, E., Change, P., Smith, K. and Tobias, C., *Radiat. Res.*, 1982, 71, 678–682.
24. Keene, J. P., *Radiat. Res*, 1964, 22, 1–13.
25. Antoku, S., *Radiat. Res.*, 1977, 71, 678–682.
26. Ewing, D., Koval T. M. and Walton, H L., *Radiat. Res.*, 1986, 106, 356–365.
27. Simone, G. and Quintiliani, M., *Int. J Radiat. Biol.*, 1977, 31, 1–10.
28. Tibly, M. J., Loverock, P. S. and Fielden, E. M, *Radiat. Res.*, 1982, 89, 488–502.
29. Singh, S. P. and Kesavan, P. C., *Curr. Sci.*, 1992, 62, 486–488
30. Ogawa, M. and Uritani, J., *Radiat. Res.*, 1970, 41, 342–351.
31. Ropp, M., Courgeon, A. M, Clavayrae, R. and Best Belpomme, M., *Can. J. Biochem. Cell. Biol.*, 1983, 61, 456–461.

ACKNOWLEDGEMENT. A.B.T is thankful to the Council of Scientific and Industrial Research, New Delhi for financial assistance.

Received 31 May 1993; revised accepted 23 February 1994

Tertiary structural categories of leiurotoxin and some other scorpion venom toxins

P. Narayanan
Department of Life Sciences, University of Bombay, Bombay 400 098, India

Similarly-placed cysteine residues (and in-register disulphide bridges) in CnII-11 (C. noxius), noxiustoxin, charybdotoxin and leiurotoxin I, and amino acid sequence homology among these scorpion venom toxins enable classification of these molecules under CnII-11 type tertiary structural category under scorpion-toxin type proteins. Similar structural motif is also found in bee venom toxin apamin and apamin-type peptides. Neurotoxin P2 (Amm P2) should be classified under the I5A type tertiary structure.

THE unique folding of macromolecules is due to the cooperative process of packing interactions of side chains, motifs, modules and domains. Therefore, prediction of tertiary structures of proteins from their amino acid sequence data is an extremely complex and challenging task. Prediction of secondary structural elements (helix, sheet and turns) of proteins from their primary structural data is one of the strategies towards the goal of predicting their tertiary structures (vide literature). But these procedures have inherent limitations because structural topologies (motifs, modules, domains, etc.), and not amino acid sequence homologies, are better conserved in folding (evolution). Even in cases where no obvious amino acid sequence homologies may be found, the unknown structures can be modelled based on structural motifs and topologies. To emphasize, the essence of structure prediction is one of pattern matchings and, therefore, the logical way of addressing this complex problem is to identify proteins by motifs, modules and shapes and align the amino acid sequences to fit the topologies. Prediction of tertiary structure (protein folding) from the primary structure data can be attempted on certain classes of proteins with better success. One of the examples is immunoglobulins. On the basis of comparative studies of known antibody structures and application of energy constraints and distance-geometry methods, the repertoire of conformations of the antibody-combining site for a given amino acid sequence of the hypervariable loop in immunoglobulins could be modelled[1,2].

Another class of proteins/peptides where the natural constraints, imposed by disulphide bridges, would simplify the protein folding problem is that of disulphide-containing proteins/peptides. In addition to empirical rules governing the packing interactions that occur between and among secondary structural elements to form motifs and modules, the incorporation of the structural role and hierarchies of disulphide bonds, where S-S bonds have predominant influence on the folding processes (to which belong the bee, scorpion, several of snake and sea snail venom toxins, hormones, growth factors, insect defensins, etc.), would improve the rate of success of structure prediction methods in the disulphide-containing proteins/peptides. The empirical 'knowledge' one could discern in the case of disulphide-containing molecules is: (i) S-S bridges are found as integral parts of structural motifs, creating hydrophobic moieties (hydrophobic effect) and (ii) there exists structural hierarchy of S-S bridges in stabilizing the structural moieties and tertiary folding. Emphasizing this empirical 'knowledge' and based on the structural motif that exists in the scorpion venom toxin, CsEV3[3], all the 'scorpion-toxin' type proteins can be classified under five tertiary structural categories from their amino acid sequence data[4]. Energy constraints, minimum accessibility of hydrophobic moieties to solvent and other procedures can be employed to model individual tertiary structures from these 'canonical' tertiary structural categories.