Antimutagenic activity of tryptophan and alanine

A. K. Handique and H. Aprem*

Department of Applied Botany and Biotechnology, Gauhati University, Guwahati 781 014, India
*Present address: Quality Control Unit, Biochemistry Division, MAHYCO, Kamadod 581 115, India

Antimutagenic activity of the amino acids tryptophan and alanine has been studied using ethyl methyl sulphonate and dimethyl sulphate-induced chromosomal aberration in onion root tip cells. Both the amino acids exhibited considerable antimutagenic activity by way of reducing the level of chromosomal aberrations. Duration of pre-treatment with the antimutagens was found to be important. On a comparative basis, tryptophan was found to be more efficient than alanine.

EVER since Muller first discovered the mutagenic action of X-ray in 1927 followed by the discovery of chemical mutagens by Auerbach in 1944, a large number of chemical substances have been added to the list, many of which are routinely used in plant breeding programmes. Even unsuspecting agents like laser beams, viruses and drugs like streptomycin are known to be mutagenic[1]. According to a conservative estimate[2], about 70,000 man-made chemicals are in everyday use and over a thousand new chemicals are put to use every year. Therefore the list and concern over mutagenic substances is never-ending. Environmental mutagens have generally two broad category effects. Firstly, they cause toxicity to the organism exposed, which manifests in the form of various ailments to mortality. The other is a long-term effect – they cause mutations which are mostly deleterious and these are transmitted to the future generations who will suffer even if they are not exposed. Against this background, antimutagens which can neutralize the mutagenic effect, deserve serious attention. Particularly certain vitamins and pro-vitamins like α-tocopherol, ascorbic acid, β-carotene; amino acids like tryptophan and cystein, metal salt of selenium, etc. are reported to have considerable antimutagenic property[3–5]. Since the concept of antimutagen developed in the 1950s, about 200 compounds are known to have varied degree of antimutagenic property[3]. The potential use of antimutagen is envisaged in plant tissue culture where chromosomal aberration is very common and which is one of the major reasons for somaclonal variation. Antimutagens can also be used for long-term cryopreservation of seeds, embryo, somatic embryo, tissue, etc. to provide the much needed genetic stability. The present study was undertaken to investigate the antimutagenic potency of amino acids – tryptophan and alanine, using chromosomal aberration study as the criterion, with emphasis on duration of pre-treatment (Tables 1, 2).

For the present study, onion root tips were taken the study material. The mutagens used were ethyl met ane sulphonate (EMS) and dimethyl sulphate (DMS) concentrations of 0.05%, 0.1%, 0.2% and 0.3%. F tryptophan and alanine, 500 ppm solutions were use Healthy onions were rooted on moist sand for one da Thereafter the roots were washed with distilled wa

Table 1 *a*. Decline in chromosomal aberrations due to pre-treatm with tryptophan followed by EMS treatment

EMS (%)	Tryptophan pre-treatment (500 ppm)	Chromosomal aberration: Laggards	Breakages	Bridges	Total aberration
0.00	Nil	0	0	0	0
0.05		0	0	6	6
0.10		0	0	8	8
0.20		0	0	9	9
0.30		0	1	11	12
0.00		0	0	0	0
0.05	10 h	0	0	2	2
0.10		0	0	4	4
0.20		0	0	5	5
0.30		0	0	5	5
0.00		0	0	0	0
0.05		0	0	0	0
0.10	20 h	0	0	0	0
0.20		0	0	0	0
0.30		0	0	1	1

Duration of EMS treatment = 8 h.
CD for tryptophan pre-treatment: At 5% probability level = 0.544
1% probability level = 0.733.

Table 1 *b*. Decline in chromosomal aberrations due to pre-treatn with tryptophan followed by DMS treatment

DMS (%)	Tryptophan pre-treatment (500 ppm)	Chromosomal aberration: Laggards	Breakages	Bridges	Total aberration
0.00	Nil	0	0	0	0
0.05		0	0	4	4
0.10		0	0	5	5
0.20		0	0	7	7
0.30		0	0	8	8
0.00		0	0	0	0
0.05	10 h	0	0	1	1
0.10		0	0	2	2
0.20		0	0	3	3
0.30		0	0	3	3
0.00		0	0	0	0
0.05		0	0	0	0
0.10	20 h	0	0	0	0
0.20		0	0	1	1
0.30		0	0	1	1

Duration of DMS treatment = 8 h.
CD for tryptophan pre-treatment: At 5% probability level = 0.384
1% probability level = 0.517.

Table 2 *a*. Decline in chromosomal aberrations due to pre-treatment with alanine followed by EMS treatment

EMS (%)	Alanine pre-treatment (500 ppm)	Chromosomal aberration			Total aberration (%)
		Laggards	Breakages	Bridges	
0.00		0	0	0	0
0.05		0	0	6	6
0.10	Nil	0	0	8	8
0.20		0	0	9	9
0.30		0	1	11	12
0.00		0	0	0	0
0.05		0	0	3	3
0.10	10 h	0	1	4	5
0.20		0	0	5	5
0.30		0	0	6	6
0.00		0	0	0	0
0.05		0	0	1	1
0.10	20 h	0	0	2	2
0.20		0	0	2	2
0.30		0	0	3	3

Duration of EMS treatment = 8 h.
CD for alanine pre-treatment: At 5% probability level = 1.247, At 1% probability level = 1.680.

Table 2 *b*. Decline in chromosomal aberrations due to pre-treatment with alanine followed by DMS treatment

DMS (%)	Alanine pre-treatment (500 ppm)	Chromosomal aberration			Total aberration (%)
		Laggards	Breakages	Bridges	
0.00		0	0	0	0
0.05		0	0	4	4
0.10	Nil	0	0	5	5
0.20		0	0	7	7
0.30		0	0	8	8
0.00		0	0	0	0
0.05		0	0	4	4
0.10	10 h	0	1	5	6
0.20		0	0	7	7
0.30		0	0	8	8
0.00		0	0	0	0
0.05		0	0	3	3
0.10	20 h	0	0	4	4
0.20		0	0	4	4
0.30		0	0	5	5

Duration of DMS treatment = 8 h.
C.D for alanine pre-treatment: At 5% probability level = 0.430, At 1% probability level = 0.579.

and treated with tryptophan and alanine solutions for 10 h and 20 h respectively. Subsequently the roots were again washed and treated with different concentrations of the mutagens for 8 h after which the roots were thoroughly washed in running tap water. The root tips were collected and fixed in aceto-alcohol for 2 h and then transferred to 70% ethanol. Root tip squash was made in aceto-carmine and then observed for chromosomal aberrations. For each treatment, 100 dividing cells were observed and each treatment was replicated thrice. The control sets were treated with distilled water followed by mutagenic treatment. The data were statistically analysed using Fisher's method for determination of variance ratio.

Chromosomal aberrations were found in the form of laggards, breakages and bridges in early anaphase stage. Tryptophan exhibited pronounced antimutagenic activity. In case of tryptophan pre-treatment for 10 h followed by EMS treatment, the range of aberration came down to 2–5% against 6–12% in control. But when the duration of pre-treatment was increased to 20 h, there was virtually no chromosomal aberration, except 1% aberration in case of 0.3% EMS treatment. The impact of duration of tryptophan pre-treatment has been found to be highly significant (CD = 0.733; $P > 0.01$). Tryptophan pre-treatment was similarly effective against DMS also. Pre-treatment with tryptophan for 10 h followed by DMS treatment resulted in chromosomal aberration 1–3% as against 4–8% in control. On increasing the pre-treatment duration to 20 h, there was no aberration at lower concentration of DMS treatment; only at higher concentration of DMS treatment 1% aberration was observed. The observation has been found to be highly significant (CD = 0.517; $P > 0.01$).

Alanine pre-treatment for 10 h followed by EMS treatment brought about nearly 50% reduction in chromosomal aberration. Increasing the duration of treatment to 20 h resulted in remarkable reduction in chromosomal aberration, when the range of aberration was 1–3% as against 6–12% in control. The effect of increasing duration of alanine pre-treatment on reducing chromosomal aberration has been found to be highly significant (CD = 1.68; $P > 0.01$). However, alanine pre-treatment for 10 h against DMS treatment failed to reduce chromosomal aberration. But a higher duration, i.e. 20 h pre-treatment of alanine was effective when the range of aberration was 3–5% as against 4–8% in control. Statistically it has been found to be highly significant (CD = 0.579; $P > 0.01$).

From the present study it is evident that tryptophan has remarkable antimutagenic activity by way of protecting the chromosomes against physical damage by mutagens. Moreover, another amino acid alanine, previously untested, has been found to have considerable antimutagenic activity although its efficacy is comparatively lesser than tryptophan. Cherkasov[6] studied the anti-mutagenic activity of tryptophan by treating the seeds of *Allium cepa* and found considerable antimutagenic activity. However, his study showed that variation in concentration of tryptophan made little difference. In the present study the emphasis was on duration of pre-treatment and the results clearly show that longer duration of pre-treatment, i.e. 20 h is remarkably effective. Moreover, tryptophan is equally effective against both the mutagens EMS and DMS.

Unlike tryptophan, alanine is not much effective against DMS, although it is considerably effective against EMS. But like tryptophan, in case of alanine also, the duration of pre-treatment made considerable difference. While a 10 h pre-treatment was simply ineffective against DMS, the same concentration at longer duration, i.e. 20 h pre-treatment could considerably reduce chromosomal aberration. Similar observations regarding greater efficacy of antimutagen following pretreatment for longer duration like 20 h were reported by Abutalybov *et al.*[7] working with *Allium fistulam* using ionol and Alekperov[15] working with *Crepis capillaris* using α-tocopherol as antimutagen. It appears that different antimutagens are not equally effective against various mutagens. But an ideal antimutagen should be the one which is universally effective against all mutagens or at least a broad spectrum of mutagens. Tryptophan deserves further and detailed investigation to ascertain whether it can be called a broad spectrum antimutagen or not. Apart from a chromosomal aberration test in onion root tip, the efficacy of antimutagens has been tested in the prokaryotic system, viz. *Salmonella typhimurium* by Bala and Grover[8], Grover and Bala[9] using citrus and myroblan fruit juice as source of antimutagen which are known to be very rich in ascorbic acid. Antimutagenic activity of polyphenols like caffeic acid and gallic acid has been demonstrated by Chan *et al.*[10] using the Salmonella test system.

Apart from tryptophan and alanine, at least another amino acid, cystein is known to have antimutagenic effect as demonstrated by Garina and Nurzhanova[11] and Ionaitis Sokolov and Ranchyalis[12]. It is not understood how a simple amino acid molecule can protect the chromosomes against mutagenic treatment damage. Kada *et al.*[13] classified antimutagens into two major groups: desmutagen and bioantimutagen. Desmutagens are defined as those which inactivate mutagen *in vitro* before they reach the interior of cell. It is likely that antimutagens like tryptophan and alanine neutralize the free radicals generated by the mutagens, thereby rendering them ineffective. Strelchk[14] detected antimutagenic activity of the extracts of *Eleutherococcus senticosum* against EMS by post treatment. However the extract had a protective effect only in the first four hours after post treatment. Obviously the mutagens do all the damage in the first four hours after treatment and therefore post treatment with antimutagen beyond this period is of no avail.

The present study clearly shows that apart from tryptophan, another amino acid alanine also has antimutagenic property, although tryptophan is superior to alanine as antimutagen.

1. Handique, A. K., *Everyman's Science*, 1990, **25**, 161–162.
2. Das, R. K., *Sambalpur Univ. J. Sci. Technol.*, Silver Jubilee Volume, 1991, pp 33–45.
3. Alekperov, U. K., *Science in USSR*, 1975, **5**, 17–19.
4. Alekperov, U. K., *Pyirodia (USSR)*, 1982, **12**, 24–28.
5. Medzhidov, M. M., Abutalybov, M. G. and Alekperov, U. K., *Ref. Zhur.*, 1977, **2T**, 327.
6. Cherkasov, O. A., *Tsitol. Genet.*, 1977, **11**, 66–68.
7. Abutalybov, M. G., Bagirova, A. D. and Akekperov, U. K., *Ref. Zhurnal.*, 1975, **6T**, 424.
8. Bala, S. and Grover, I. S., *Mutat. Res.*, 1989, **222**, 141.
9. Grover, I. S. and Bala, S., *Indian J. Exp. Biol.*, 1992, **30**, 339–341.
10. Chan, R. I. M., San, R. H. C. and Stitch, H. F., *Cancer Lett.*, 1986, **31**, 27.
11. Garina, K. D. and Nurzhanova, A. A., *Ref. Zh.*, 1982, **2**, 65–86.
12. Ionaitis Sokolov, E. K. and Ranchyalis, V. P., *Ref. Zh.*, 1982, **2**, 685–683.
13. Kada, T., Inoue, T. and Namiki, M., *Environmental Mutagenesis, Carcinogenesis and Plant Biology*, (ed. Klekowski Jr. J.), Praeger, New York, 1982, p. 133.
14. Strelchk, S. I., *Tsitol. Genet.*, 1987, **21**, 136–139.
15. Alekperov, U. K., *Tsitol. Genet.*, 1976, **10**, 37–39.

Received 24 September 1996; revised accepted 3 March 1997

Anti-staphylococcal activity of *Pseudomonas aeruginosa*

G. Arunkumar, S. Gowrish Rao and P. G. Shivananda

Department of Microbiology, Kasturba Medical College, Manipal 576 119, India

Anti-staphylococcal activity of *Pseudomonas aeruginosa* was studied on a total of 118 strains of staphylococci. The results revealed the existence of a highly active anti-staphylococcal *Pseudomonas aeruginosa* metabolite which appears to be pyocyanine. It was even active against methicillin resistant *Staphylococcus aureus* (MRSA) and coagulase negative staphylococci (CONS).

PSEUDOMONAS represents the major group of non-differentiating microorganisms producing antibiotics[1]. The practical use of antibiotics from *Pseudomonas* sp. dates back to the period before the antibiotic era. Emmerich and Low[2] reported that the cell-free culture fluid of *Pseudomonas aeruginosa*, concentrated to one tenth of its original volume, killed several kinds of bacteria. This was extensively used in the therapy of diphtheria, influenza and meningitis during the first two decades of the century[1]. During the antibiotic era, approximately 50 different antibiotic substances from *Pseudomonas* sp. were discovered. Of these compounds, pyocyanine and pyrrolnitrin were most abundantly present. We failed to find in literature any study regarding anti-staphylococcal activity of *P. aeruginosa*, except a report on the biosyn-