

colour. Thus the present isolate *A. brassicicola* (Schew) Wiltshire is quite different from *A. brassicae* (Berk.) Sacc.

The pathogen has been deposited under accession No. ITCC 2939 with the courtesy of Dr J. N. Kapoor.

The conidial suspension in sterilized distilled water was atomized on taramira plants which were kept in moist chambers (36 hr before and after inoculation). Characteristic symptoms of leaf blight developed on the inoculated plants within 5–7 days and the fungus *A. brassicicola* (Schew) Wiltshire was recovered from the diseased leaves.

The symptoms start appearing during the last week of January and become abundant in the last week of March.

The author thanks Prof. K. G. Mukerji, of Delhi University, Delhi for the confirmation of fungal isolate.

19 September 1984; Revised 30 May 1985

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INFLUENCE OF CHEMICAL ENVIRONMENT ON THE FIDELITY OF RIBOSOMAL PROTEIN SYNTHESIS

A. JANA and S. P. SEN

Department of Botany, Kalyani University,
Kalyani 741 235, India.

THAT the presence of metabolites other than those which are involved in RNA synthesis, have both qualitative and quantitative effects on nuclear RNA synthesis in plants has already been reported¹. We report here that common organic acids at low concentrations can introduce errors in the poly(U) programmed reactions as indicated by the increased incorporation of leucine with respect to phenylalanine. Since aminoacids themselves are incorporated in proteins, the effect of aminoacid mixtures was not studied.

Misreading of polynucleotide messengers of the ribosomal protein synthesizing machinery has been reported by several workers^{2–5}. Misreading of the

genetic code by antibiotics like streptomycin, kanamycin, neomycin and nucleic acid base analogues like 5-bromo uracil has also been reported^{6,7}. Apparently this is due to errors in the selection of appropriate aminoacyl tRNAs by the ribosomes influenced by environmental conditions or as a result of mutational events^{8,9}. The possibility of interaction of a tRNA with both specific and nonspecific codons with slightly different nucleotide sequences has been recognized. The error frequency is also known to be controlled by Mg^{2+} and elongation factors¹⁰. Gravrilova *et al*¹¹ have shown that elongation factor Tu along with GTP reduces the leucine to phenylalanine ratios of the poly (U)-mediated translation process; this error reducing effect is observed only at low but not at high concentrations of Mg^{2+} .

Ribosomes were isolated from wheat germs (Valejo, California, U.S.A.), purified according to the method of Marcus *et al*¹² and the top three-fourths of the 78,000 g centrifugation constituting the S-100 fraction was made $10^{-3}M$ with respect to dithiothreitol. There were seven control sets. From five of them the following were excluded from the complete system—Poly U, GTP, ATP, energy generating system and the S-100 fraction; the sixth set included RNase and the seventh one contained the complete incubation mixture without any organic acids or sugars.

Table 1 shows that the presence of organic acids (succinic, malic, fumaric and citric) and sugars (fructose, glucose, sucrose) in the incubation mixture markedly affected the poly (U)-mediated ³H-phenylalanine incorporation into protein. A $10^{-6}M$ mixture of organic acids enhanced phenylalanine incorporation by about 80%; however, when the concentration was raised to $10^{-5}M$, an inhibition of the order of 40% was observed. No promotion was observed with sugars at either of the concentrations tried. In the control sets the incorporation was only 1.03–5.17% of that obtained with the complete system and the differences among them were statistically insignificant. The incorporation was least when poly U or the S-100 fraction were omitted from the incubation mixture; the slightly higher incorporation in the presence of the S-100 fraction in the control sets was probably due to the presence in small quantities of the factors required for protein synthesis.

To test whether the inhibition could be a result of misreading of the triplet codon in the presence of organic acids and sugars, the effect of these metabolites on the incorporation of ³H-leucine (codons UUA, UUG, CUU, GUC, CUA or GUC) and ³H-alanine (codons CGU, GCC, GCA and GCG) in the

Table 1. Effect of two different concentrations of mixtures of organic acids and sugars on poly U-directed incorporation of ^3H -phenylalanine, ^3H -leucine and ^3H -alanine in the TCA precipitable fraction of the wheat germ ribosomal system.

Treatment	Radioactivity incorporated in the acid-insoluble fraction (cpm)			
	Expt I	Expt II		
	Phenylalanine	Phenylalanine	Alanine	Leucine
Complete (with penicillin and chloramphenicol)	16,262	6851	238	839
" - Poly U	169	-	-	-
" - GTP	841	-	-	-
" - ATP	746	-	-	-
" - Energy generating system	627	-	-	-
" - S-100 fraction	249	-	-	-
" + RNase	662	-	-	-
" + 10^{-6}M organic acids	28,596	12,142	239	2097
" + 10^{-5}M organic acids	9,814	4,304	268	3129
" + 10^{-6}M sugars	13,735	6,066	277	289
" + 10^{-5}M sugars	12,184	4,572	268	565
S.E.	376	272	31	26
LSD at P = 0.05	1,036	693	88	74
LSD at P = 0.01	1,926	1,126	152	127

The incubation mixture contained $10\ \mu\text{M}$ tris-acetate buffer (pH 8.1), $0.4\ \mu\text{mole}$ ATP, $3.2\ \mu\text{moles}$ creatine phosphate, $16\ \mu\text{g}$ creatine phosphokinase, $0.01\ \mu\text{mole}$ GTP, $0.9\ \mu\text{mole}$ dithiothreitol, $17.5\ \mu\text{mole}$ KCl, $0.84\ \mu\text{mole}$ Mg acetate, ribosomes (= $200\ \mu\text{g}$ RNA) from wheat germ, $0.12\ \text{ml}$ of S-100 fraction from wheat germ, $10\ \mu\text{g}$ poly (U), $5\ \text{uCi}$ each of either ^3H -phenylalanine or ^3H -leucine or ^3H -alanine of identical specific activity (sp. activity $198\ \text{mCi/m mole}$) penicillin ($10\ \text{U/ml}$) and chloramphenicol, ($10\ \mu\text{g/ml}$) along with $0.03\ \mu\text{mole}$ each of 19 other non-radioactive aminoacids. Total incubation mixture was $0.4\ \text{ml}$. RNase when added was included at $50\ \mu\text{g/ml}$. Reaction continued for $30\ \text{min}$ at 25°C . Reaction was stopped by adding equal volume of 5% warm TCA. The protein precipitate was collected after repeated washing by suction on a membrane filter disc and counted.

same poly (U)-mediated systems was studied in a subsequent experiment. Leucine incorporation into protein by the poly (U)-mediated system was also found to be affected by organic acids and to some extent by sugars, although the degree of incorporation was less as compared to the incorporation of phenylalanine. The incorporation of leucine into protein increased with increasing concentrations of organic acids. The presence of sugars in the incubation mixture was markedly inhibitory. Alanine incorporation, however, was practically unaffected. If the UUU codon is misread for any of the triplets for leucine, then the variations in the incorporation of leucine in the poly (U)-directed protein synthesis in presence of organic acids can be explained. This conclusion is strengthened by the fact that the alanine codons being much different from UUU could not be misread significantly, with and without the organic acids or sugars.

The aminoacid incorporation into proteins cannot be due to bacterial metabolism, since sterilized glasswares were used throughout and the incubation mixture contained penicillin and chloramphenicol, an inhibitor of prokaryotic protein synthesis. In the absence of further information we refrain from speculating on the probable causes of the alteration observed. Interaction of organic acids with poly (U), the tRNAs present in the wheat germ S-100 fraction and the ribosomes are possibilities which should be considered. The binding of aminoacyl tRNAs to several enzymes has been reported¹³. The low concentrations of the organic acids used is unlikely to affect seriously the pH of the incubation mixture.

It is interesting to note that a strain of *E. coli* inducible for β -galactosidase when grown in the presence of high concentrations of asparagine and glutamic acid in addition to lactose has been reported to produce a second β -galactosidase, in which there

was internal aminoacid replacement, indicating translational errors due to the presence of high concentrations of aminoacids¹⁴.

The authors thank the University Grants Commission for providing financial support. We are grateful to Dr S. N. Seal of the Institute for Cancer Research, Philadelphia, USA, for his help and interest in the work.

26 December 1984; Revised 23 May 1985

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EFFECT OF CERTAIN PROANTHOCYANIDINS ON THE CHLOROPHYLL CONTENT OF *LEMNA PAUCICOSTATA* HEGELM.

S. SEETA RAM RAO and K. V. N. RAO*

Department of Botany, P. G. College of Science, Osmania University, Saifabad, Hyderabad 500004, India.

* *Department of Botany, Osmania University, Hyderabad 500007, India.*

PLANT growth regulators like cytokinins, gibberelins auxins and abscisic acid play an important role in the maintenance of chlorophyll levels in plants and literature in this aspect has been reviewed by Thimann¹. Proanthocyanidins (formerly referred to as leucoanthocyanins) are an important group of growth regulating phenolic compounds²⁻⁷. The present study aims at evaluating the effect of proanthocyanidins on the chlorophyll contents of *Lemna paucicostata* Hegelm plants. The extraction and isolation of various compounds used in this work have been reported earlier^{6,8}.

Clonal and axenic cultures of *L. paucicostata*, maintained on modified Bonner and Devirian medium⁹ were used as inoculum. The basal medium (100 ml, without sucrose) poured into 250 ml Erlenmeyer flasks was autoclaved and supplemented with filter-sterilized proanthocyanidin solution at five concentrations (0.01, 0.05, 0.1, 0.5 and 1 ppm). Ten *Lemna* plants each with 3 fronds were introduced aseptically into each flask and the cultures were maintained under a light intensity of 5000 lux, at 25 ± 1 C. On the 10th day chlorophyll was extracted from 5 mg plant material in 5 ml 96% methanol and the chlorophyll content was estimated adopting the following formulae given by Holden¹⁰

$$\begin{aligned} \text{Chlorophyll a (mg/l)} &= 16.5 D_{665} - 8.3 D_{650}, \\ \text{Chlorophyll b (mg/l)} &= 33.8 D_{650} - 12.5 D_{665}, \\ \text{Total Chlorophyll (mg/l)} &= 25.5 D_{650} + 4.0 D_{665}. \end{aligned}$$

All the compounds enhanced the levels of chlorophylls in *Lemna* cultures (table 1). The earlier studies on the growth promoting activities of proanthocyanidins^{6,7} and the results obtained in this study showed that there was a good correlation between the enhancement of growth and chlorophyll content in *Lemna* by proanthocyanidins. Leucocyanidin from *Tamarindus indica* (0.1 ppm), procyanidin from *Phoenix sylvestris* (0.1 ppm), procyanidin from *Anona squamosa* (0.5 ppm) enhanced the growth to a maximum extent⁶ and these compounds also caused the