The A_{260} of the rRNA preparations from both *E. coli* and *M. barkeri* ribosomes did not change during the recovery of activity of the enzymes, indicating that rRNA did not denature or degrade within that time.

As seen earlier, the recovery of enzyme activity depended on the state of protein denaturation. Even when samples were picked up at definite times from denaturation reaction the residual activities varied to some extent in different experiments. The ribosome-mediated recovery of enzyme activity also varied accordingly. However, denatured enzyme samples having the same residual activity would always exhibit the same level of recovery of activity. The data presented in Figures 1–3 are from one set of experiments. Although the experiments were repeated many times, the graph could not be plotted with average of a number of data for the reason just mentioned. As we can see from Figure 3, the rRNA-mediated recovery of activity was poor compared with the recovery in presence of 70S ribosome. We do not have an explanation for this at this moment. It was not due to degradation of that rRNA. The reason hopefully would come when the individual rRNA molecule is checked for protein-folding activity.

In this report, therefore, we have shown some generalized protein-folding activity in the ribosome and total ribosomal RNA. The protein-folding activity of RNA seems to be specific for ribosomal RNA only and is not dependent on any nonspecific secondary structure containing extensive hairpins and loops since transfer RNA from *E. coli* having double-stranded and looped regions failed to reactivate the denatured enzymes at about 40 times higher molar concentration. This activity on rRNA deserves special attention, since these findings point towards the evolution of the protein-folding activity of ribosome even before its translational activity evolved, as the latter activity also needed the ribosomal proteins. It should be noted that RNAs exhibit not only enzymatic activity on nucleic acids, but also could take part in refolding the polypeptides after their synthesis on ribosome surface. Recently, folding of nascent polypeptide on ribosome surface has been demonstrated in *in vitro* translation system in crude cell extracts from wheat germ and *E. coli*[10].

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Myoinositol trisphosphate sensitive calcium stores in *Entamoeba histolytica*

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Calcium mobilization from internal stores of the parasitic protozoan *Entamoeba histolytica* was studied by fluorescence measurements of the calcium indicator quin 2 in saponin permeabilized amoeba. Both Ins (1,4,5)P_3 and Ins(2,4,5)P_3 could release calcium equally well from permeabilized *E. histolytica*. On an average, about 40% of the A23187 releasable calcium pool could be mobilized by 2 μM $InsP_3$. Neither GTP nor cAMP could influence $InsP_3$-mediated Ca^{2+} mobilization. $InsP_3$-mediated Ca^{2+} release from internal stores of *E. histolytica* occurred in an $InsP_3$ receptor-dependent manner. Differential interference contrast microscopy revealed that increased motility and pseudopod formation could be produced in *E. histolytica* through $InsP_3$ treatment.

§For correspondence

INTRACELLULAR calcium and protein kinase C play important roles in the cytolytic activities of *Entamoeba histolytica*, a parastic protozoan[1,2]. TMB-8, an intracellular calcium antagonist causes significant reduction in vesicle exocytosis in this enteric parasite[1]. However, very little else is known about calcium homeostasis in this organism.

Trypanosoma brucei, another parasitic protozoan, has very recently been shown[3,4] to possess a large extramitochondrial calcium pool and contain inositol phosphates, especially Ins(1,4,5)P_3. Both the circulating and

the intracellular forms of this parasite have these common properties. Surprisingly, Ins(1,4,5)P_3 failed to mediate calcium release from intracellular stores of both the stages of *T. brucei*. We now report that this is not essentially true for all parasitic protozoa as an Ins(1,4,5)P_3-sensitive calcium store exists in *E. histolytica*. *E. histolytica* (EC 22) was isolated from patients at the Kothari Medical Centres, Calcutta and axenized.

Differential interference constrast microscopy revealed that increased motility and pseudopod formation could be produced in permeabilized *E. histolytica* through Ins(1,4,5)P_3/Ins(2,4,5)P_3 treatment. Furthermore, calcium depletion led to loss of motility and abnormal morphological changes (Figures 1 *a–c*).

Figure 2 *a* illustrates that Ins(1,4,5)P_3 was effective in mobilizing calcium from permeabilized *E. histolytica* trophozoites. However, in intact cells Ins(1,4,5)P_3 was unable to release calcium (data not shown), indicating an intracellular site of action for InsP_3 in *E. histolytica* trophozoites. On the other hand, calcium ionophore A 23187 could mobilize calcium equally well from permeabilized (Figure 2 *a*) and intact *E. histolytica* (data not shown). Heparin (100 µg/ml) significantly ($P < 0.01$) inhibited Ins(1,4,5)P_3-mediated calcium release (Figure 2 *b*). Ins(1,4,5)P_3 released Ca^{2+} from *E. histolytica* in a dose-dependent manner and the EC50 value was determined to be 0.2 µM. The EC50 of Ins(1,4,5)P_3-induced calcium release (0.1–0.75 µM) reported for permeabilized mammalian cells[5–7] seems compatible with our finding in *E. histolytica*, demonstrating similar sensitivity to Ins(1,4,5)P_3. Other inositol phosphates (Ins-1-P and InsP_6) failed to produce any change ($P > 0.05$) in internal Ca^{2+} levels of *E. histolytica* (Figure 2 *b*). Significant reduction ($P < 0.05$) was observed in InsP_3-mediated calcium release by pretreatment with a lower concentration of Ins(1,4,5)P_3 (Figure 2 *b*), indicating desensitization towards Ins(1,4,5)P_3. Specific binding of [^{3}H]-Ins(1,4,5)P_3 was observed in *E. histolytica* crude membrane fraction by a centrifugation assay[8,9] and was estimated to be about 20 pmol/mg protein with a ligand concentration of 25 nM. [^{3}H]-Ins(1,4,5)P_3 binding was increasingly displaced by the presence of 10 nM to 10 µM of unlabelled Ins(1,4,5)P_3. These results document the release of internal calcium by the calcium-mobilizing second messenger Ins(1,4,5)P_3 in *E. histolytica*. Inhibition of Ins(1,4,5)P_3-induced calcium release by the well-known Ins(1,4,5)P_3 receptor antagonist heparin[10] indicates that the observed calcium release in *E. histolytica* is mediated by the Ins(1,4,5)P_3 receptor. Specific binding of Ins(1,4,5)P_3 to the crude membrane fraction of *E. histolytica* also supports this concept.

The vesicular nature of the InsP_3-sensitive calcium store is evident from the strong inhibition of InsP_3-mediated calcium release by prior treatment with calcium ionophore A 23187 in permeabilized amoebae. A 23187

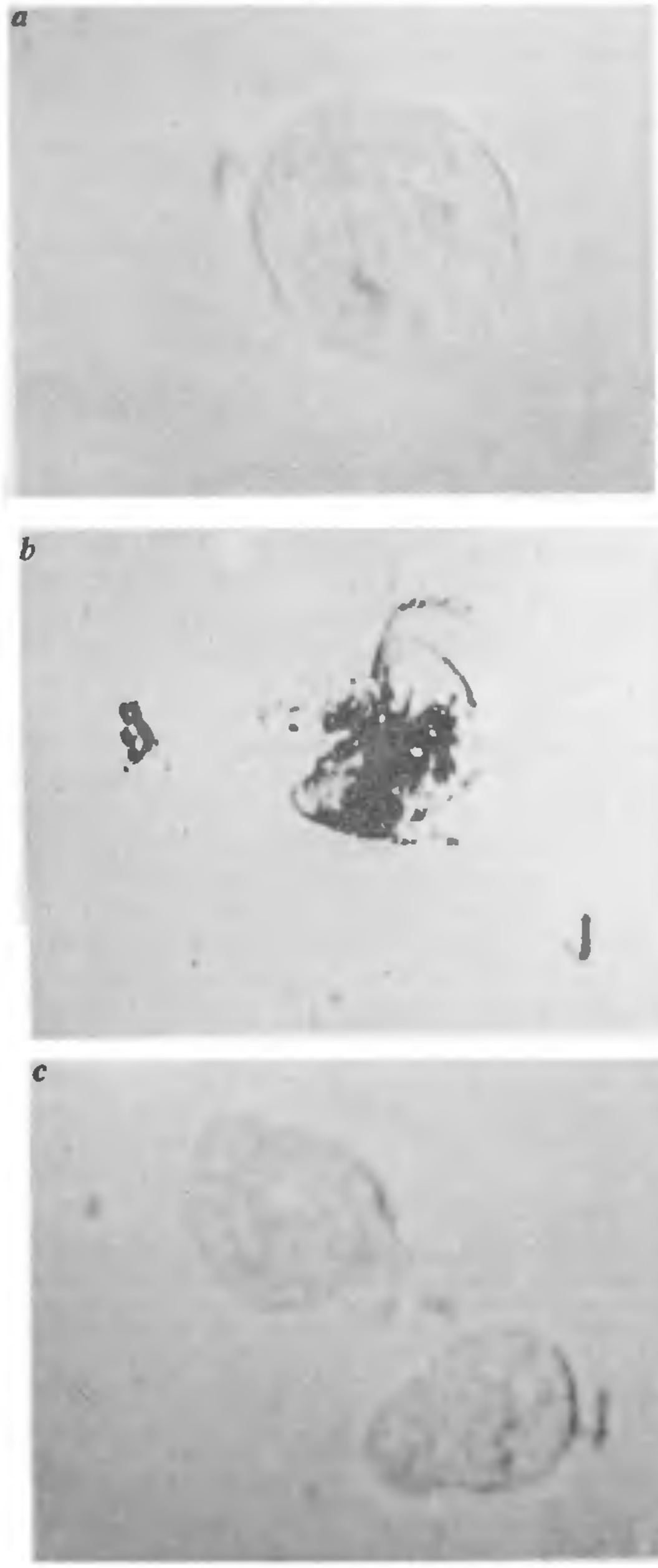
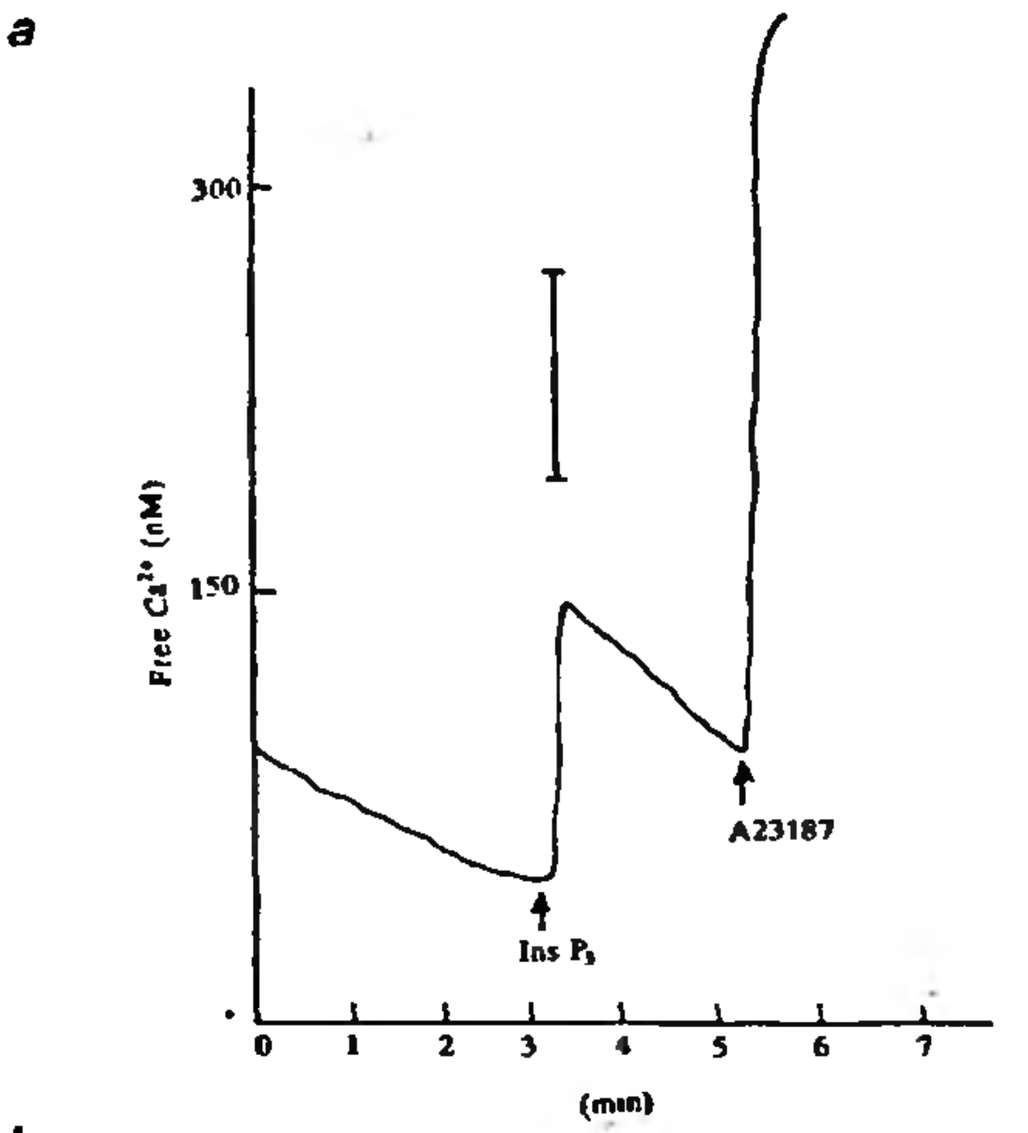


Figure 1. Differential interference contrast micrographs of *E. histolytica* trophozoites. *a*, Control-suspended in buffer without added calcium (X 850). *b*, Suspended in buffer without added calcium and treated with 20 µg/ml A23187 (X 1000) *c*, Suspended in intracellular type buffer without added calcium. Cells were permeabilized by the addition of 20 µg/ml saponin 2 mM ATP was added to the permeabilized amoebae which was followed 3 min later by 2 µM Ins(1,4,5)P_3. Cells were examined within 30 s of Ins(1,4,5)P_3 addition (X 750) Cells were examined after 1 min of A 23187 treatment



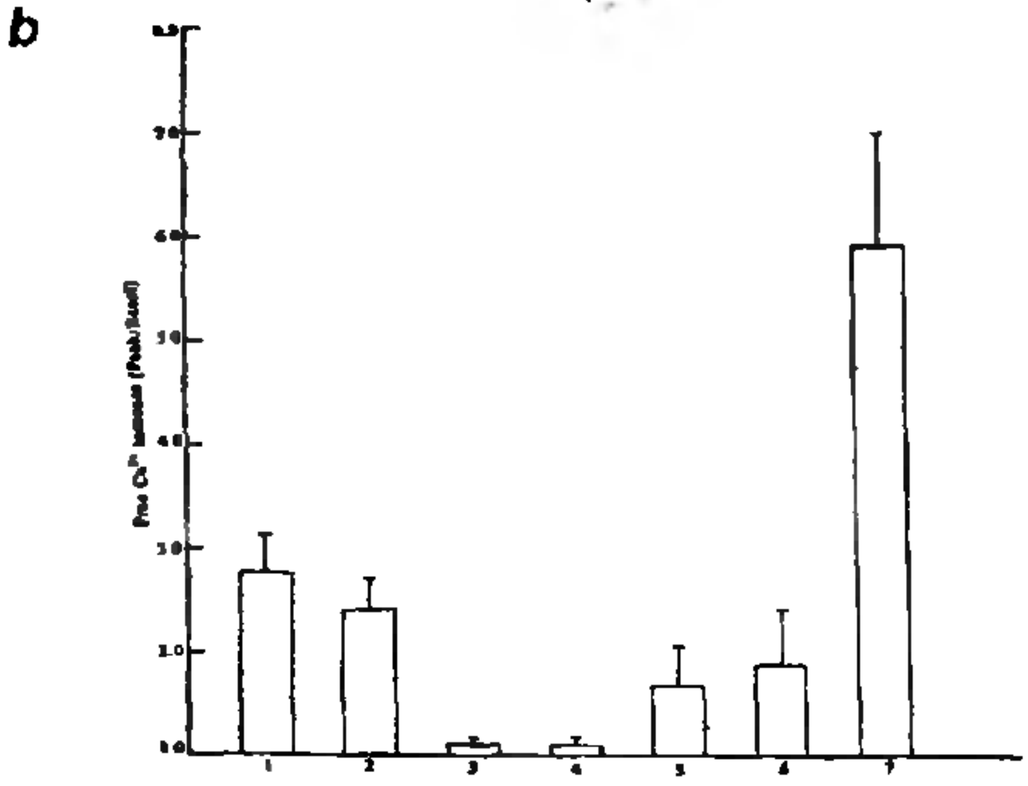


Figure 2. Calcium release by inositol phosphates and A 23187 from permeabilized *E. histolytica* trophozoites. 2*a* exhibits changes in free calcium concentration when saponin-permeabilized *E. histolytica* trophozoites (10^6/ml), suspended in a cytoplasmic-type medium (100 mM KCl, 2 mM $MgCl_2$, 10 mM NaCl, 10 mM PIPES, 10 mM Glucose, pH 7.2), were treated with 2 µM Ins(1,4,5)P_3 followed by 5 µg/ml A 23187. Calcium sequestration prior to the addition of Ins(1,4,5)P_3 was initiated at the zero time point by the addition of 2 mM ATP. Results are representative of 4 experiments. Free and total calcium were estimated from the fluorescence of calcium indicator dye quin 2 (100 µM) as previously described in literature[17, 18]. The calibration bar in the figure represents 1 nmol/ml Ca^{2+}. 2*b* demonstrates peak/basal values for free calcium concentrations in permeabilized *E. histolytica* suspensions after treatments with inositol, phosphates, A 23187 and heparin. Experimental conditions are similar to 2*a*. Data are presented as mean ± SD of 3–6 experiments. Peak [Ca^{2+}] was taken as the maximum Ca^{2+}-level reached within the first 25 s after the addition of test substance. Basal [Ca^{2+}] was that measured just before addition of the first test substance. Column 1,2 µM Ins(1,4,5)P_3, column 2,2 µM Ins(2,4,5)P_3; column 3,10 µM Ins P_6; column 4,10 µM Ins-1-P; column 5,2 µM Ins(1,4,5)P_3 added after 4 min-preincubation with heparin (100 µg/ml); column 6,2 µM Ins(1,4,5)P_3 added 2 min after 0.2 µM Ins(1,4,5)P_3; column 7 A 23187 (5 µg/ml). Correction for autofluorescence due to A 23187 was not made.

(5 µg/ml) mobilized a significantly higher ($P < 0.01$) amount of calcium than 2 µM Ins(1,4,5)P_3 (Figure 2 *b*). On an average, about 40% of the A 23187 releasable calcium pool could be mobilized by 2 µM Ins(1,4,5)P_3. This could compare favourably with the value of 30–50% reported for mammalian cells[11]. In contrast however, is the ability of a saturating concentration of Ins(2.4.5)P_3 which is known to be less potent in releasing calcium in mammalian cells[12, 13], to release almost similar amounts ($P > 0.05$) of calcium as Ins(1,4.5)P_3 in *E. histolytica* (Figure 2 *b*). In this context, the findings of Schultz *et al.*[14] that the $InsP_3$ receptor from *Neurospora crassa* is less specific and responds equally well to various Ins(1,4,5)P_3 analogues, could be relevant. Also neither GTP[15] nor cAMP[16] which influences $InsP_3$-mediated calcium release in mammalian cells had any effect on $InsP_3$-mediated calcium release in *E. histolytica*.

In view of the absence of Ins(1,4,5)P_3-mediated signalling mechanisms in *T. brucei*, the existence of Ins(1,4,5)P_3-sensitive calcium stores in *E. histolytica* adds an important dimension to our knowledge of cellular signalling mechanisms in simpler eukaryotes, particularly in parasitic protozoa. Since pseudopod formation and marked morphological changes in trophozoites are recorded in Ca^{2+} loaded and depleted parasites respectively, the characterization of different mechanisms by which *E. histolytica* can mobilize Ca^{2+} might help in designing future drugs for this amoeba. This aspect of work is now in progress.

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DAHP synthase of phenylalanine non-producing and phenylalanine producing mutant strains of *Arthrobacter globiformis*

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3-Deoxy-D-arabinoheptulosonate 7-P (DAHP) synthase from wild type, tryptophan and tyrosine double auxotrophic mutant and double auxotrophic multianalogue resistant mutant of *Arthrobacter globiformis* were assayed. Maximum enzyme activity was observed in late exponential phase and TT-39 PTMN^r-7 retained maximum activity at late stationary phase of growth. Tryptophan, tyrosine and phenylalanine repressed the enzyme synergistically but TT-39 PTMN^r-7 was less sensitive to repression. Tryptophan, tyrosine and phenylalanine synergistically inhibited the enzyme but on their own did not produce sufficient inhibition.

BIOSYNTHESIS of aromatic amino acids begins with condensation of phosphoenolpyruvate and erythrose 4P by the enzyme DAHP synthase (EC. 4.1.2.15). This is the main feedback control point of the branched pathway and the aromatic amino acids control the activity of this enzyme. Micro-organisms show considerable diversity in the feedback regulation of DAHP synthase[1–4]. In *E. coli*[5–7], *Neurospora crassa*[8], *Salmonella typhimurium*[9], *Claviceps paspali*[10] and *Saccharomyces cerevisae*[11, 12], the reaction was carried out by three isozymes, the activity of which are inhibited by L-tyrosine, L-phenylalanine and L-tryptophan respectively. In *Bacillus subtilis*[2, 13] chorismate and prephenate (branched point intermediate) inhibited DAHP synthase whereas aromatic amino acids did not. Other control patterns reported are cumulative feedback inhibition found in *Hydrogenomonas*[2] sp, concerted feedback inhibition found in *Rhodomicrobium vanneili*[14], unimetabolite control found in *Pseudomonas*[2] sp where only tyrosine and in *Streptomyces aureofaciens*[15] where only L-tryptophan inhibited the enzyme. Synergistic inhibition has been reported in *Brevibacterium flavum*[16] and *Corynebacterium glutamicum*[17]. By mutagenesis with NTG, the present authors were able to obtain both double auxotrophic mutants[18,19] and double auxotrophic multianalogue-resistant mutants of *Arthrobacter globiformis*. Both these mutants produced L-phenylalanine which is an essential amino acid for human nutrition, now widely used as constituent for the dipeptide sweetener 'Aspartame'[20].

The present paper reports the activity of DAHP synthase under different phases of growth in wild type (A8), double auxotrophic mutant (TT-39) and double auxotrophic multianalogue resistant mutant (TT-39PTMN^r-7) of *Arthrobacter globiformis*. The pattern of regulation of the DAHP synthase of different strains has also been compared.

A. globiformis (A8), a glutamate producer, was isolated from Burdwan soil[21]. The tryptophan and tyrosine double auxotroph (TT-39) and double auxotrophic multianalogue-resistant mutant of TT-39PTMN^r-7 of *A. globiformis* were isolated by mutagenesis with NTG treatment[19]. The strains were maintained on Alfoldis[22] agar slant with supplements as required. The organisms were grown in shaken flask using the same liquid medium on a rotary shaker at 30°C. Growth was measured turbidimetrically and extra-cellular phenylalanine estimated by paper chromatography and micro-biological assay[18, 19]. An aqueous suspension of bacteria from a 24 h old culture on agar slant (10^6 cells ml^{-1}, 2% v/v) was to inoculate the flasks. The cells were harvested by centrifugation of 1000 *g* for 45 min in cold centrifuge and washed twice with 0.04 M cold potassium phosphate buffer (pH 7.0). Washed cells were resuspended in the above buffer at a cell density of 500 mg of fresh weight per ml and disrupted by sonication for 4 min on an ice bath using ultrasonic disintegrator (Soniprep-20) and the extract was centrifuged at 10000 g for 20 min at 2°C. The extract was dialysed overnight using the same buffer and its protein content was measured[23]. The DAHP synthase was assayed[24] by measuring the absorbance at 549 nm (the β-formyl-pyruvic acid formed) with UV-visible double beam spectrophotometer (Shimadzu).

The activity of DAHP synthase in wild type (A8), double auxotroph (TT-39) and double auxotrophic multianalogue-resistant mutant (TT-39 PTMN^r-7) increased gradually with growth (Table 1). In all the three types (A8, TT-39 and TT-39 PTMN^r-7), the DAHP synthase activity was maximum at late exponential phase of growth. The enzyme activity of A8 and TT-39 declined 40% and 25% of the maximal activity respectively during the late stationary phase of growth but TT-39PTMN^r-7 retained about 90% of the maximal activity at this stage. TT-39 and TT-39PTMN^r-7 showed 60% and 200% greater activity of the DAHP synthase respectively over the phenylalanine non-producing *A. globiformis* (A8). Shetty *et al.*[25] reported a similar increase of DAHP synthase activity when the cells of phenylalanine producing *Bacillus polymyxa* approach the stationary