Phosphate transport in *Claviceps* sp. strain SD-58

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Summary. Phosphate uptake in *Claviceps* sp. strain SD-58 was found to be linear for 20 min, proportional to cell density in mg/ml, energy dependent, and taking place against a concentration gradient with a K_m value of 45.45×10^{-5} M. Osmotic shock treatment to the cell caused a reduction in phosphate uptake associated with the release of binding protein. Partial restoration of uptake was observed on incubation of osmotically shocked cells with shock fluid. The results are discussed with reference to the effect of phosphate on alkaloid synthesis in *Claviceps* sp. strain SD-58.

Key words. Claviceps; fungus, ergot; ergot fungus; phosphate uptake.

Soon after the discovery that ergot alkaloids can be produced under saprophytic conditions, numerous investigations of their fermentative production using *Claviceps* spp. were performed[1,2]. It has long been known that high concentrations of phosphate inhibit biogenesis of many secondary metabolites, including alkaloids[3–6]. However, our understanding of the transport of phosphate in the cell and its effect at the cellular level remains poor.

Recent work in this laboratory has provided evidence that lower tryptophan accumulation and higher ATP generation due to carbon catabolic shift[7], repression of chlamydospore formation[8], inhibition and repression of phosphatases[6,9] are some of the factors causing inhibition of alkaloid production under conditions of high phosphate concentration. In continuation of our work on the physiology of alkaloid production[6–10], we wish to report here the kinetics of the phosphate uptake system in *Claviceps* sp. strain SD-58.

Materials and methods. The ergot fungus *Claviceps* sp. strain SD-58 (ATCC 26019), was obtained from the American Type Culture Collection, Maryland, USA. The organism was maintained by subculturing every fortnight on potato dextrose agar (PDA) slopes, incubated at 25 °C for an initial period of five days and subsequently stored at 5 °C.

Inoculum preparation, composition of NL-406 medium and shake cultivation conditions were the same as described earlier[6,7,10].

Uptake studies were carried out by following the method of Desai et al.[11,12], a modification of the method of Brown and Romano[13]. The rate of uptake was calculated from the initial values. The intracellular concentration of phosphate was calculated on the basis of 4 µl water/mg dry cell weight[11,12,14].

The two-stage osmotic shock treatment of Wiley[15], with the modification of Desai and Modi[11], was employed to isolate binding protein from the cells. Activity of phosphate binding protein was determined by the equlibrium dialysis technique as described earlier[11,12,16]. A dialysis sac with 0.5 ml of protein sample was placed in a test tube containing, in µmoles; tris HCl (pH 7.2) 200; 2-mercaptoethanol 4; chloroform, 0.5%, and phosphate, 10; the final volume was 4 ml. A control sac contained 0.5 ml of buffer instead of protein sample. The dialysis was performed for 24 h at 4 °C. The material from the dialysing sac was removed and after heat-treatment for 30 min at 60 °C analyzed for inorganic phosphate concentration. The binding activity was evaluated by comparing phosphate concentration with the control sac, and this value was confirmed by comparing phosphate concentrations inside and outside the dialysis sac. Protoplasts were prepared by following the method of Robbers et al.[17].

Inorganic phosphate and protein were estimated according to the methods of Fiske and Subba Row[18] and Lowry et al.[19], respectively. The results reported here are average values from at least three independent experiments.

Results and discussion. Figure 1 shows that phosphate uptake by *Claviceps* sp. strain SD-58 is linear with time for about 20 min, and proportional to the cell density up to about 1 mg/ml dry cells. (fig. 2). The rate of phosphate uptake is inhibited by 75% on addition of KCN (5 mM), 2,4-dinitrophenol (1 mM) and NaN_3 (10 mM), indicating the dependence of the uptake process on energy. As shown in the table the level of intracellular concentration of phosphate was about 90 times higher than the extracellular concentration. This indicates that the entry of

Level of intracellular and extracellular phosphate during the uptake studies in *Claviceps* sp. strain SD-58

External conc. (mM) Initial	Final	Pi uptake*	Intracellular conc. (mM)	Intracellular** / Extracellular concentration
0.4	0.08	0.33	7.5	93.75
0.5	0.15	0.35	12.0	80.00
0.6	0.2	0.41	15.0	75.00

* Expressed as µmoles/mg dry cells/20 min. ** Indicated amount of phosphate was added during the uptake studies and after 20 min of incubation intracellular concentration was calculated on basis 4 µl water/mg dry cells[12,14].

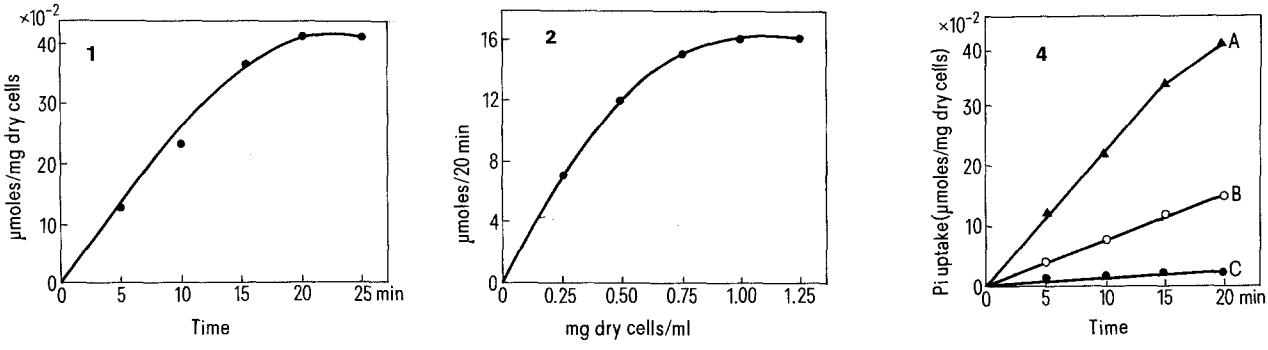

Figure 1. Phosphate uptake by *Claviceps* sp. strain SD-58 as a function of time.

Figure 2. Effect of cell density on phosphate uptake by *Claviceps* sp. strain SD-58.

Figure 4. Effect of osmotic shock on phosphate uptake by *Claviceps* sp. strain SD-58. Cells were harvested and divided into three parts. Two part of cells received osmotic shock treatment, while untreated cells were taken as control. phosphate uptake by control (▲), osmotically shock treated cell in presence (○) and absence (●) of shock fluid (10 mg protein).

phosphate into the cell is against the concentration gradient. Phosphate uptake in relation to the substrate concentration yielded a hyperbolic curve (fig. 3) suggesting the entry of phosphate by a saturable system. When these data were replotted as a Lineweaver-Burk plot a K_m value of 45.45×10^{-5} M was found. The results satisfy the requirements of an active transport system.

Over the past few years considerable attention has been given to a new class of protein called binding proteins. It is now a well-established fact that the transport of many substances in bacterical cells requires proteins of a specific type, present in the periplasmic region of cells, from which they can be selectively released by a cold osmotic shock treatment[20-22]. We have presented evidence for the presence of binding proteins for phosphate[12] and glucose[11,16] in *A. nidulans*. Binding proteins for tryptophan[15] and sulphate[23] have also been reported in *N. crassa* and *A. nidulans*, respectively. To examine the possible role of binding protein in the phosphate uptake in *Claviceps* sp. strain SD-58, cells were subjected to osmotic shock treatment, which caused a significant decrease in the uptake of phosphate. Partial restoration of uptake was observed by the addition of shock fluid to shocked cells (fig. 4). On analysis of shock fluid, the phosphate binding activity was reduced by 80–90% on incubation with protease. This suggested that the component involved in transport of phosphate which is released by an osmotic shock is a protein.

The protoplast preparation of cells was found to be retarded in phosphate uptake ability by about 80%, which confirms the involvement of periplasmic binding protein.

Several transport systems have been shown to be regulated by inhibition of uptake by high concentration of substrate or the operation of distinct transport systems depending upon the concentration of substrates[15,19,24,25]. The phosphate uptake system in *Claviceps* sp. strain SD-58 was not inhibited by high concentration of phosphate (10 mM), nor did it show the existence of two transport systems (fig. 3). Thus, under conditions of high phosphate concentration, the cell will continue to accumulate phosphate. Accumulation of a high amount of phosphate in the cell will in turn affect the cell physiology leading to a higher rate of TCA cycle operation[7] and a greater biomass, with concomitant inhibition of alkaloid synthesis.

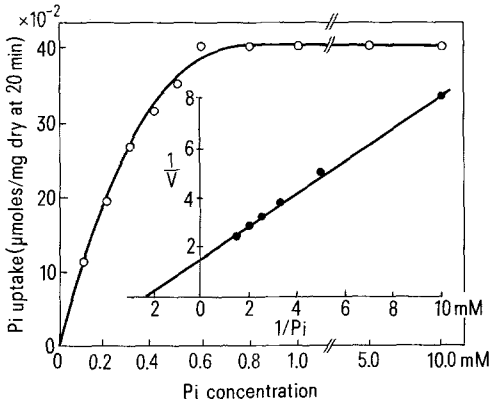

Figure 3. Substrate dependent uptake of phosphate by *Claviceps* sp. strain SD-58. Inset figure: Line weaver – Burk plot of phosphate uptake.

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Endogenous splenic colonies and megakaryopoiesis in methylcellulose treated irradiated mice[1]

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Summary. Endogenous splenic colonies are increased in methylcellulose-treated irradiated mice, 10 days after sublethal irradiation (450 R). The spleen shows an enhancement of megakaryopoiesis, especially localized around foam-cell foci. This suggests that the macrophage system, activated through phagocytic activity against methylcellulose, affects megakaryopoiesis by a microenvironmental mechanism.

Key words. Mouse, irradiated; irradiation, sublethal; mouse spleen; splenic colonies, endogenous; methylcellulose treatment; megakaryopoiesis; macrophage system.