6 Haskins, W.T., and Luttermoser, G.W., J. Pharmac. exp. Ther. *109* (1953) 201.
7 Bernstein, E.H., Pienta, P.W., and Gershon, H., Toxicol. appl. Pharmac. *5* (1963) 599.
8 Berggren, L., and Hansson, O., Clin. Pharmac. Ther. *9* (1968) 67.
9 Tateishi, J., Kuroda, S., Saito, A., and Otsuki, S., Lancet *2* (1971) 1263.
10 Jack, D.B., and Riess, W., J. pharmac. Sci. *62* (1973) 1929.
11 Ogata, M., Watanabe, S., Tateishi, J., Kuroda, S., and Otsuki, S., Lancet *1* (1973) 1248.
12 Ogata, M., Watanabe, S., Tateishi, J., Kuroda, S., and Otsuki, S., Lancet *1* (1973) 1325.
13 Kasai, M., Kanamitsu, M., Katada, T., Tokumitsu, Y., Itaya, K., and Ui, M., Toxicology *8* (1977) 327.
14 Hayakawa, K., Imanari, T., Tamura, Z., Kuroda, S., Ikeda, H., and Tateishi, J., Chem. pharmac. Bull. *25* (1977) 2013.
15 Yamanaka, N., Imanari, T., Tamura, Z., and Yagi, K., J. Biochem. *73* (1973) 993.
16 Yagi, K., Ohishi, N., Takai, A., and Hattori, S., Experientia *30* (1974) 1430.
17 Tamura, Z., Jap. J. med. Sci. Biol. *28* (1975) 69.
18 Inouye, B., and Ogata, M., Physiol. Chem. Phys. *11* (1979) 49.
19 Tamura, Z., Yoshioka, M., Imanari, T., Fukaya, J., Kusaka, J., and Samejima, K., Clinica chim. Acta *47* (1973) 13.
20 Yagi, Y., Matsuda, M., and Yagi, K., Experientia *32* (1976) 905.
21 Ohtsuka, K., Ohishi, N., Eguchi, G., and Yagi, K., Experientia *38* (1982) 120.
22 Okada, T.S., Itoh, Y., Watanabe, K., and Eguchi, G., Dev. Biol. *45* (1975) 318.
23 Ohkawa, H., Ohishi, N., and Yagi, K., Analyt. Biochem. *95* (1979) 351.
24 Munro, H.N., and Linder, M.C., Physiol. Rev. *58* (1978) 317.
25 Aisen, P., and Listowsky, I., A. Rev. Biochem. *49* (1980) 357.
26 Tappel, A.L., Fedn Proc. *32* (1973) 1870.
27 Plaa, G.L., and Witschi, H., A. Rev. Pharmac. Toxic. *16* (1976) 125.
28 Recknagel, R.O., and Glende, E.A. Jr., in: Handbook of Physiology, Section 9, p. 591. Eds D.H.K. Lee, H.L. Falk, S.D. Murphy and S.R. Geiger. Waverly Press Inc., Baltimore 1977.
29 Peterkofsky, B., and Prather, W., J. Cell Physiol. *90* (1977) 61.
30 Prasad, K.N., Sinha, P.K., Ramanujam, M., and Sakamoto, A., Proc. natn. Acad. Sci. USA *76* (1979) 829.
31 Haugaard, N., Physiol. Rev. *48* (1968) 311.
32 Hiramitsu, T., Hasegawa, Y., Hirata, K., Nishigaki, I., and Yagi, K., Experientia *32* (1976) 622.
33 Yagi, K., Matsuoka, S., Ohkawa, H., Ohishi, N., Takeuchi, Y.K., and Sakai, H., Clinica chim. Acta *80* (1977) 355.

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Effect of pyrophosphate and orotidine monophosphate on cytosine deaminase regulatory properties

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Summary. The maximal velocity of the reaction (V_{max}) and the half-saturation constant ($K_{0.5}$) values of the *S. typhimurium* cytosine deaminase were altered in the presence of its effectors, pyrophosphate and orotidine monophosphate. From the kinetics of orotidine monophosphate inhibition of cytosine deaminase, it was characterized as a mixed-type noncompetitive inhibitor.
Key words. Cytosine deaminase; kinetics; pyrophosphate; orotidine monophosphate.

The deamination of cytosine to uracil with the concomitant release of ammonia is catalyzed by the enzyme cytosine deaminase (cytosine aminohydrolase, EC 3.5.4.1)[2]. Cytosine deaminase in *Salmonella typhimurium* is an anabolic salvage enzyme in pyrimidine metabolism whose synthesis is repressed by pyrimidines[3]. In *S. typhimurium*, cytosine deaminase has been purified and is tetrameric in structure with its subunits being identical[4]. It is active at high temperatures with a neutral pH optimum and a substrate specificity for cytosine or 5-fluorocytosine. The cytosine deaminase reaction, which exhibits Michaelis-Menten kinetics, can be activated by pyrophosphate as well as inhibited by orotidine monophosphate. The present report examines the influence of pyrophosphate and orotidine monophosphate on *S. typhimurium* cytosine deaminase regulatory properties.

Materials and methods. Cytosine deaminase from *S. typhimurium* was purified from strain HD11-AE2 as previously described[4]. The preparation showed a single protein band after 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis[5]. The specific activity of the enzyme specimen was 21.4 μmoles uracil formed/min/mg protein. The cytosine deaminase assay used in this study measured enzyme activity by utilizing the difference in molar extinction coefficients between cytosine and uracil in acid at 295 nm ($2.38 \times 10^3\ M^{-1} \cdot cm^{-1}$). The 0.5 ml assay mixture, which contained 50 mM Tris-HCl buffer (pH 7.3), 1.34 μg/ml purified enzyme and cytosine, was incubated at 37 °C for 10 min. The reaction was terminated by the addition of 1 N perchloric acid (1 ml) at 4 °C. Enzyme activity is expressed as nmoles uracil formed/min/ml.

Results and discussion. Pyrophosphate is best characterized as a nonessential activator of cytosine deaminase since the deamination proceeds in its absence. In the table, it can be observed that the half-saturation constant ($K_{0.5}$) for cytosine decreased from 0.77 mM with no pyrophosphate present to 0.43 mM in the presence of 10 mM pyrophosphate. The concentration of activator giving 50% of maximal stimulation is 4.27 mM pyrophosphate. The maximal velocity of the reaction (V_{max}) values were also noted to increase for the deamination of cytosine as the pyrophosphate concentration was elevated (table). Therefore, the affinity for cytosine and the rate of product formation by cytosine deaminase was enhanced as pyrophosphate was added. The cytosine deaminase from *Serratia marcescens*, another enteric microorganism, has been purified[6] and pyrophosphate was noted to stimulate its enzyme activity[7]. Interestingly, the stimulation by pyrophosphate of the *S. marcescens* deaminase increased its V_{max} while its affinity for cytosine remained constant.

Influence of effectors on kinetic properties of cytosine deaminase

Effector	Concentration (mM)	$K_{0.5}$ (mM)	V_{max} (μmoles/min)
None	–	0.77	48.93
Pyrophosphate	2	0.63	52.94
	4	0.55	56.87
	10	0.43	62.12
Orotidine monophosphate	0.2	1.20	36.15
	1.0	2.00	25.57

Cytosine deaminase was assayed as stated in text. Values were derived from plots of velocity versus substrate concentration and Hill plots[15].

Purified cytosine deaminase from *Pseudomonas aureofaciens*[8] also has been found to undergo stimulation in the presence of pyrophosphate[9]. As with the *S. marcescens* enzyme, only V_{max} increased for the deaminase reaction. Thus, the *S. typhimurium* deaminase differs from these other deaminases with respect to pyrophosphate activation since its $K_{0.5}$ for cytosine changes.

The best inhibitor of *S. typhimurium* cytosine deaminase activity was determined to be orotidine monophosphate[4]. It can be noted in the table that both the $K_{0.5}$ and V_{max} values are altered as the orotidine monophosphate concentration is increased. The $K_{0.5}$ for cytosine increased from 0.77 mM in the absence of inhibitor to 2.00 mM after 1 mM orotidine monophosphate was added. In contrast, the V_{max} decreased approximately 50% following the addition of 1 mM orotidine monophosphate. These data indicated that orotidine monophosphate is a mixed-type noncompetitive inhibitor of cytosine deaminase. Nucleotides have also been determined to be allosteric inhibitors of the cytosine deaminases isolated from *S. marcescens* and yeast. In *S. marcescens*, cytosine deaminase activity was significantly inhibited by dGMP[7]. This noncompetitive inhibitor of the deaminase affected only the V_{max}. Purified yeast deaminase is inhibited by a variety of purine and pyrimidine nucleotides that have been characterized as mixed-type competitive or noncompetitive inhibitors[10].

The regulatory nature of the *S. typhimurium* cytosine deaminase could be intrinsic to its role in the salvage of pyrimidine bases. Along with a CMP-degrading activity and uracil phosphoribosyltransferase, cytosine deaminase occupies a central role in the recycling of RNA degradation products, such as CMP, in *S. typhimurium*[11]. Depending upon intracellular concentrations of pyrophosphate and orotidine monophosphate, salvage of such nucleotide products might be regulated at the level of enzyme activity. Under pyrimidine nucleotide limiting conditions, the de novo pyrimidine ribonucleotide biosynthetic pathway will be synthesizing UMP at a maximal rate to ensure cellular survival[12]. This pathway will also be producing significant levels of pyrophosphate[13] which may stimulate cytosine deaminase activity. This promotes the salvage of pyrimidine bases since it allows their conversion to the nucleotide level. If pyrimidine nucleotides are present in excess within the bacterial cell, orotidine monophosphate, an intermediate of the de novo pyrimidine ribonucleotide biosynthetic pathway, will most likely be elevated in concentration due to orotidine monophosphate decarboxylase inhibition by UMP[14]. Therefore, cytosine deaminase activity will be inhibited which correlates with the lack of pyrimidine base salvage biosynthesis under such conditions. This type of regulation complements the repression of cytosine deaminase synthesis by pyrimidines in *S. typhimurium* since it ensures an immediate response by the bacterial cell as effector concentrations vary.

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2 Chargaff, E., and Kream, J., J. biol. Chem. *175* (1948) 993.
3 West, T.P., and O'Donovan, G.A., J. Bact. *149* (1982) 1171.
4 West, T.P., Shanley, M.S., and O'Donovan, G.A., Biochim. biophys. Acta *719* (1982) 251.
5 Laemmli, U.K., Nature *227* (1970) 680.
6 Sakai, T., Yu, T., Tabe, H., and Omata, S., Agric. biol. Chem. *39* (1975) 1623.
7 Yu, T., Sakai, T., and Omata, S., Agric. biol. Chem. *40* (1976) 543.
8 Sakai, T., Yu, T., Taniguchi, K., and Omata, S., Agric. biol. Chem. *39* (1975) 2015.
9 Yu, T., Sakai, T., and Omata, S., Agric. biol. Chem. *40* (1976) 551.
10 Ipata, P.L., Marmocchi, F., Magni, G., Felicioli, R., and Polidoro, G., Biochemistry *10* (1971) 4270.
11 Beck, C.F., Neuhard, J., Thomassen, E., Ingraham, J.L., and Kleker, E., J. Bact. *120* (1974) 1370.
12 Williams, J.C., and O'Donovan, G.A., J. Bact. *115* (1973) 1071.
13 Wilkins, A.S., J. Bact. *110* (1972) 616.
14 Creasy, W.A., and Handschumacher, R.E., J. biol. Chem. *236* (1961) 2058.
15 Ribereau-Gayon, G., Sabraw, A., Lammel, C., and Preiss, J., Archs Biochem. Biophys. *142* (1971) 675.

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Manipulating the activity of immobilized enzymes with different organo-smectite complexes[1]

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Summary. Enzymes may be immobilized on hydrophobic surfaces of organo-smectite complexes. The immobilized enzyme may be active, partially active, or inactive depending on the nature of the organic surface. These materials may be useful as models for enzymes in natural systems, and in medicine and industry.
Key words. Immobilized enzymes; smectite; organo-smectite complex; hydrophobic bonding; urease; arginase.

Understanding the basic biochemistry of enzymes may be facilitated by studies of immobilized enzymes, for in living cells enzymes exist largely in this state[2]. In nature, enzymes may also exist in the immobilized form by sorption onto natural clay-organic matter complexes[3]. Immobilized enzymes also have many important practical applications in industry and medicine[4,5].

The solid matrices used to immobilize enzymes vary widely in their nature[2,4,5]. The type of enzyme attachment to the matrix material may be through covalent bonding, adsorption, microencapsulation, and matrix entrapment. Adsorption of enzymes on layer silicates, such as smectite[6], may involve a number of interactions, the most energetic of which is ionic bonding[6,7]. This involves the exchange of cations on the mineral surface with positive sites on the enzyme or protein structure.

Our work reported here and in two earlier publications[8,9] has shown that enzymes may be immobilized by a unique process which involves the use of synthetic organo-smectite complexes. These complexes are prepared by saturating the exchange capacity of smectite clay with organic cations such as hexadecyltrimethylammonium$^+$ (HDTMA). This creates a surface of alkyl groups (in the case of HDTMA) which changes the mineral surface from one of a hydrophilic to hydrophobic nature. Enzymes are believed to be immobilized by hydrophobic bonding to the organic surface of the organo-smectite complex as shown schematically in figure 1. Hydrophobic portions of the enzyme interact with the hydrophobic alkyl groups creating an adsorption which is pH independent.

We now describe results which provide the basis for development of 'tailor-made' immobilized enzyme systems based on the use of different organo-smectite complexes. We have found that the enzymes glucose oxidase[8] and urease[9] were strongly bound to HDTMA-smectite and exhibited activities similar (60–