here, the first antiserum (anti-E2) was monospecific. However, a method could be conceived which requires only partially purified enzyme: A first oligospecific serum can be reacted with a prepurified antigen solution. The precipitation band of the desired antigen is isolated (e.g. from immunoelectrophoresis slides) and used for immunization. Further investigations on another enzyme of arginine biosynthesis will be reported in a subsequent publication[12].

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Immunochemical studies on acetylornithine 5-aminotransferase from Pseudomonas aeruginosa

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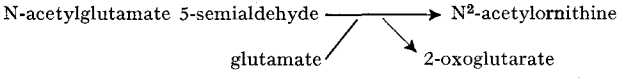
Summary. Mouse antibodies with specificity towards acetylornithine 5-aminotransferase (ACOAT) from Pseudomonas aeruginosa were used to study the structural similarities of serveral isofunctional enzymes from different sources. With the antibody directed against ACOAT, the amounts of enzyme present in cells grown under different conditions were determined. These experiments established that the enzyme is induced by arginine and is subject to repression by carbon sources.

In a previous paper[3] immunochemical studies on N-acetylglutamate 5-phosphotransferase, the second enzyme of arginine biosynthesis were presented. This communication deals with N^2-acetylornithine 5-aminotransferase (ACOAT, EC 2.6.1.11) which catalyses the fourth step in arginine biosynthesis.



A couple of important questions could be answered by immunological experiments.

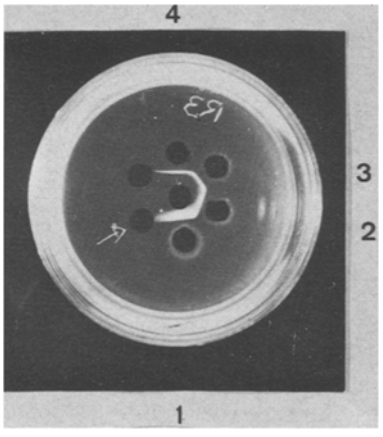


Fig. 1. Ouchterlony assay of pure ACOAT against mouse anti-ACOAT. 1 through 4, dilution series of mouse anti-ACOAT with PBS 1:1, 2, 4, 10; center well, pure ACOAT (0.5 mg/ml).

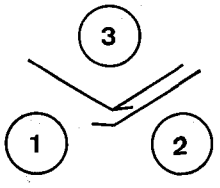


Fig. 2. Ouchterlony assay of 1, pure ACOAT preparation; 2, crude extract from Pseudomonas aeruginosa PAO1 against; 3, mouse anti-ACOAT.

Antibodies were raised in 3 mice using the procedure described previously[3]. ACOAT which was purified[4] by acid fractionation, ammonium sulfate precipitation, gelfiltration on sephadex G-100, and ionexchange chromatography on DE-52 (90% pure) served as immunogen. 1.3 ml of antiserum were obtained. The antibody displayed 1 sharp precipitation band in Ouchterlony double diffusion assay[5] when run against pure ACOAT (figure 1). Development against the immunogenic solution as well as against crude extracts revealed 2 distant bands indicating non-monodispersity of the solutions (figure 2). The second weak antibody population with specificity against a non-ACOAT antigen, however, did not disturb the experiments to be reported.

Specificity and cross-reactivity of the antiserum were examined. The P. aeruginosa wildtype strains PAC1 and PAT2 possessed an antigen which completely cross-reacted with ACOAT from strain PAO1. This result is indicative for the structural identity of the enzymes from different sources. Since the aminotransferases from E. coli and P. aeruginosa display similar structural and kinetic properties ([4,6], unpublished observations from this laboratory), it was of interest to learn about common antigenic specificities of the enzymes. No visible immunoprecipitate was formed with extracts of E. coli K12. This result indicates that the surfaces of the enzymes from the 2 species are not very similar. The question is legitimate whether or not at least the catalytic sites of the isofunctional enzymes have closely related shape. These common antigenic determinants should lead to cross-reactivity towards a specific antiserum. Immunochemical and enzymological studies demonstrate that both the antigen binding sites of immunoglobulins, as well as active sites of enzymes, are concave in shape, thus explaining why antibodies against active sites are not likely to be raised.

1 Acknowledgment. We wish to thank Prof. J. Lindenmann for helpful discussions.
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4-Aminobutyrate aminotransferase (EC 2.6.1.19) from P. aeruginosa PAO1 which also transaminates acetylornithine and ornithine was purified to electrophoretic homogeneity as judged by gel electrophoresis[7]. ACOAT and 4-aminobutyrate aminotransferase show similar catalytic properties. It was therefore interesting to determine whether the 2 enzymes have a common subunit or at least related surface structures. No cross-reactivity with 4-aminobutyrate aminotransferase could be detected.

Precipitates of ACOAT with anti-ACOAT were formed by incubating 20 µl of mouse anti-ACOAT antiserum with 130 µl of phosphate buffered saline and 50 µl of enzyme solution containing 25 µg of ACOAT. 63% of the original enzyme activity was found in the precipitate while 2% remained in the supernatant. This result resembles the one found for N-acetylglutamate 5-phosphotransferase from the same organism[3]. Enzymatic activity was assayed as described previously[4].

Another interesting finding resulted from experiments using Ouchterlony double diffusion. Purified ACOAT and ACOAT from a crude extract run alongside displayed a pattern of partial identity with a spur forming towards the well of the crude preparation (figure 2). This observation indicates that the structure of the enzyme might have been changed somewhat during the purification. Such an alteration could be caused by the loss of a ligand or of the cofactor.

ACOAT appears to be induced by arginine and to be repressed by various carbon sources[4]. In order to understand the regulation of the enzyme, it was crucial to determine whether the control is exerted at the level of enzyme activity or of enzyme synthesis. The radial monodiffusion technique described by Mancini[8] offered a convenient means to measure the concentration of a specific protein, not depending on the catalytic activity. Mancini assay confirmed the induction of enzyme synthesis by arginine and its repression by different carbon sources. The 2% agar gels contained 2% of antiserum. The relative content of ACOAT of an extract from strain PAO1 grown on mineral salts medium with arginine as the only source of carbon and nitrogen was 100. The same strain cultivated with arginine and citrate revealed a relative enzyme content of 21, while the amount of enzyme in uninduced cells grown on glutamate as the sole C and N source was 6. Thus the enzyme is induced by arginine at least 16–17fold. The data obtained by this approach agree very well with measurements of enzyme activity.

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The effect of pyrophosphate and diphosphonates on calcium transport in red cells

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Summary. The effect of 0.5 mM pyrophosphate (PPi), disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP) and disodium dichloromethane diphosphonate (Cl_2MDP) on the ATP-dependent Ca^{2+} extrusion from the human red cell ghosts was studied. PPi and Cl_2MDP had no effect, when introduced into the cells or added outside to the medium. EHDP slightly increased the calcium concentration in the released cells and slightly decreased the rate constant of the calcium transport, having opposite effects when it was inside or outside the cells. PPi and the 2 diphosphonates were not found to move easily across the red cell membrane.

Pyrophosphate (PPi[2]), a product of many biosynthetic reactions[3], inhibits apatite formation[4] and dissolution[5]; and it has therefore been suggested to regulate bone turnover[5]. The diphosphonates EHDP[2] and Cl_2MDP[2] are compounds structurally related to PPi, containing a P-C-P instead of a P-O-P bond. In contrast to PPi, they are resistant to hydrolysis and are more potent in vivo than PPi. They are powerful inhibitors of ectopic calcification[6], bone calcification[7] and bone resorption[7,8]. These effects might be explained by the action on crystal

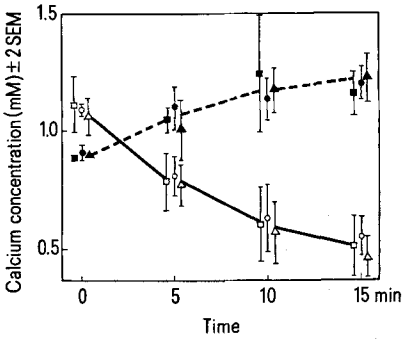

Fig. 1. Ca^{2+} transport of human red cells in the presence of 0.5 mM Cl_2MDP. Open signs represent Ca^{2+} concentration in the cells; filled signs represent Ca^{2+} concentration in the incubation medium; control (○, ●); Cl_2MDP within the cells (□, ■); Cl_2MDP in the incubation medium (△, ▲). Values are given ± 2 SEM.

1 Acknowledgments. We thank Dr H. J. Schatzmann for introducing us to the methods of calcium transport out of human red cells. We are very grateful to Miss M. Leu for skilled assistance and Mrs B. Gyger for preparing the manuscript. – This work has been supported by the Swiss National Science Foundation (3.725.76) and by the Procter & Gamble Company, Cincinnati, USA.
2 Abbreviations: PPi, pyrophosphate; EHDP, disodium ethane-1-hydroxy-1,1-diphosphonate; Cl_2MDP, disodium dichloromethane diphosphonate.
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