

columns No.5 are identical. On the other hand, in cell-free systems, results are compatible with a model according to which some tRNA species, e.g. leucyl-tRNA, become limiting (extracts from Interferon-treated cells)^{6,7}. Leucyl-tRNAs have been shown to be unstable in extracts prepared from Ehrlich ascites cells treated with Interferon¹¹. An explanation for these conflicting results may be that the impaired function of certain tRNAs has no structural correlate which can be detected with our chromatographic means.

Despite the fact that the deviations of the isotope ratio in the figure are somewhat larger than allowed by the counting statistics, we think – based on the similarity of all profiles – that Interferon has probably no significant effect on tRNA methylation in our system. Kroath et al.²⁵ came to the same conclusion by analyzing the pattern of methylated nucleotides of tRNA of CEF. Even if methylase activities or concentrations are stimulated as reported¹³, the normal levels may be sufficient fully to methylate homologous tRNAs. The concept of rate-limiting tRNA-function or concentration provoked by Interferon need not be wrong, but it would not be based on changes in methylation extent or pattern.

- 1 Work supported by the Swiss National Science Foundation, grants 3.1050 and 3.540.
- 2 Institute of Medical Microbiology, University of Berne, Switzerland.
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- 4 J.P. Garel, J. theor. Biol. 43, 211 (1974).
- 5 W.F. Anderson, Proc. natl Acad. Sci. USA 62, 566 (1969).
- 6 R. Falcoff, B. Lebleu, J. Sanceau, J. Weissenbach, G. Dirheimer, J.P. Ebel and E. Falcoff, Biochem. biophys. Res. Commun. 68, 1323 (1976).
- 7 J. Content, B. Lebleu, A. Zilberstein, H. Berissi and M. Revel, FEBS Lett. 41, 125 (1974).
- 8 S.L. Gupta, M.L. Sopori and P. Lengyel, Biochem. biophys. Res. Commun. 57, 763 (1974).

- 9 F.L. Riley and H.B. Levy, Virology 76, 459 (1977).
- 10 I. Sela, S.E. Grossberg, J.J. Sedmak and A.H. Mehler, Science 194, 527 (1976).
- 11 G.C. Sen, S.L. Gupta, G.E. Brown, B. Lebleu, M.A. Rebello and P. Lengyel, J. Virol. 17, 191 (1976).
- 12 J.A. Lewis, E. Falcoff and R. Falcoff, Path. Biol. 25, 9 (1977).
- 13 K.R. Rozee, L.J. Katz and E.S. McFarlane, Can. J. Microbiol. 15, 969 (1969).
- 14 A. Peterkofsky, C. Jesensky and J.D. Capra, Cold Spring Harb. Symp. quant. Biol. 31, 515 (1966).
- 15 U.Z. Littauer, M. Revel and R. Stern, Cold Spring Harb. Symp. quant. Biol. 31, 501 (1966).
- 16 Reagents, abbreviations: Medium Eagle MEM is minimum essential medium-Hanks (BBL, USA); Medium 199 – Hanks (Difco, USA); Fetal calf serum (Flow, Scotland); [³H]-uridine, uniformly labelled, 3.5–6.3 Ci/mmole, [¹⁴C]-uridine, generally labelled, 500 mCi/mmole, [¹⁴C-methyl]-L-methionine, 60 mCi/mmole, all from Amersham, England; adenosine, actinomycin D and tRNA coli (Calbiochem, Switzerland); ethylenediamine-tetraacetate (EDTA) and benzoylated DEAE-cellulose (BD-cellulose) (Schuchardt, Germany); Kieselgur (Merck, Germany); phenol (redistilled) (Fluka, Switzerland); polyvinylsulfuric acid, K salt (PVS) (Eastman, USA); sodium dodecyl sulfate (SDS) (Serva, Germany).
- 17 H. Koblet, U. Kohler and R. Wyler, Appl. Microbiol. 24, 323 (1972).
- 18 Ph. Dossenbach, H. Koblet and R. Wyler, Experientia 32, 1514 (1976). The authors wish to thank Prof. H. Zuber, ETH Zurich, for his preparation 10/1. 1 unit is defined as that amount of protein, which reduces plaque counts or uridine incorporation into viral RNA to 50% of the control value.
- 19 J. Lindenmann and G.E. Gifford, Virology 19, 302 (1963).
- 20 D. Bernhardt and J.E. Darnell, Jr, J. molec. Biol. 42, 43 (1969).
- 21 N. Sueoka and T. Yamane, Proc. natl Acad. Sci. USA 48, 1454 (1962).
- 22 L. Gillam, S. Millward, D. Blew, M. von Tigerström, E. Wimmer and G.M. Tener, Biochemistry 6, 3043 (1967).
- 23 M. Klagsbrun, Virology 44, 153 (1971).
- 24 C. Colby, E.C. Penhoet and C.E. Samuel, Virology 74, 262 (1976).
- 25 H. Kroath, H.J. Gross, C. Jungwirth and G. Bodo, Nucl. Acids Res. 5, 2441 (1978).

Polyamines as activators of AMP nucleosidase from *Azotobacter vinelandii*

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Summary. Polyamines at physiological concentrations activate AMP nucleosidase from *Azotobacter vinelandii*. Biological significance of the activation is discussed in relation to the control of adenylate energy charge and the purine nucleotide synthesis in prokaryotes.

A vast amount of literature has accumulated on the role of polyamines related to increased cellular proliferation in animal and bacterial cells^{1,2}. Polyamine accumulation is accompanied by an increase in the rate of RNA-synthesis as well as protein synthesis²⁻⁵. Furthermore, polyamines were demonstrated to be involved in the control of the activities of several enzymes⁶⁻¹⁰. Recently, we reported that polyamines at physiological concentrations activate the rat liver AMP deaminase (EC 3.5.4.6)¹¹, which may be important to stabilize the adenylate energy charge¹² and in the conversion of adenine nucleotides to inosine or guanine nucleotides¹³. Polyamines were suggested to participate in the stabilization of energy charge and the synthesis of purine nucleotides during cell proliferation of liver¹¹. On the other hand, AMP nucleosidase (EC 3.2.2.4) is responsible for the regulation of the energy charge¹⁴ and conversion of AMP to IMP in *Azotobacter vinelandii*^{15,16} and

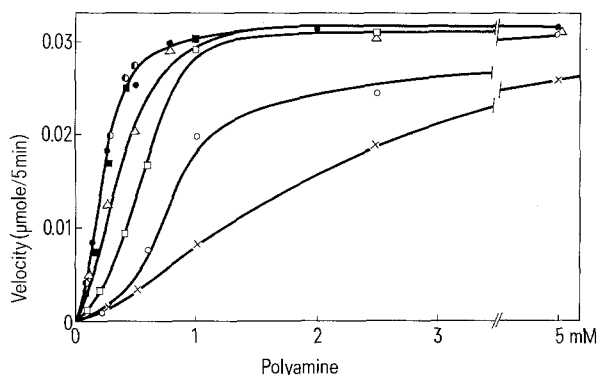
probably also in other prokaryotic cells^{12,14}. It is, thus, reasonable to assume that polyamines can activate the *Azotobacter* AMP nucleosidase, and this assumption was verified in the present paper. The physiological significance of the results is discussed in relation to the control of the energy charge and the purine nucleotide synthesis in bacterial cell growth.

The effect of polyamines and diamines, which differ from each other in the number of carbon atoms separating the 2 amino groups, on the AMP nucleosidase activity was examined. All the polyamines including diamines acted as powerful activators of the enzyme (figure). The activating effect of polyamines was suggested to depend upon the spatial separation of the primary amino groups: diamines separated by 5 or more carbon atoms as well as spermine and spermidine were the most effective activators of the enzyme. Polyamines are known to replace Mg²⁺ entirely or

partially in several Mg^{2+} -requiring reactions⁶⁻⁸ and AMP nucleosidase also requires Mg-ATP or Mg-PPi absolutely¹⁷. However, Mg^{2+} ions are suggested to be entirely replaced by polyamines in AMP nucleosidase reaction, since the maximal velocities attained in the presence of saturated concentrations of polyamines and Mg^{2+} ions were almost identical (data not shown).

The present study shows that polyamines express a powerful activating effect on the activity of AMP nucleosidase. Of polyamines tested, spermine, spermidine and some diamines, which are generally found in bacterial cells^{1,2}, showed a most striking activation of the enzyme, and furthermore, the concentrations necessary for 50% activation of the enzyme activity for these physiological amines were in the range 0.2–0.5 mM. It is thus reasonable to assume that these amines are physiological regulators of the

enzyme in *A. vinelandii*. AMP nucleosidase may be a regulatory enzyme in the conversion of adenine nucleotides to inosine or guanine nucleotides^{15,16} and to stabilize the adenylate energy charge¹⁴ in *A. vinelandii*. The role of the enzyme in the control of adenine nucleotide metabolism is strengthened by the lack or very low activity of AMP deaminase and 5'-nucleotidase (EC 3.1.3.5)^{12,14,15}. The present result suggests that the increase in polyamines activates the activity of AMP nucleosidase, which will result in the purine nucleotide synthesis and stabilization of energy charge in the exponential stage of bacterial growth. It should be emphasized that polyamines can regulate the purine nucleotide synthesis and the adenylate energy charge through the activation of AMP nucleosidase and AMP deaminase in prokaryotes and eukaryotes, respectively.



Effect of concentrations of polyamines and diamines on the activity of AMP nucleosidase. The enzyme was purified from *A. vinelandii*, strain 0, as described previously¹⁵ with a slight modification: protamine treatment was replaced by aminohexyl-Sepharose (Pharmacia) chromatography. Enzyme activity was measured by estimating production of ribose 5-phosphate¹⁸. The reaction mixture contained 0.5 mM ATP, 1 mM AMP, 5 mM Tris-HCl buffer, pH 8.0, various concentrations of polyamines and the enzyme in a final volume of 0.2 ml. The reaction was carried out at 37°C for 5 min and terminated by the addition of the reducing sugar reagent of Dygert et al.¹⁸. ●: Spermine, ■: spermidine, ●: diaminoethane, △: cadaverine, □: putrescine, ○: propylenediamine, ×: ethylenediamine.

- 1 D.R. Morris and R.H. Fillingame, *A. Rev. Biochem.* 43, 303 (1974).
- 2 H. Tabor and C.W. Tabor, *Adv. Enzymol.* 36, 203 (1972).
- 3 W.G. Dykx, Jr. and E.J. Herbst, *Science* 149, 428 (1965).
- 4 C.M. Calderera, B. Barbiroli and G. Moruzzi, *Biochem. J.* 97, 84 (1965).
- 5 J.E. Kay and V.J. Lindsay, *Exp. Cell. Res.* 77, 428 (1973).
- 6 S. Yoshida, S. Masaki and T. Ando, *J. Biochem.* 79, 895 (1976).
- 7 H. Fukuyama and S. Yamashita, *FEBS Lett.* 71, 33 (1976).
- 8 H. Tanigawa, M. Kawamura and M. Shimoyama, *Biochem. biophys. Res. Commun.* 76, 406 (1977).
- 9 C.C. Levy, W.E. Mitch and M. Schmukler, *J. biol. Chem.* 248, 5712 (1973).
- 10 Y. Tashima, M. Hasegawa and H. Mizunuma, *Biochem. biophys. Res. Commun.* 82, 13 (1978).
- 11 M. Yoshino, K. Murakami and K. Tsushima, *Biochim. biophys. Acta* 542, 177 (1978).
- 12 A.G. Chapman and D.E. Atkinson, *J. biol. Chem.* 248, 8309 (1973).
- 13 C.L. Zielke and C.H. Suelter, in: *The Enzymes*, vol. 4, 3rd ed., p. 47. Ed. P.D. Boyer. Academic Press, New York and London 1971.
- 14 V.L. Schramm and H. Leung, *J. biol. Chem.* 248, 8313 (1973).
- 15 M. Yoshino, *J. Biochem.* 68, 321 (1970).
- 16 M. Yoshino and N. Ogasawara, *J. Biochem.* 72, 223 (1972).
- 17 J. Hurwitz, L.A. Heppel and B.L. Horecker, *J. biol. Chem.* 226, 525 (1957).
- 18 S. Dygert, L.H. Li, D. Florida and J.A. Thoma, *Analyt. Biochem.* 13, 367 (1974).

Ethanol metabolism in *Drosophila melanogaster*

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Summary. A quantitative study of the transformation of ethanol into acetaldehyde shows that, in *Drosophila melanogaster*, the mitochondrial ethanol oxidizing system is not very active but that the part played by catalase appears more important than expected. For a strain without alcoholdehydrogenase, ethanol is highly toxic. The presence of acetaldehyde in the culture medium is toxic for all the strains studied. But, since even a strain without any aldehydeoxidase lives normally, the metabolic production of acetaldehyde does not seem dangerous.

The physiological and ecological importance of ethanol tolerance for *D. melanogaster* has been emphasized by many authors¹⁻⁶. Adapting itself to temperate climates, this species is considered to have modified its ecological niche in order to exploit food sources characterized by a higher ethanol concentration, as e.g. in vinification caves and breweries. A nutritional value of ethanol has even been

demonstrated, since the survival time of flies without food is greater in presence of ethanol^{7,8}.

Ethanol tolerance appears to be related in some way to the presence of alcohol dehydrogenase (ADH)⁹⁻¹³. The survival time without food but in presence of ethanol is greater for flies from strains with a higher ADH level¹⁴. The correlation between survival time and ADH level is however not a