steric activator AMP on the activity of glycogen phosphorylase. The intersection of the curves on the $1/v$ axis demonstrates the competitive inhibition between AMP and 5-hydroxytryptamine in connection with the phosphorylase *b*. The inhibition of phosphorylase *a* proved to be of a mixed type.

The results suggest that phosphorylase *b* is inhibited by the stabilization of an inactive conformation, according to allosteric theory. The effect of 5-hydroxytryptamine on phosphorylase *a* seems to be a little more complicated. This form of the glycogen phosphorylase has no absolute requirement for AMP and inhibition was not competitive with AMP. We suggest an indirect interaction, very likely due to a conformational change in the enzyme structure. The strong electronic field of the 5-hydroxytryptamine molecule makes this suggestion plausible.

Comparing the above findings to the data of other authors, we concluded that it is impossible to increase the glycogen mobilisation by 5-hydroxytryptamine directly, because of the inhibiting effect of 5-hydroxytryptamine on the key enzyme of glycogenolysis. The following scheme is proposed for the indirect effect of 5-hydroxytryptamine:

5-Hydroxytryptamine → adrenal gland → adenyl cyclase → 3′,5′-AMP → active kinase →
→ active phosphorylase → increased glycogenolysis

The experimental data which support this scheme will be presented elsewhere.

Department of Pathophysiology,
University Medical School,
Debrecen (Hungary)

JÁNOS SÜMEGI
SÁNDOR DAMJANOVICH
BÉLA CSABA

1 S. GARATTINI, *Serotonin*, Elsevier, Amsterdam, 1965, p. 157.
2 T. E. MANSOUR, E. W. SUTHERLAND, T. W. ROLL AND E. BUEDING, *J. Biol. Chem.*, 235 (1960) 466.
3 J. P. COLOMBO, J. W. WEBER, A. GUIDOTTI, D. KANAEISHI AND P. FOA, *Endocrinology*, 67 (1960) 693.
4 M. VAUGHAM, *J. Biol. Chem.*, 235 (1960) 3019.
5 E. H. FISCHER AND E. G. KREBS, *J. Biol. Chem.*, 231 (1958) 65.
6 E. H. FISCHER AND E. G. KREBS, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 5, Academic Press, New York, 1962, p. 369.
7 S. DAMJANOVICH, T. SANNER, AND A. PIHL, *European J. Biochem.*, 1 (1967) 347.

Received March 25th, 1968

Biochim. Biophys. Acta, 159 (1968) 545–547

BBA 63326

Enzymic basis for a genetic suppression: Accumulation and deacylation of *N*-acetylglutamic γ-semialdehyde in enterobacterial mutants

Double mutants[1] of *Escherichia coli* W, blocked in the path of proline synthesis (before glutamic γ-semialdehyde) and in the path of arginine synthesis at acetylornithine δ-transaminase[2] (α-*N*-acetyl-L-ornithine:2-oxoglutarate aminotransferase, EC 2.6.1.11) grow slowly on minimal medium. It was suggested[1] that growth without

added proline occurs because *N*-acetylglutamic γ-semialdehyde, an intermediate of arginine synthesis, accumulates and yields glutamic γ-semialdehyde, a precursor of proline; growth without added arginine presumably is due to a low-level activity of some transaminase other than acetylornithine δ-transminase, which allows the formation of arginine, although in growth-rate-limiting amounts. The remaining enzymes of the arginine path[3–6] are, therefore, derepressed. Acetylornithine δ-transaminase-deficient mutants[7,8] (*argD*⁻ mutants) have been found among (phenotypic) revertants of suitable *pro*⁻ auxotrophs. An *argD*⁻ mutation thus can be regarded as a suppressor for *pro*⁻. On minimal medium supplemented with repressive amounts of arginine, the accumulation of *N*-acetylglutamic γ-semialdehyde appears to be curtailed, and *pro*⁻ *argD*⁻ mutants show a growth requirement for proline[1]. From such double mutants, we have isolated triple mutants that do not require proline in the presence of arginine; the third mutation (*argR*⁻) affects the regulatory gene[7] of the arginine system and leads to derepressed levels of the (nonmutant) arginine enzymes. Similar results have been obtained with *E. coli* K12 (E. URM, S. BAUMBERG AND H. J. VOGEL, unpublished). It is now shown that suppression of *pro*⁻ by *argD*⁻ can occur in *Salmonella typhimurium* as well as in *E. coli*, and the enzymic mechanism for the "internal cross-feeding" by *N*-acetylglutamic γ-semialdehyde has been analyzed in both species.

Strain L1, isolated as a revertant of a *proB* mutant[9] (*proB25*) derived from *S. typhimurium* LT2, grows slowly on minimal medium. On minimal agar supplemented with arginine (but not proline), L1 yields rare colonies; strain AR is an isolate from one of them. Levels of acetylornithine δ-transaminase[2] and of acetylornithinase[3,10] (α-*N*-acetyl-L-ornithine amidohydrolase) in extracts of the mutants grown under various conditions of supplementation were measured, and genotypes were inferred (Table I).

It appeared possible that the conversion of *N*-acetylglutamic γ-semialdehyde to glutamic γ-semialdehyde, thought to be involved in the suppression of *pro*⁻ by *argD*⁻, is catalyzed by acetylornithinase, because this enzyme deacylates such compounds as *N*-acetyl-L-methionine[10] which, like *N*-acetylglutamic γ-semialdehyde, can be considered related to N^{α}-acetyl-L-ornithine, with a modification in the region of the δ carbon. *In vitro* experiments with extracts of the *S. typhimurium* mutants (Table I) and of analogous mutants derived from *E. coli* W, grown under various conditions of repression–derepression, indeed demonstrated an *N*-acetylglutamic γ-semialdehyde deacylase activity that is directly proportional to acetylornithinase activity; extracts of auxotrophic mutants devoid of acetylornithinase activity are also devoid of *N*-acetylglutamic γ-semialdehyde deacylase activity. Assays for *N*-acetylglutamic γ-semialdehyde deacylase were performed like those for acetylornithinase[10], except that *N*-acetylglutamic γ-semialdehyde was used as substrate and the glutamic γ-semialdehyde formed was determined through its color reaction with *o*-aminobenzaldehyde[2]. The results obtained with the *E. coli* mutants are given in Fig. 1. It is seen that 100 units (ref. 10) of acetylornithinase correspond to approx. 0.33 unit (see the legend of Fig. 1) of *N*-acetylglutamic γ-semialdehyde deacylase. Direct proportionality of the two activities was also observed with extracts of the *S. typhimurium* mutants; in this case, 100 units of acetylornithinase correspond to 0.28 unit of *N*-acetylglutamic γ-semialdehyde deacylase. *In vivo* experiments confirmed the role of acetylornithinase in the suppression of *pro*: *pro*⁻*argD*⁻*argR*⁻ strains, on arginine-containing medium, were shown to display a growth requirement for

TABLE I

LEVELS OF ACETYLORNITHINE δ-TRANSAMINASE AND ACETYLORNITHINASE IN EXTRACTS OF *S. typhimurium* MUTANTS GROWN UNDER VARIOUS CONDITIONS OF SUPPLEMENTATION

Cultures were grown in Medium E (ref. 10) containing 0.5% glucose and supplemented, as indicated, with L-proline (15 μg/ml) and L-arginine hydrochloride (50 μg/ml). Incubation was at 37° for 15 h, with shaking. Organisms were collected, suspended in 0.1 M potassium phosphate (pH 7.0) containing 1.0 mM glutathione, and subjected to ultrasonic disruption. Acetylornithine δ-transaminase[2] and acetylornithinase[10] in the resulting extracts were assayed as described in terms of the units given. Protein was determined according to LOWRY *et al.*[11]. Results are expressed as units/mg protein; a value of 0 indicates no detectable activity.

Strain	*Inferred genotype*	*Supplement*	*Transaminase (units/mg)*	*Acetylornithinase (units/mg)*
proB25	*pro⁻argD⁺argR⁺*	Pro	1.10	39
proB25		Pro + Arg	0.06	21
L1	*pro⁻argD⁻argR⁺*	None	0	119
L1		Pro + Arg	0	16
AR	*pro⁻argD⁻argR⁻*	None	0	144
AR		Pro + Arg	0	121

Abbreviations: Pro, proline; Arg, arginine.

proline if a mutation (*argE⁻*) causing loss of acetylornithinase activity was also present*.

Thus, the suppression of *pro⁻* reflects an "abnormal" action of a biosynthetic enzyme (acetylornithinase) on a metabolite (*N*-acetylglutamic γ-semialdehyde) whose

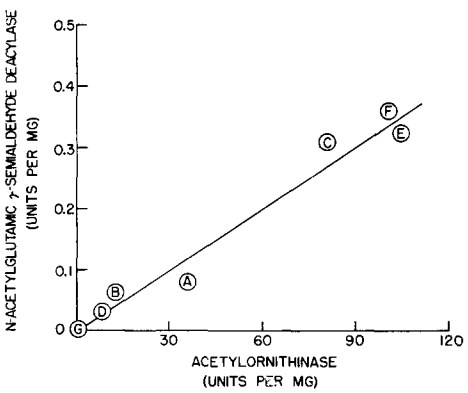

Fig. 1. Relation between *N*-acetylglutamic γ-semialdehyde deacylase and acetylornithinase activities of extracts of *E. coli* W mutants. The strains used and their genotypes are: W2, *pro⁻*; 21, *pro⁻argD⁻*; 215, *pro⁻argD⁻argR⁻*; 160-37-12, *argE⁻argR⁻*. See the legend of Table I for cultivation, preparation of extracts, assays of acetylornithinase and protein, and abbreviations. The points plotted correspond to strains and to supplements, as follows: A, W2, Pro; B, W2, Pro + Arg; C, 21, none; D, 21, Pro + Arg; E, 215, none; F, 215, Pro + Arg; G, 160-37-12, Arg. For *N*-acetylglutamic γ-semialdehyde deacylase assays, the enzymic reaction mixture is as for acetylornithinase assays[10], the substrate, however, being 1.1 mM *N*-acetylglutamic γ-semialdehyde (L isomer, prepared by enzymic transamination of N^{α}-acetyl-L-ornithine and purified by ion-exchange chromatography (S. BAUMBERG AND H. J. VOGEL, unpublished). After incubation at 37°, reaction mixtures and suitable blanks receive a mixture of 0.3 ml 6 M HCl and 1.0 ml 3.6 M sodium acetate, followed by 0.2 ml 0.033 M aq. *o*-aminobenzaldehyde[2], and the absorbance (light path, 1 cm) at 440 mμ is measured. One unit of *N*-acetylglutamic γ-semialdehyde deacylase is defined as that amount of enzyme which will yield an absorbance of 0.100 per 10 min of incubation of the enzymic reaction mixture, under the conditions given.

accumulation is occasioned by a mutation (*argD*$^-$) and aided by regulatory conditions (derepression). From the standpoint of evolution, it is noteworthy that, although *N*-acetylglutamic γ-semialdehyde is a common precursor of arginine and proline in the suppressed mutants, the corresponding wild-type organisms (*cf.* ref. 3) have maintained separate and individually controllable paths to these two amino acids from glutamate.

The portion of this work performed at C. W. Post College was supported in part by Public Health Service grant 1 RO1-GM 12938 to Dr. M. DEMEREC. The work done at Rutgers University was aided by the Public Health Service, National Scienc Foundation, and Damon Runyon Fund**.

Department of Biology,
C. W. Post College,
Greenvale, N.Y. (U.S.A.) — H. ITIKAWA***

Department of Genetics,
University of Leeds (Great Britain) — S. BAUMBERG

Institute of Microbiology,
Rutgers University,
New Brunswick, N.J. (U.S.A.) — H. J. VOGEL

1 D. F. BACON AND H. J. VOGEL, *Federation Proc.*, 22 (1963) 476.
2 A. M. ALBRECHT AND H. J. VOGEL, *J. Biol. Chem.*, 239 (1964) 1872.
3 H. J. VOGEL, *Proc. Natl. Acad. Sci. U.S.*, 39 (1953) 578.
4 H. J. VOGEL AND D. F. BACON, *Proc. Natl. Acad. Sci. U.S.*, 55 (1966) 1456.
5 S. BAUMBERG, D. F. BACON AND H. J. VOGEL, *Proc. Natl. Acad. Sci. U.S.*, 53 (1965) 1029.
6 H. J. VOGEL, S. BAUMBERG, D. F. BACON, E. E. JONES, L. UNGER AND R. H. VOGEL, in H. J. VOGEL, J. O. LAMPEN AND V. BRYSON, *Organizational Biosynthesis*, Academic Press, New York, 1967, p. 223.
7 H. J. VOGEL, D. F. BACON AND A. BAICH, in H. J. VOGEL, V. BRYSON, AND J. O. LAMPEN, *Informational Macromolecules*, Academic Press, New York, 1963, p. 293.
8 D. F. BACON AND H. J. VOGEL, *Cold Spring Harbor Symp. Quant. Biol.*, 28 (1963) 437.
9 K. E. SANDERSON AND M. DEMEREC, *Genetics*, 51 (1965) 897.
10 H. J. VOGEL AND D. M. BONNER, *J. Biol. Chem.*, 218 (1956) 97.
11 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.

Received February 12th, 1968
Revised manuscript received April 11th, 1968

* The strains required for these tests were constructed as follows. From an *E. coli* W Hfr donor strain (kindly provided by Dr. D. F. BACON), which carries *phe*$^-$ and *try*$^-$ mutations and transfers markers with the orientation *leu–met–ilva*, a *phe*$^-$*try*$^-$*pro*$^-$*argD*$^-$*argR*$^-$ derivative having the Hfr character was prepared by the successive introduction of *pro*$^-$, *argD*$^-$, and *argR*$^-$, as indicated in the opening paragraph. This derivative was mated with an *E. coli* W recipient strain, *his*$^-$*pro*$^-$*argE*$^-$*argR*$^-$ (the *pro*$^-$ mutation corresponding to a block before glutamic γ-semialdehyde), and *phe*$^+$*try*$^+$*his*$^+$ recombinants were selected, 40% of which proved to have the desired *argE*$^-$-*pro*$^-$*argD*$^-$*argR*$^-$ constitution verified by relevant enzyme assays.

** T. KUO AND B. A. D. STOCKER (personal communication) have encountered similar "arginine-inhibitable" (phenotypic) revertants of *proA* and *proAB* deletion mutants of *S. typhimurium*, strain LT2, and infer from their growth responses and from genetic analysis that they are mutants deficient in acetylornithine δ-transaminase.

*** Present address: Department of Biology, Tokyo Metropolitan University, Setagaya-ku, Tokyo, Japan.