On the mechanism of activation of acetyl glutamic acid

The catalytic role of carbamyl glutamic acid or related compounds for citrulline biosynthesis has been shown by us[1]. However the mechanism of activation leading to compound X* or carbamyl phosphate has not been clarified. Experiments leading to the mechanism of activation of carbamyl glutamic acid showed that the hydrogen atoms attached to the carbon atoms were not involved in the primary activation[1]. There was, however, indication of a primary activation at the carbamyl

TABLE I

THE EFFECT OF NH_3 REPLACEMENT ON CITRULLINE SYNTHESIS, CO_2 FIXATION, AND INORGANIC PHOSPHATE LIBERATION WITH BACTERIAL AND MAMMALIAN ENZYMIC PREPARATIONS

Expt.	*Conditions*	*$^{14}CO_2$ fixed c.p.m. (thousands)*	*Phosphate liberation μmoles*	*Citrulline synthesis μmoles*
A	NH_3 excluded	0	0	0
A	NH_2OH replacing NH_3	0	6.5	0
A	Complete system	315	9.3	6.3
B	NH_3 excluded	0	0	0
B	NH_2OH replacing NH_3	0	0.09	0
B	Complete system	48	1.01	1.06

The following components expressed in μmoles were incubated at 38° for 20 min in a final volume of 3.5 ml: L-ornithine, 15; ATP, 13; $NaH^{14}CO_3$, 25 (50,000 c/μmole); $MgSO_4$, 30; Tris buffer (pH 7.5), 160; acetyl glutamate, 20 and, when used NH_3, 50; NH_2OH, 150. In A, water extracts corresponding to 20 mg of acetone powder rat liver mitochondrial preparations were used. In B, 1 mg of bacterial preparation was used[3].

TABLE II

THE EFFECT OF PREINCUBATION OF COMPONENTS AND OF HEXOKINASE ON CITRULLINE BIOSYNTHESIS WITH BACTERIAL AND MAMMALIAN SYSTEMS

Conditions	*Liver*		*Conditions*	*Bacteria*
	A	*B*		
	μmole citrulline			*μmole citrulline*
No preincubation	0.330	0.128	No preincubation	0.210
AG + ATP preincubated	0.530	0.240	ATP preincubated	0.210
No preincubation hexokinase added	0.014	--	ATP + CO_2 preincubated	0.204
AG + ATP preincubated hexokinase added	0.11	--		

The following components expressed in micromoles per 2 ml final volume were incubated with 15 mg rat liver mitochondrial acetone powder extracts: ATP, 8; Tris (pH 7.5), 100; $MgSO_4$, 25; $NaHCO_3$, 100; acetyl glutamate, 40; NH_4Cl, 50; L-ornithine, 10. Preincubation of the enzyme preparation with the components indicated in the table, 5 min at 38°. All tubes were brought to 30° for expt. A and to 25° for expt. B, and completed at definite intervals with the rest of the components and incubated for exactly 30 sec, followed by deproteinization. When hexokinase was used the samples were incubated for 2 min with enough hexokinase to transfer 25 μmoles of ATP phosphate in 2 min to insure removal of ATP and only 4 μmoles of ATP were used. The preincubation time was also reduced to 2 min and the final incubation was conducted at 38° for 2 min. When hexokinase was used 100 μmoles of glucose were added.

With the bacterial preparation[3] 1 mg was used and 1 mg of pyruvic kinase was added as well as 10 μmoles of PEP. The ATP was decreased to 2 μmoles, the NH_3 increased to 100 μmoles, and the pH of the buffer was 8.5. The preincubation was conducted at 38° for 5 min and the final incubation at 22° for exactly 30 sec.

* The following abbreviations are used throughout this paper: AG, acetyl glutamate; ATP, adenosine triphosphate; PEP, phosphoenol pyruvate; compound X, the active intermediate for citrulline synthesis or related reactions formed from AG or related compounds[1].

glutamic acid level as shown by the production of inorganic phosphate when carbamyl glutamate reacted with ATP[2]. It was also shown that hydroxylamine increased this inorganic phosphate liberation without a concomitant formation of a citrulline-like compound[1]. These experiments have been repeated using $^{14}CO_2$ and are shown in Table I. They clearly confirmed and extended the previous observations and also show that the bacterial system unlike the mammalian system does not react with hydroxylamine. We have found further indications for an active precursor of X and/or carbamyl phosphate. As shown in Table II when ATP is preincubated with acetyl glutamic acid there is an increase in citrulline synthesis. This effect is not demonstrable with CO_2 or NH_3 and ATP or CO_2, NH_3 and ATP. Also hexokinase will not react or reacts slowly with the formed intermediate. These experiments are interpreted as indication for the formation of an active phosphoryl intermediate from ATP and acetyl glutamic acid. The chemical synthesis of such an intermediate is under investigation. This work was done during the tenure of an Established Investigatorship of the American Heart Association by the senior author. This work was supported by Grants No. H-1925, National Institutes of Health, No. 67, The Helen Hay Whitney Foundation, Life Insurance Medical Research Fund and the Kansas Heart Association.

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[1] S. GRISOLIA AND R. O. MARSHALL, in W. D. MCELROY AND B. GLASS, *Symposium on Amino Acid Metabolism*, Johns Hopkins Press, Baltimore, 1955, p. 258.
[2] S. GRISOLIA, in W. D. MCELROY AND B. GLASS, *Phosphorus Metabolism*, Vol. I, Johns Hopkins Press, Baltimore, 1951, p. 619.
[3] S. GRISOLIA, H. J. GRADY AND D. P. WALLACH, *Biochim. Biophys. Acta*, 17 (1955) 277.

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Strahlenchemische Bildung von Aminen und Aminocarbonsäuren

Bei Bestrahlung kristallisierter Aminosäuren und ihrer wässrigen Lösungen mit Röntgenstrahlen können neben anderen Verbindungen flüchtige Spaltprodukte gefunden werden, von denen Kohlendioxyd, Wasserstoff, Kohlenwasserstoffe und Ammoniak mit Ionenausbeuten zwischen *ca.* 0.1 und 4 stets in bemerkenswerter Menge auftreten[1,2,3,4]. Auf Grund eigener Untersuchungen, wie auch der anderer Autoren (z.B.[5]), kamen wir weiterhin zu dem Schluss, dass sich bei genügend intensiver Bestrahlung organisch chemischer Verbindungen in dem bestrahlten System zwischen ab- und aufbauenden strahlenchemischen Reaktionen ein Gleichgewicht einstellt. Daher war zu erwarten, dass es möglich sein musste, durch Einwirkung von ionisierender Strahlung auf die durch dieselbe Strahlung aus einfachen Aminosäuren abgespaltenen kleinsten Moleküle (NH_3, CO_2, H_2, CH_4 usw.) umgekehrt wieder u.a. Aminosäuren und verwandte Verbindungen zu synthetisieren. Zugleich aber war das Studium der Strahlenchemie solcher und ähnlich zusammengesetzter Atmosphären so z.B. auch eines Gasgemisches bestehend aus NH_3, H_2, CH_4 und H_2O, wie es von OPARIN[6] und UREY[7] für die Erdatmosphäre eines frühen Stadiums angenommen wird, von allgemeinem Interesse.

Nachdem es nunmehr MILLER[8,9,10] geglückt ist, die Bildung von Aminosäuren durch elektrische Entladungen in einer von OPARIN und UREY vorgeschlagenen Atmosphäre zu beobachten, war daher ein ähnliches Ergebnis bei Anwendung ionisierender Strahlung nicht unwahrscheinlich.

Zur Strahlenerzeugung benutzten wir die Dermopanröntgenanlage von Siemens (45 kV, 25 mA), Dosisleistung in 1 cm Abstand vom Be-Fenster $2.4 \cdot 10^5$ *r*/min. Als Bestrahlungsgefässe für die Gase verwendeten wir kegelförmige Glasgefässe von 2 l Inhalt. Sie waren ähnlich wie Saugflaschen geformt, hatten jedoch zur Zu- und Ableitung der Gase zwei seitliche Ansätze; die obere Öffnung wurde durch 7 μ starke Polyäthylenfolie verschlossen. Im Mittel ergab sich so für das bestrahlte Gas eine Dosisleistung von $2 \cdot 10^4$ *r*/min. Bei der durchschnittlich aufrechterhaltenen Bestrahlungszeit von 180 min erhielten wir auf diese Weise eine Gesamtdosis von *ca.* $3.5 \cdot 10^6$ *r*. Die Versuchstemperatur war 20° C. Es wurden Gase folgender Zusammensetzungen bei Normaldruck bestrahlt: