Pyrroline-5-carboxylate Synthesis from Glutamate by Rat Intestinal Mucosa*

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The mitochondria of rat intestinal mucosa were found to have an enzymatic activity that converts radioactive glutamate to pyrroline-5-carboxylate (P5C) in the presence of ATP, NADPH, and MgCl₂. The product of this enzyme was identified as P5C by the fact that it was converted to proline by chemical reduction with NaBH₄, or by enzymatic reduction with NADH in the presence of purified yeast P5C reductase. The product was demonstrated to be P5C rather than pyrroline-2-carboxylate by thin layer chromatography. The presence of the activity in mitochondria prepared from intestinal mucosa of germ-free rats proved that this activity is of mammalian origin.

Omission of either ATP, NADPH, or MgCl₂ from the reaction mixture resulted in little or no activity. The apparent Hill coefficient for NADPH appeared to be about 7.0 under the conditions used. Substrate saturation curves in the presence of an ATP and an NADPH regeneration system gave apparent Kᵥ values of 2.5 mM for glutamate, 0.19 mM for ATP, and 0.5 µM for NADPH in the presence of 20 mM MgCl₂. The mitochondrial preparation usually produced P5C at a rate of 1.2 to 1.6 nmol/mg/min at 20°C when incubated with 1 mM glutamate, 3 mM ATP, 0.2 mM NADPH, and 20 mM MgCl₂.

Nutritional studies first suggested that conversion of glutamate to proline occurred in mammalian tissues (1-3). It was then demonstrated that rats or guinea pigs were able to convert intraperitoneally or orally administered radioactive glutamate to proline (4-6). The carbon chain of proline can also be derived from glutamine via glutamate in HeLa cells (7) and in human lung fibroblasts (8) and other cultured human cell lines (9). In addition, a proline auxotroph derived from Chinese hamster ovary cells was obtained that did not grow in proline-free medium (10). Although the addition of ornithine or arginine to the proline-free medium allowed these cells to grow, growth was slower than that observed in the presence of proline. However, addition of P5C in place of ornithine or arginine to the proline-free medium allowed these cells to grow at the maximal rate. These results indicate that the mutation had affected protein(s) that catalyze the conversion of glutamate to glutamate γ-semialdehyde while the pathway from ornithine to proline was intact. Although the data above as well as the established presence of P5C reductase in various tissues (11) suggests that P5C is a direct precursor of proline in proline biosynthesis from glutamate, there has been little well documented evidence for the enzymatic conversion of glutamate to P5C in homogenates of mammalian tissues.

In bacteria, on the contrary, the metabolic pathway glutamate → P5C → proline was demonstrated in Escherichia coli mutants (12) and Neurospora mutants (13). Data from the laboratories of Moses (14-16) and Baich (17-19) as well as a paper by Hayzer and Leisinger (20) have suggested that the conversion of glutamate to P5C may require two enzymatic reactions: a γ-glutamyl kinase and a γ-glutamylphosphate reductase; these enzymes have not been extensively purified.

Three papers have been published providing evidence that rat intestinal mucosa has the enzyme(s) that synthesize P5C from glutamate. Windmueller and Spaeth (21, 22) found that rat intestine perfused with radioactive glutamine, either vascularly or luminally, produced a significant amount of radioactive proline, ornithine, and citrulline. Ross et al. (23) were able to obtain for the first time a homogenate of rat intestinal mucosa that formed ornithine from glutamate when ATP, Mg, glutamate, and NADPH were present. Ornithine would be produced from glutamate γ-semialdehyde, the presumed product of a step requiring these substrates, and glutamate by the ornithine aminotransferase of the intestinal mucosa. In neutral solutions, glutamate γ-semialdehyde is in equilibrium with P5C; therefore, the formation of ornithine from glutamate strongly supported the enzymatic conversion of glutamate to P5C by this tissue.

The present paper describes an identification of P5C as the product of this enzyme(s), and a direct assay for P5C formation from glutamate (in the presence of ATP, NADPH, and MgCl₂) by rat intestinal mucosa mitochondria as well as preliminary kinetic studies using this method. The following paper (24) will describe evidence for mitochondrial localization of this activity and the temperature-labile properties of this enzyme(s).

### Experimental Procedures

1 Portions of this paper (including "Experimental Procedures" and Figs. 2-6) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-0861, cite authors, and include a check or money order for $4.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
RESULTS

Throughout the present investigations, we have included regenerating systems for both ATP and NADPH in assay mixtures in the hope that rapid consumption of these two substrates by other enzymes in the crude homogenates would be avoided. Without these regenerating systems, the amount of product formed was 50% of that obtained in the presence of the regenerating systems.

The assumption that P5C and/or glutamate γ-semialdehyde can be synthesized from glutamate in the intestinal mucosa was originally based on the observation that ornithine is formed from glutamate by a homogenate of rat intestinal mucosa (23). Ornithine synthesis from glutamate γ-semialdehyde is catalyzed by ornithine aminotransferase (25). Glutamic γ-semialdehyde also undergoes spontaneous intramolecular cyclization to form P5C, which can be converted to proline by P5C reductase. The demonstration of proline synthesis from glutamate by a homogenate of this tissue would confirm the existence of an enzyme synthesizing P5C in the intestinal mucosa.

We found that a 33% (w/v) homogenate of rat intestinal mucosa was able to convert glutamate to proline when incubated with MgCl₂ plus ATP, NADPH, and NADH with their respective regenerating systems at 37 °C for 90 min. The reaction was terminated with perchloric acid and deproteinized, as described under "Experimental Procedures." The deproteinized supernatant was analyzed by cation exchange chromatography as described under "Experimental Procedures." Microanalytical procedures were used to determine the amounts of the products in the reaction mixtures. The amounts of ammonia, proline, and P5C in the reaction mixtures were determined by color developed with ninhydrin (50). The amount of the reaction product formed by the mitochondrial extract was also converted to proline by chemical treatment with NaBH₄ and reducing mixture (see "Experimental Procedures"). The product of the mitochondrial extract was also converted to proline by chemical treatment with NaBH₄ and reducing mixture (see "Experimental Procedures"). The product of the mitochondrial enzyme(s) which converted glutamate to P5C are in mitochondria (24). Mitochondria, we developed a modification from the conventional procedure of subcellular fractionation (24), primarily developed for liver, to suit an intestinal mucosa homogenate. This improved method enabled us to ascertain that enzyme(s) which converted glutamate to P5C must exist in the 12,000 × g pellet.

In order to ascertain whether this enzyme is located in mitochondria, we developed a modification from the conventional procedure of subcellular fractionation (24), primarily developed for liver, to suit an intestinal mucosa homogenate. This improved method enabled us to ascertain that enzyme(s) which converted glutamate to P5C must exist in the 12,000 × g pellet. The enzymatic product formed by the mitochondrial extract possessed the characteristics expected for P5C. The elution profile for the radioactive product on the cation exchange column was determined and compared with that of authentic L-P5C and proline (Fig. 1). When the incubation mixture did not contain P5C reductase (Fig. 1A), the product appeared mostly at the position of authentic P5C; the amount of P5C formed was 141 nmol. A minor radioactive peak of proline suggests a slight contamination of the mitochondrial pellet with the cytosolic P5C reductase. When purified yeast P5C reductase was added to the reaction mixture, the enzyme radioactive product formed now eluted as the carrier proline;
terminating the activity of the mitochondrial enzyme(s); 172 nmol of proline were formed. As P5C has been reported to be reduced almost entirely to proline under the condition used (28), this experiment also demonstrates that the product formed by the mitochondrial enzyme(s) was P5C.

To confirm that the product was not P2C, an isomer of P5C which has been recently proposed as a direct precursor of proline via ornithine in plants (29), we compared the enzymatic product with L-P5C, DL-P5C, P2C, and proline on thin layer chromatograms (Table I). With all six solvents used, the product formed, as visualized by o-aminobenzaldehyde reagent, migrated exactly as DL-P5C and L-P5C on the thin layer plates. When the product was mixed with synthetic P5C, a single spot was observed. L-P5C and DL-P5C and the enzymatic product were clearly separated from P2C and proline.

Since reduction of P5C to proline by P5C reductase is an essentially irreversible process with proline formation being favored (28), advantage of this fact was taken to develop an assay system for P5C. Using an excess of yeast P5C reductase, P5C was converted to proline which was then separated from other metabolites by cation exchange chromatography. Addition of 1 unit of P5C reductase (24) to the mixture was found to be sufficient for maximal proline formation from P5C. This is a coupled enzyme assay, the formation of proline increased linearly for 25 min at 25 °C but slowed down to some extent after 10 min at 37 °C. The production of P5C was also proportional to the mitochondrial proteins at 20 °C; at 37 °C, product formation was low when less than 1 mg of mitochondrial protein was used but was linear at protein concentrations above this amount (24).

All the data including the substrate and pH effects were similar whether proline synthesis (with the coupled enzyme system) or P5C synthesis was measured, only the data from the direct assay system where the product is P5C are presented here. Mitochondria contain ornithine ami

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<th>Solvents</th>
<th>Proline</th>
<th>DL-P5C</th>
<th>L-P5C</th>
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<td>Methanol/pyridine/H2O (120:6:30)</td>
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<td>1-Propanol/formate/H2O (75:25)</td>
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<tr>
<th>Proline formation in homogenates prepared from</th>
<th>P5C or proline formation in homogenates prepared from</th>
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<tr>
<td>Conventional rat</td>
<td>Germ-free rat</td>
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<td>nmol/20 min/mg protein</td>
<td>nmol/20 min/mg protein</td>
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<td>PSC formation</td>
<td>Complete system</td>
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<tr>
<td>- ATP</td>
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<td>- NADPH</td>
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<td>ATP, MgCl2, and NADPH</td>
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<tr>
<td>P2C formation</td>
<td>Complete system</td>
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<td>- P5C reductase</td>
<td>2.6</td>
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<td>- P5C reductase + NaBH4</td>
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germ-free rats were very close, 1.30 and 1.25 nmol/mg of protein/min, respectively, and the proline formed when yeast P5C reductase was added was 1.27 and 1.10 nmol/reg/min, respectively. Although it is impossible to compare these two different preparations directly since the germ-free rats were younger than conventional ones and were not starved before preparation of the mitochondria, these two sets of essentially identical values suggest that the observed activities were primarily derived from the mucosal mitochondria of rat intestine even when conventional animals were used.

2 L- or DL-P5C and P2C frequently had a major and a minor spot or showed some streaking in solvents where polymerization (26) or esterification with alcohol would be expected.
The optimal pH was pH 7.0 (Fig. 2) with a rather steep drop of activity in the acidic region. Activity was negligible below pH 6 or above pH 9. When proline formation was measured using unlysed mitochondria in the absence of NP-40, the same pH profile was obtained except that the entire curve was shifted toward the acid region so that the optimum pH was 6.5.

The effect of varying the substrate concentration on the initial rate of P5C formation is shown in Figs. 3–6. Fig. 3 shows the effect of varying the ATP concentration on the activity, when equimolar amounts of MgCl₂ and ATP were added in addition to the basal concentration of 20 mM MgCl₂. The apparent Kₐ for ATP was calculated to be 0.19 mM and the Vₘₐₓ was 1.6 nmol/mg of protein/min. When a similar study was conducted using the proline assay which has more glutamate (3 rather than 1 mM), the values obtained were 0.27 mM and 2.4 nmol/mg of protein/min, respectively. Concentrations of ATP greater than 10 mM were inhibitory. Fig. 4 shows the effect of varying the MgCl₂ concentration when the ATP concentration was set at 3 mM. About 20 mM MgCl₂ gave the highest rate; higher concentrations were inhibitory. A similar observation was obtained with the proline assay system.

The effect of NADPH concentration (Fig. 5) shows that the apparent Kₐ for NADPH, 6.5 μM, and the Vₘₐₓ was 1.2 nmol/mg of protein/min. In the proline assay system, these values were 1.3 and 2.6, respectively. High concentrations of NADPH, greater than 400 μM, were inhibitory for proline synthesis as they also were for ornithine synthesis from glutamate (31). The effect of glutamate concentration was also examined (Fig. 6). The calculated values were 2.5 mM for the apparent Kₐ and 4.5 nmol/mg of protein/min for Vₘₐₓ. The similar experiment using the proline assay system gave a higher apparent Kₐ of 7.1 mM and 5.2 nmol/mg of protein/min for Vₘₐₓ.

Among the buffers tested at a concentration of 100 mM and at pH 7.4, triethanolamine-, imidazole-, and Tris-chloride buffers gave activities similar to that observed with potassium-Hepes, pH 7.4, whereas potassium phosphate buffer of the same pH was inhibitory so that the P5C formed was only 40% that formed using the other buffers. The lysis of mitochondria with NP-40 usually increased the activity observed up to 2-fold, suggesting that this treatment allows the substrates to be more accessible to the enzyme(s). The activity was inhibited when additional KCl was added: 19% at 75 mM, 37% at 150 mM, and 63% at 300 mM KCl. As was mentioned above, some P5C (with glutamate as CO-substrate) could have been converted to ornithine by ornithine aminotransferase. Since canaline is known to be a potent irreversible inhibitor of the transferase (32), we examined the effect of the addition of canaline on P5C synthesis. Generally, the effect of additional KC1 was added: 19% at 75 mM, 37% at 150 mM, and 63% at 300 mM KCl.

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We also tested the effect of rotenone which inhibits the NAD-dependent P5C dehydrogenase of rat liver mitochondria (33) and might, therefore, increase P5C accumulation by inhibiting P5C reoxidation to glutamate. No significant activation was observed when rotenone was added in concentrations ranging from 1 to 25 μg/ml. This is consistent with the reported poor activity of P5C dehydrogenase in rat intestine (34) and with the fact that we did not add any oxidized form of pyridine nucleotides to the mixture. Finally, we examined, in a preliminary way, the effect of L-proline, L-ornithine, and DL-P5C on the enzyme activity. The P5C synthetic activity was inhibited 97% by 5 mM ornithine, 28% by 3.4 mM DL-P5C, and 8% by 5 mM proline while ammonia (10 mM, neutralized with Hepes) did not affect the activity. The latter observation demonstrates that the P5C inhibition was not due to the ammonia present in the P5C preparation; a similar result was observed with the E. coli system (14).

DISCUSSION

Fig. 7 shows the accepted metabolic pathways interconverting glutamate, proline, and ornithine (27, 35, 36) in mammals.

The first enzymatic step, the conversion of glutamate to glutamate γ-semialdehyde (and P5C), is the only enzyme reaction that has not been well characterized. Vogel and Davis (12) first reported evidence that the intermediate between glutamate and proline must be P5C. They identified P5C by: 1) finding that the migration of the unknown substance on paper chromatograms was identical with that of chemically synthesized standard P5C; 2) showing that the substance gave a yellow color with o-aminobenzoaldehyde and that the absorption spectrum was the same as that observed when o-aminobenzoaldehyde reacts with authentic P5C; and 3) showing that the chemically synthesized P5C supported the growth of proline-auxotrophic E. coli strain 55-25. Although these studies proved that glutamate is converted to P5C by intact E. coli cells, there have been only a few papers describing the enzymology of this reaction because cell-free extracts have had little or no activity (11, 37–39).

In analogy to the conversion of aspartic acid to aspartic semialdehyde (40) and the conversion of N-acetylglutamate to N-acetylglycine (80) and γ-semialdehyde, Baich (41) considered that the reduction of glutamate to glutamyl γ-semialdehyde/P5C might occur in two steps: namely, a phosphorylation of the γ-carboxyl group of glutamate utilizing ATP followed by a reduction, utilizing NAD or NADP, of the acylphosphate to the aldehyde. Since P5C excretion from E. coli is inhibited by proline in the medium (18, 38), Baich (17) looked for and found a proline-inhibitable γ-glutamyl kinase in E. coli extracts. This observation was confirmed by Hayzer and Moses (16) who found that E. coli mutants unable to convert glutamate to P5C lacked the proline-inhibitable glutamate kinase activity. A similar activity has been partially purified from Pseudomonas aeruginosa (42); proline, proline analogs, and L-methionine-DL-sulfoximine inhibit this kinase. Baich (19)
presented indirect evidence for the second reaction, the conversion of γ-glutamylphosphate to PSC. This activity was observed in the reverse (optical biosynthetic) reaction as a P
-dependent reduction of NADP⁺ in the presence of PSC (16, 19-20); activity is absent in the appropriate mutants (18, 21).

Although the evidence cited above indicates the possibility that in bacteria two separate enzymes, a γ-glutamyl kinase and a γ-glutamylphosphate reductase, may be required to convert glutamate to PSC, there is no direct proof that γ-glutamylphosphate is the product of the kinase or that it is the substrate for a second enzyme which converts it to PSC.

The discovery that perfusion of the intestine with glutamate (21, 22) leads to production of ornithine from glutamate encouraged this laboratory to develop conditions where a cell-free homogenate of the intestinal mucosa would synthesize ornithine from glutamate (23, 31). This synthesis would require the initial conversion of glutamate to γ-glutamyl γ-semialdehyde (and PSC). The prediction that the intestinal mucosa has the ability to convert glutamate to PSC was verified in this paper by isolating radioactive proline from an incubation mixture containing a cell-free homogenate of intestinal mucosa, radioactive glutamate, ATP, NADPH, and MgCl₂.

The synthesis of both proline (this paper) and ornithine (23, 31) from glutamate by cell-free homogenates of intestinal mucosa led us to develop conditions where PSC itself would accumulate. A difficulty in detecting PSC production was the high PSC reductase activity in this tissue (34) and the fact that the equilibrium for the reductase favors proline formation. Ornithine aminotransferase is also present; however, the equilibrium of this reaction is toward PSC (25). The first of these difficulties turned out to be avoidable by our discovery that the enzyme(s) converting glutamate to PSC had a different subcellular localization from PSC reductase. The assignment of the PSC-synthesizing activity to the mitochondria was made possible by developing a modified procedure of subcellular fractionation for intestinal mucosal cells (24).

The identification of the product as PSC was accomplished in three ways. Purified yeast PSC reductase converted the product, which eluted as authentic DL-PSC on a cation exchange chromatogarm, to proline. The same conversion was observed when the product was treated with NaBH₄ under conditions selected for optimal reduction of PSC to proline (26). The behavior on thin layer chromatography of the product was identical with that of DL-PSC or L-PSC, but different from that of the isomeric P₂C. These results clearly demonstrated that the product was PSC. We did not attempt to use the colorimetric reaction of the crude product with o-aminobenzaldehyde, since reduced pyridine nucleotides interfere with this assay for PSC (31, 43).

We have developed two quantitative assay procedures for the PSC-synthesizing enzyme(s): first, an indirect measurement using purified yeast PSC reductase to convert PSC to proline, and second, by directly isolating PSC. The coupled assay is more advantageous than the direct assay when the enzyme preparations (or crude homogenates) contain endogenous PSC reductase or ornithine aminotransferase. The direct assay, however, is to be preferred when one wishes to characterize this enzyme. Both assays have revealed that the enzyme synthesizing PSC is temperature labile (24).

The experiment with germ-free rats proved that the activities we have studied are of mammalian origin. The dependence of the reaction on ATP, Mg²⁺, glutamate, and NADPH was demonstrated; NADH could not replace NADPH. The mitochondrial preparations contained a trace of PSC reductase. The pH profile showed that the optimum pH was 7.0 with a steep drop of activity as the pH was lowered, while a more gradual loss is observed in the alkaline region. The small difference in the optimal pH for the coupled assay (now 6.5) may be attributed to the different incubation temperature (37 °C in contrast to 20 °C), absence of NP-40, or addition of NADH and the lactate dehydrogenase regeneration system for NADH. When the pH of the lysed mitochondria was raised to 9 for 10 min and then lowered to 7.4, the activity was lost, suggesting that the enzyme protein was unstable under these conditions. However, when the pH was lowered to 6 for 10 min and then raised to 7.4, 87% of the activity remained.

Preliminary apparent Kₘ values for the substrates indicate that this enzyme activity can function well in vivo in view of reported intramitochondrial concentration values for the substrates (44, 45). Substrate inhibition was observed for all substrates except glutamate. The lower Kₘ for glutamate in the direct assay may reflect a temperature effect for binding of a substrate or an effect on enzyme stability and/or conformation.

Although most of the buffers tested were equal in that the enzyme activity was almost the same from one buffer to another, phosphate buffer was inhibitory. The possibility that this may be due to product inhibition (Fig. 7) was not tested. The activity was not inhibited by the presence of NADH and ornithine (23, 31) from glutamate by cell-free homogenates of intestinal mucosa led us to develop conditions where PSC itself would accumulate. A difficulty in detecting PSC production was the high PSC reductase activity in this tissue (34) and the fact that the equilibrium for the reductase favors proline formation. Ornithine aminotransferase is also present; however, the equilibrium of this reaction is toward PSC (25). The first of these difficulties turned out to be avoidable by our discovery that the enzyme(s) converting glutamate to PSC had a different subcellular localization from PSC reductase. The assignment of the PSC-synthesizing activity to the mitochondria was made possible by developing a modified procedure of subcellular fractionation for intestinal mucosal cells (24).

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Smith et al. (48) could not find any P5C-synthesizing activity using either Hesper or Tris buffer in the absence of P. They implied that their inability to find saturation with glutamate had to be re-examined with a purer enzyme source. In contrast, we find reasonably similar glutamate saturation curves with either crude homogenates or the mitochondrial fraction. We would presume that the assay procedure in which o-aminobenzaldehyde was utilized to trap the P5C, the use of P, buffer, or, possibly, the use of proline as agent to inhibit further consumption of P5C during incubation may be the cause of the differences between their and our studies.

Since the P5C-synthesizing activity and ornithine aminotransferase (49) are both localized in mitochondria while P5C reductase is a cytosolic enzyme (50), the inhibitory effect of ornithine on P5C synthesis may play an important role in metabolic regulation of this enzyme activity in situ. Lodato et al. (51) have also found that ornithine inhibits P5C synthesis. Inhibition of this enzyme(s) by P5C is most easily explained as being due to product inhibition. The lack of inhibition by proline is in marked contrast to the strong inhibition of the γ-glutamyl kinase activities in E. coli (17) and P. aeruginosa (42) by proline. It is worth noting that proline was also a good inhibitor of prolinc synthesis from glutamate in a mammalian tissue culture system (9) because it inhibits P5C reductase.

The reported K_m values of ornithine aminotransferase in the direction toward ornithine synthesis were 25 mm for glutamate and 1.4 mm for dL-P5C (47). The K_m values for P5C reductase were reported to be 0.21 mm for dL-P5C and 0.25 mm for NADH (50). These values together with the favored direction (toward P5C) of ornithine aminotransferase and P5C reductase (toward proline) suggest that the mitochondrial localization of the P5C-synthesizing activity may be essential for ornithine aminotransferase to be able to convert P5C to ornithine before it diffuses to the cytosol to be trapped as proline in the irreversible P5C reductase reaction.
PROCEDURE

PREPARATION OF MITOCHONDRIA

Well-washed preparations of mitochondria of intestinal mucosa were obtained from rats of 250 to 265 g. Preparations were centrifuged at 15,000 × g for 5 min and the precipitate was used for the assay. If not used immediately, the preparations were resuspended in a physiological saline solution, and stored at 4°C.

RESULTS

The results are shown in Table I. A significant increase in P5C synthesis was observed in the presence of Glu-1 in the presence of NaF and Pi. The Glu-1 concentration required for half maximal stimulation was about 10 μM. The effect of Glu-1 was maximal at about 100 μM, and was not significantly increased at higher concentrations. The stimulation of P5C synthesis by Glu-1 was not affected by the presence of NaCl, NaHCO3, or KCl.

DISCUSSION

The results indicate that Glu-1 is a potent activator of P5C synthesis in intestinal mucosa. The mechanism of action of Glu-1 is not yet known, but it may involve an inhibition of the degradative pathway for P5C. Further studies are needed to elucidate the molecular mechanisms underlying the stimulation of P5C synthesis by Glu-1.

REFERENCES


Supplementary Material

Supplementary Material for "Proline-5-carboxylase Synthesis by Intestinal Mucosa" on page 3871.

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Pyrroline-5-carboxylate Synthesis by Intestinal Mucosa

Fig. 2. pH dependence of the PSC synthesising activity. Mitochondrial protein (2.08 mg) was incubated under the standard conditions except 5 mM ATP and 25 mM MgCl₂ were used. All buffers were 100 mM in the water. The pH of the incubation were measured at the middle of the incubation time. Buffers used were: Hepes, glycine, x. glycylglycine, glycyglycine-KOH; HEPES-KOH; Glycyglycine-KOH.

Fig. 3. Effect of ATP concentration on PSC synthesis. Mitochondrial protein (2.02 mg) was incubated under the standard condition except an equimolar mixture of ATP and MgCl₂ (as indicated) was added in addition to the basal 20 mM MgCl₂. The inset represents a double reciprocal plot.

Fig. 4. Effect of MgCl₂ concentration on PSC synthesis. Mitochondrial protein (2.17 mg) was incubated under the standard condition except the concentration of MgCl₂ was varied as indicated. ATP was 3 mM.

Fig. 5. Effect of NADPH concentration on PSC synthesis. Mitochondrial protein (2.19 mg) was incubated under the standard condition except the concentration of NADPH was varied as indicated. The inset represents a double reciprocal plot.

Fig. 6. Effect of glutamate concentration on PSC synthesis. Mitochondrial protein (2.77 mg) was incubated under the standard condition except the concentration of glutamate was varied as indicated. The inset represents a double reciprocal plot.
Pyrroline-5-carboxylate synthesis from glutamate by rat intestinal mucosa.
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