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The Fate of Arginine and Proline Carbon in Squid Tissues[1]

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ABSTRACT: The metabolism of proline and arginine was investigated in kidney, gill, and heart of the pelagic squid, *Symplectoteuthis*. The rates of CO_2 release from ^{14}C-proline exceeded the rates from ^{14}C-arginine. The metabolic rate of arginine and proline was assessed by monitoring the incorporation of arginine-derived carbon into various intermediates. Arginine was metabolized, through ornithine, to proline as well as to glutamate and various subsequent derivatives (alanine, octopine, aspartate, and carboxylic acids). The same components became labeled using ^{14}C-proline as the starting substrate, but only the gill was capable of converting proline to arginine via the urea cycle. In addition, ^{14}C-proline oxidation rates were high enough to exceed those of ^{14}C-glucose in at least three tissues, kidney, heart, and inner mantle muscle.

AT LEAST IN PART because of the large pool size of free amino acids in cephalopod muscles (e.g., see Hochachka, French, and Meredith 1978), interest recently has been focusing on their possible roles in energy metabolism. During metabolic studies on the 1979 *Alpha Helix* Cephalopod Expedition, relatively high rates of CO_2 release from arginine and proline were observed in tissues of the octopus, *Octopus macropus* (Hochachka and Fields, this issue), the squid, *Nototodarus* (Hochachka, unpublished data), and the chambered nautilus, *Nautilus pompilius* (Fields and Hochachka, this issue). In mammals and bacteria (Adams and Frank 1980), proline and arginine are metabolized through common intermediates, such as pyrroline-5-carboxylate, glutamate, and ornithine, so it was of interest to assess these potentials in cephalopod tissues. In this paper, we report data for heart, gill, and kidney from the squid, *Symplectoteuthis*, showing the capacity for arginine conversion to proline. The conversion of proline to arginine was measurable only in the gill. Although qualitatively similar to results obtained with other species, these data also show some important, tissue-specific differences (Mommsen et al. 1983; Mommsen and Hochachka 1981). Nevertheless, the available pathways of proline and arginine metabolism appear to be conserved among the cephalopod groups thus far examined, with main differences arising from the way arginine and proline metabolic potentials are expressed in different tissues.

MATERIALS AND METHODS

Animals and Chemicals

Pelagic squid of the species *Symplectoteuthis oualaniensis* were jigged at nightlights off the leeward side of Oahu, Hawaii, during the Cruise FIDO XVII of the R/V *Kana Keoki* in June 1980. Biochemicals were purchased from Sigma Chemical Co., St. Louis, Missouri; radiochemicals came from New England Nuclear, Boston, Massachusetts.

Tissue Slice Experiments

Heart, kidney, and gills from freshly killed animals were blotted dry of blood, cut into

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small blocks, and carefully sliced with razor blades into 0.5–1-mm-thick slices. The slices (about 100–200 mg) were taken up in 2.10 ml of medium A (0.35 M NaCl, 8 mM KCl, 31 mM $MgCl_2$, 8 mM $CaCl_2$, 16.2 mM $MgSO_4$, 20 mM Tris, adjusted to pH 7.50 at 23°C) and preincubated for 10 min with unlabeled substrate (final concentration 2.0 mM), using 25-ml flasks in a metabolic shaker. The flasks were capped, and 0.2 ml of medium A containing 0.5 μCi of ^{14}C-labeled substrate was injected through each cap. After 30 min at 23°C, the reaction was stopped by the injection of 0.25 ml trichloroacetic acid (20%), and the $^{14}CO_2$ was collected on glass fiber filters, suspended above the reaction medium, and soaked with 0.15 ml of phenylethylamine (Iverson, Bittaker, and Myers 1977). The flasks were shaken for an additional 90 min, and then the filters were removed and counted in toluene-based scintillation fluid.

Amino Acid Analysis

The contents of the flasks were homogenized with a Polytron tissue grinder, centrifuged, and the supernatant adjusted to pH 1.95 with crystalline lithium hydroxide. Two aliquots of the supernatant were separated on a Beckman 118C amino acid analyzer: the first run was reacted with ninhydrin; in the second run, the column eluate was collected in 2.0-ml samples and 1.0-ml aliquots were counted. Amino acid analyses of tissues were performed as above, after homogenizing the tissue in 10 vol ice-cold sulfosalicylic acid (3.75%, pH 1.95). Specific activities were calculated for the start of the experiments only. We showed that for heart, fin, and gill, the production of $^{14}CO_2$ from ^{14}C-labeled glucose, proline, and arginine under the above conditions was linear with time for at least 60 min.

RESULTS AND DISCUSSION

All three tissues under consideration produced significant amounts of $^{14}CO_2$ when supplied with either [U-^{14}C]proline or [U-^{14}C]arginine. The results presented in Table 1 indicate that arginine is metabolized at fast rates in both kidney and gill, with low rates of turnover in heart, which is consistent with the activity distribution of arginase in tissues of squid (Mommsen and Hochachka 1981) and *Octopus* (Gaston and Campbell 1966). Proline is metabolized at high rates, 5–6 times higher than arginine in both heart and kidney.

TABLE 1

CO_2 PRODUCTION FROM PROLINE AND ARGININE IN *Symplectoteuthis* TISSUES

TISSUE	[U-^{14}C]PROLINE	[U-^{14}C]ARGININE
Kidney	4.37 ± 0.65 (4)	0.71 ± 0.40 (4)
Heart	4.04 ± 0.77 (4)	0.075 ± 0.026 (4)
Gill	1.74 ± 0.53 (8)	0.55 ± 0.21 (4)

NOTE: Data expressed in micromoles of CO_2 produced from the respective L-amino acid per gram of fresh tissue in 1 hr at 23°C ± S. D. The number of experiments is given in parentheses.

TABLE 2

DISTRIBUTION OF LABEL FROM [U-^{14}C]ARGININE PRECURSOR IN TISSUE SLICES OF *Symplectoteuthis*

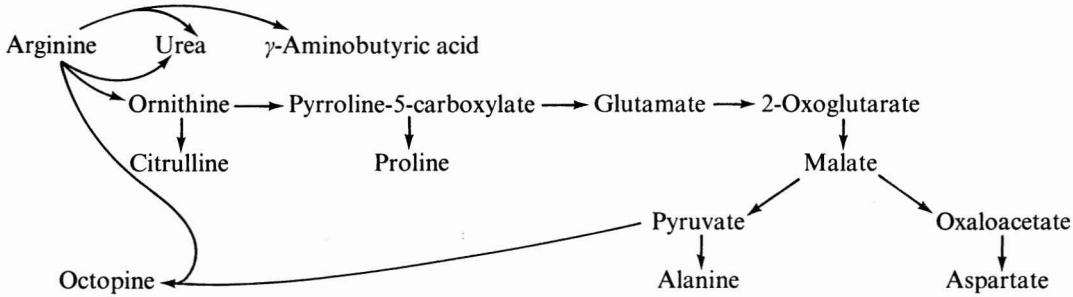
METABOLITE	GILL	HEART	KIDNEY
Carboxylic acids	4.3	4.9	3.9
Urea	0.84	0.22	3.9
Aspartate	0.4	0.4	0.7
Glutamate/Glutamine	1.2	1.2	4.1
Proline	4.2	0.5	1.7
Alanine	0.14	0.2	0.6
Citrulline	0.08	0.09	1.3
Octopine	0.6	1.0	0.8
γ-Aminobutyric acid	0.4	0.5	0.5
Ornithine	1.8	0.11	5.4
Arginine	85.9	93.0	76.6
Recovery	99.8	102	97.9

NOTE: Label recovered in the respective compound in percent of label (DPM) loaded onto the amino acid analyzer. For conditions of incubations see the text.

Arginine Metabolism

The percent distribution of radiolabel that was extractable from tissue slices after incubation with uniformly labeled ^{14}C-arginine is shown for gill, kidney, and heart in Table 2. ^{14}C-Arginine-derived carbon could be found in urea, ornithine, glutamate/glutamine, proline, citrulline, alanine, octopine, and aspartate. Furthermore, a small percentage of label

could be localized in γ-aminobutyric acid (GABA), indicating that at least a small portion of arginine is degraded by an L-amino acid oxidase rather than by arginase. The occurrence of this pathway and the identification of intermediates and enzymes involved have been described by van Thoai (1965) and Baret et al. (1965). The observed distribution of labeled carbon in the squid is consistent with the following reaction scheme, which is also thought to be operative in other cephalopods (Mommsen and Hochachka 1981), mammals, and bacteria (Adams and Frank 1980):



Although urease has been demonstrated in a variety of mollusks, mainly in terrestrial snails (Campbell and Speeg 1968; Florkin 1966; Hammen, Miller, and Geer 1966), we failed to detect any urease activity in tissues of the short-finned squid, *Illex illecebrosus*. Our data in Table 2 indicate that urease is also absent in the three tissues from *Symplectoteuthis*, but the definitive proof has yet to be supplied. It should also be emphasized that label can be distributed into octopine through two different pathways. In addition, occurrence of label in citrulline, particularly in the kidney and gill (see below), implies the presence of a functional urea cycle; in our experiments, the concurrent synthesis of arginine from endogenous precursors would lead to a drop in specific activity of [U-^{14}C]arginine, which we did not follow up in this paper.

Proline Metabolism

Using proline as the ^{14}C-labeled substrate, the same metabolites as already mentioned in Table 2 became labeled during incubation of squid tissue slices, with the exception of urea and γ-aminobutyric acid (Table 3). In the squid gill, the distribution of label is consistent with the scheme given below:

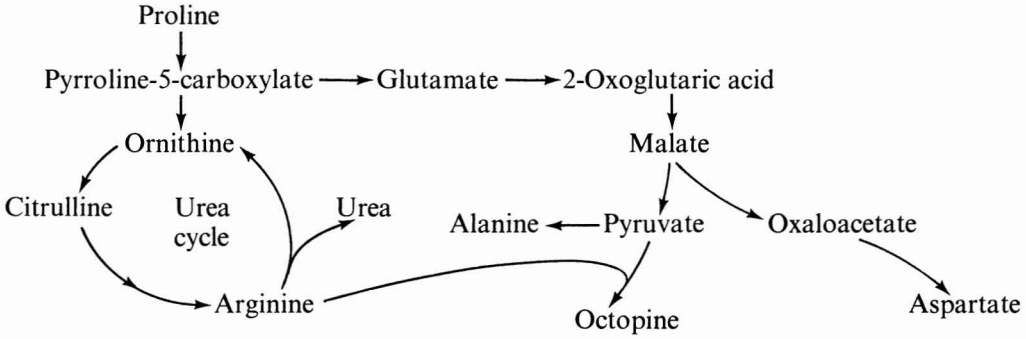


TABLE 3
DISTRIBUTION OF LABEL FROM [U-^{14}C]PROLINE PRECURSOR IN TISSUE SLICES OF *Symplectoteuthis*

METABOLITE	GILL	HEART	KIDNEY
Carboxylic acids	1.2	1.7	0.9
Aspartate	1.2	0.2	0.7
Glutamate/Glutamine	1.7	2.9	1.2
Proline	93.7	91.2	95.4
Alanine	0.4	0.3	0.25
Citrulline	0.05	0.02	0
Octopine	0.3	0.2	0.6
Ornithine	0.12	0.04	0.04
Arginine	0.51	0	0
Recovery	98.9	96.6	99.0

NOTE: Label recovered in the respective compound in percent of total label loaded (DPM) onto the amino acid analyzer. For conditions of incubations see the text.

Under our experimental conditions, gill was found to be the only organ to possess a fully operative urea cycle, but our failure to detect it in kidney may be due to the considerable dilution of label at the ornithine level in this tissue (Table 4), where the concentration of ornithine is more than an order of magnitude higher than in gill or heart. Physiologically, some of these reactions are the reverse of those in arginine metabolism, but they are drawn separately, because they are catalytically distinct and may be localized in separate compartments in the cell (Mommsen and Hochachka 1981, Phang, Yeh, and Hagedorn 1981). The fact that the tissue slices produce labeled alanine from both [U-^{14}C]arginine and [U-^{14}C]proline indicates that the three tissues possess the potential to truly oxidize these two amino acids and that at least some of the recovered $^{14}CO_2$ is liberated through malic enzyme (malate dehydrogenase: NADP-linked, decarboxylating, EC 1.1.1.40).

Carbon Dioxide Release from Proline as Compared to Glucose

The importance of the oxidation of amino acids—especially proline—for the overall energy production of the squid can be seen when comparing oxidation rates of proline with oxidation rates of uniformly labeled ^{14}C-glucose in a variety of squid tissues (Table 5). Generally, glucose seems to be the preferred substrate, but kidney and especially heart clearly prefer proline. The picture presented in Table 5 might change dramatically in favor of proline if the stationary concentration of the different metabolites is taken into consideration. Although cephalopods seem to accumulate large glycogen stores (Hochachka and Fields, this issue), the resting concentrations of free glucose (Mommsen, unpublished data) are lower than those generally found for proline, particularly in kidney, heart, and muscles (Table 4). Thus, the data in Table 5 may underestimate actual rates of proline oxidation.

Finally, an important specialization of mantle muscle into inner and outer oxidative bands plus a middle, more anaerobic band has been identified by us in at least five species of squid (Mommsen et al. 1981*b*) and by Bone, Pulsford, and Chubb (1981) in *Alloteuthis*. In *Symplectoteuthis*, this specialization is modified into inner oxidative and middle/outer anaerobic muscle bands (Mommsen et al. 1981). It is interesting to note that the ratio of CO_2 released from glucose/CO_2 released from proline is less than unity for the oxidative inner mantle, but is about 2.5 for the middle mantle. Tentacle muscle, which enzymatically is more similar to the middle mantle (Mommsen, unpublished data), displays a ratio of about 3 (Table 5). These results imply that not only does the oxidative potential vary between inner and outer mantle, but so also do substrate preferences for oxidative metabolism.

Implications

These experiments clearly establish the potential for metabolic functions for arginine and proline additional to those usually ascribed to them (see Hochachka and Fields, this issue). In the case of arginine, not only is it a potentially important source of metabolic CO_2 and waste nitrogen (as urea), it may also play a role in augmenting proline reserves, should they become depleted in times of prolonged performance. Because the pool size of proline is typically large, the appearance of ^{14}C-arginine-derived proline in tissues such as the heart is considered particularly significant.

TABLE 4

FREE AMINO ACIDS IN *Symplectoteuthis* TISSUES

AMINO ACID	GILL	HEART	KIDNEY	BRAIN	TENTACLE	MIDDLE MANTLE	INNER MANTLE	DIGESTIVE GLAND
His	0.32	0.84	0.20	0.31	0.55	6.95	4.98	0.15
Arg	3.56	1.87	2.27	4.35	16.80	37.40	28.84	1.56
Orn	0.17	0.13	2.22	0.94	0.23	0.83	0.73	0.14
Pro	1.60	5.95	15.96	7.35	10.10	14.70	18.88	1.93
Gly	2.12	1.14	3.00	3.58	9.20	14.61	12.74	1.35
Ala	3.82	23.28	11.26	7.71	7.83	11.66	13.27	4.23

NOTE: Data expressed in micromoles per gram of tissue. Amino acid analyses were performed on tissue pooled from four animals. Three independent heart samples from three or four pooled animals were analyzed, and the maximum standard deviation found was below 15% of the mean given in the table. No measurable amounts of pyrroline-5-carboxylate or citrulline could be detected in any of the amino acid analyses.

TABLE 5

CO_2 PRODUCTION FROM PROLINE AND GLUCOSE IN TISSUES OF THE SQUID *Symplectoteuthis oualaniensis*

TISSUE	PROLINE	GLUCOSE	GLUCOSE/PROLINE RATIO
Kidney	4.37 ± 0.65 (4)	2.10 ± 0.27 (4)	0.5
Heart	4.04 ± 0.77 (4)	0.70 ± 0.19 (4)	0.2
Gill	1.74 ± 0.53 (8)	2.82 ± 0.84 (4)	1.6
Digestive gland	0.092 ± 0.030 (4)	1.22 ± 0.23 (4)	13.3
Middle mantle	0.15 ± 0.06 (4)	0.36 ± 0.12 (4)	2.4
Inner mantle	0.41 ± 0.17 (4)	0.36 ± 0.19 (4)	0.9
Tentacle	0.19 ± 0.078 (4)	0.57 ± 0.023 (4)	3.0
Fin (center)	0.57 ± 0.25 (3)	0.81 ± 0.15 (3)	1.4
Brain	2.74 ± 0.90 (4)	8.43 ± 1.51 (4)	3.1

NOTE: Data expressed in micromoles of CO_2 produced from proline or glucose per gram of fresh tissue in 1 hr at 23°C ± S.D. The number of experiments is given in parentheses.

Although different fates of proline are not yet fully quantitated, the spread of label among various intermediates implies the potential for a complex metabolism leading mainly to alanine, octopine, and aspartate. Tentatively, we assume that the pathways for proline metabolism in these tissues are comparable to those observed in insects (Weeda et al. 1980), except for the complicating interactions with pathways of arginine metabolism.

The main questions that remain to be more closely analyzed are the following: (1) How do these various metabolic interactions change following arginine–phosphate depletion and octopine accumulation during anaerobic work? (2) How do the arginine–proline interactions change following exhaustive aerobic work, when proline reserves may be depleted? (3) How is proline carbon partitioned between complete oxidation and Krebs cycle augmentation? (4) How is waste nitrogen partitioned between urea and ammonia under different metabolic conditions? Further work is required before these problems can be put into clearer perspective.

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