Acta Medica Okayama

Volume 21, *Issue* 6 1967 *Article* 5

DECEMBER 1967

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Abstract

The compositions of nitrogen pools of ox liver, bladder bile, kidney and lung were analyzed with an especial bearing on their minor components, and some distinctive features of these tissues were described. DCEC and CMC were found in ox liver and kidney. Liver was low in free arginine and lysine, but high in ornithine, ethanolamine, and glutathione. Glycine was only a predominant amino acid in ox bile. All amino acids were contained moderately in kidney, but glutathione content was low. The concentrations of arginine and lysine were relatively high in lung.

*PMID: 4236511 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 21, 321—326 (1967)

STUDIES ON NITROGEN POOL OF ANIMAL TISSUES

III. OX LIVER AND BILE
IV. OX KIDNEY AND LUNG

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Received for publication, October 20, 1967

Continued from the first paper of this series[1], the nitrogen pools of ox liver, bile, kidney, and lung were analyzed with a special emphasis on their minor components.

Among cysteine conjugates[2] which have been found in urine, S-(1.2-dicarboxyethyi)-L-cysteine (DCEC)[3] and S-(carboxymethyl)-L-cysteine (CMC)[4] were found in liver and kidney.

MATERIALS AND METHODS

Ox liver (500 g), bile (500 ml), kidney (500 g), and lung (200 g) were obtained fresh from a slaughter house.

The ampholyte fractions of liver, kidney, and lung were prepared by the same method as described in the previous paper[1].

The ampholyte fractions of bile were prepared as follows. Five hundred ml of bladder bile was made weakly acidic with acetic acid and filtered. The filtrate was treated in a separatory funnel three times with the same volume of ether and then once with petroleum ether. The water layer was transferred on a column containing Diaon SK-1 (500 ml, H-form of strong cation exchanger, mesh 100), and the column was washed with 2 liters of water, 3 liters of 95% ethanol, and 3 liters of water in that order. During the ethanol washing, some white solid appeared in the effluent. The solid may be steroids and bile salts.

The Diaion SK-1 column was finally eluted with 3 liters of 2N-NH_3 and the ammonia eluate was evaporated to dryness under reduced pressure. The residue was dissolved in a proper volume of water and filtered. The ampholytes in the filtrate was divided into three Fractions (I, II, and III) on an Amberlite CG-4B column as described in the previous paper[1]. Fraction I contains mainly basic and neutral ampholytes, Fraction II acidic ampholytes, and Fraction III strong acidic ampholytes. Each fraction was analyzed on an automatic amino acid analyzer before and after hydrolysis.

If necessary, the hydrolysates of these fractions were again fractionated on Amberlite CG-4B column.

The Fraction IV of the bladder bile was prepared as follows. The effluent and washings (water and 95 % alcohol) from the above Diaion SK-1 column were combined and made weakly alkaline with dilute sodium hydroxide solution. The alkaline solution was concentrated to about one liter and filtered. Solid sodium hydroxide was added to the filtrate to make the final concentration about 10 %, and the solution was boiled with a reflux condenser on a sand bath for 24 hours. The hydrolysate was acidified with concentrated hydrochloric acid in a sparatory funnel and the precipitate appeared was removed with ethyl ether by occasionally shaking. The water layer was evaporated to dryness repeatedly to remove excess hydrochloric acid. The amino acids in the dried residue was collected by using Diaion SK-1 column. This is Fraction IV of bladder bile which contains N-covered ampholytes. The method of preparation of Fraction IV of the other tissues was described in the previous paper[1].

RESULTS

The amounts of usual amino acids and glutathione were summarized in Table 1. The values were expressed in μ moles per 100 g of wet weight or per 100 ml of bile. The values in parentheses were those obtained after hydrolysis.

I. Ox Liver

Liver contained S-(1.2-dicarboxyethyl) glutathione (DCEG)[5] and S-(1.2-dicarboxyethyl)-L-cystein (DCEC)[3] in Fraction III. The total amount (0.39 μ moles/100 g) was calculated as DCEC after hydrolysis of Fraction III. The hydrolysate of Fraction III was fractionated on Amberlite CG-4B column with 2M-acetic acid and then 2N-hydrochloric acid. DCEC was found only in the 2N-HCl eluate.

When the hydrolysate of Fraction II was fractionated on a long column containing Diaion SK-1 with 0.5N-hydrochloric acid by using a fraction collector, a small amount of S-(carboxymethyl)-L-cysteine (CMC)[4] appeared after aspartic acid. CMC was identified on paper electrophoresis and on amino acid analyzer by comparing with authentic CMC[4].

The other distinctive features of the nitrogen pool of ox liver were as follows. A negligible amount of arginine was found before hydrolysis and no remarkable increase of arginine was observed after hydrolysis. Ornithine content, however, was fairly high. This might be due to a strong arginase activity of liver. Citrulline was not detected either. Free lysine was not detected, but it appeared after hydrolysis. Histidine content was doubled after hydrolysis. Two unknown peptides were seen around carnosine and just after ammonia peak, the

Table 1

	Liver	Bile	Kidney	Lung
Aspartic Acid	103.81 (100.18)	1.56 (2.97)	89.75 (157.48)	84.82 (108.63)
Threonine	15.93 (49.63)	0.74 (1.72)	23.07 (67.21)	3.72 (13.84)
Serine	125.94 (53.00)	1.84 (2.46)	32.92 (68.80)	32.31 (28.84)
Glutamic Acid	387.60 (718.05)	10.32 (15.20)	361.50 (807.12)	311.03 (463.11)
Proline	44.32 (29.92)	1.10 (2.93)	29.88 (130.86)	27.97 (43.78)
Glycine	361.58 (779.20)	52.15 (79.37)	320.29 (735.42)	510.08 (739.65)
Alanine	211.68 (239.20)	4.49 (6.99)	78.69 (182.66)	96.47 (103.63)
Half Cystine	46.40 (242.40)	0.61 (0.76)	22.20 (140.74)	37.31 (104.03)
Valine	35.04 (52.80)	1.23 (2.74)	20.40 (39.44)	34.42 (36.00)
Methionine	+ (6.40)	0.73 (0.34)	+ (14.13)	+ (8.16)
Isoleucine	22.08 (37.60)	0.38 (1.27)	13.14 (34.18)	15.61 (19.35)
Leucine	36.40 (36.96)	1.61 (1.93)	25.92 (60.16)	36.60 (40.80)
Tyrosine	12.80 (9.92)	0.41 (0.58)	10.98 (28.04)	13.51 (12.40)
Phenylalanine	11.68 (9.60)	0.59 (0.86)	10.74 (29.04)	16.68 (17.50)
Histidine	36.16 (65.28)	0.25 (4.98)	12.54 (129.28)	10.09 (14.87)
Lysine	− (41.44)	0.46 (1.75)	18.73 (100.48)	30.14 (54.00)
Arginine	+ (7. 2)	+ (0.75)	10.97 (37.36)	20.07 (23.87)
Ornithine	52.64 (58.56)	0.38 (0.46)	10.38 (39.52)	+ (5.5)
γ-Aminobutyric Acid	+ (+)	− (−)	4.64 (14.96)	− (+)
Ethanolamine	157.19 (104.32)	2.63 (3.54)	31.48 (103.38)	47.87 (84.00)
Glutathione	193.42 (−)	− (−)	5.02 (−)	29.43 (−)

latter might be a lysine containing peptide. After hydrolysis of Fraction I, ß-alanine increased from 30.43 μ moles/100 g to 41.31 μ moles/100 g. γ-Aminobutyric acid was negligible before and after hydrolysis. The amount of carnosine-like peak (about 21 μ moles/100 gm) exceeded the increment of ß-alanine after hydrolysis. So the carnosine-like peak might not be a single peptide. Before and after hydrolysis, 1- and 3-methylhistidine were not found.

The concentrations of ethanolamine and glutathione were extremely high in liver. Several unknown peptides which disappeared after hydrolysis were seen as follows: several peptides which immigrate before aspartate in Fractions I, II, and III; one peptide before glysine in Fraction III; one peptide after glutamate in Fraction II; one peptide after serine in Fraction I.

α-Aminobutyric acid (8.5 μ moles/100 g) was seen in Fraction I and did not increase after hydrolysis.

Glutamate and glycine were prodominant amino acids in Fraction IV, and small amounts of aspartate, threonine, serine, proline, and alanine were also detected.

II. Ox Bladder Bile

The concentration of ampholytes in ox bladder bile was very low on the whole.

As in the case of liver, free arginine content was negligible. But unlike liver, ornithine content was not high. After hydrolysis of Fraction I, increments of arginine and ornithine were very low, but that of histidine was somewhat higher and of lysine was moderate. An unknown peptide which overlapped on the descending curve of lysine might be composed of lysine and histidine.

Carnosine was found in the order of 2.0 μ moles/100 ml. ß-Alanine concentration was 0.48 μ mole before hydrolysis, and increased to 2.45 μ moles after hydrolysis. γ-Aminobutyric acid was not detected before and after hydrolysis. 1-Methylhistidine was found in trace amount after hydrolysis.

Glutathione was not found in bladder bile, but it has been found occasionally in the fistula bile of some animals in this laboratory.

Many acidic peptides which appeared quite faster than aspartic acid, three peptides which appeared before and after glutamic acid, and one peptide which appeared after alanine, were seen in Fraction II. After hydrolysis of Fraction II, glycine, glutamin, cystine, threonine, and serine increased. The peptide which appeared after alanine might be cystinyl glycine.

Among amino acids in the bladder bile, glycine content was overwhelmingly high and followed by glutamic acid.

In Fraction IV, which was the hydrolysates of N-covered ampholytes, glycine content (41.7 μ moles/100 ml) was also predominant and the other amino acids were negligible. Taurine content was high only in Fraction I and was not found in Fraction IV. A greater part of taurine might be excreted in urine.

III. Ox Kidney

As reported already of guinea pig kidney[3], DCEC was found also in ox kidney in the order of 0.11 μ mole/100 g. CMC was also identified when the hydrolysate of Fraction II was fractionated on a long Diaion SK-1 column as described in the case of liver. Its amount was 0.19 μ mole/100 g.

Glutathione content of kidney was very low. This may be due to a strong glutathionase activity of kidney. A fairly large amount of carnosine (about 45 μ moles/100 g) was detected. The amount of ß-alanine was 8.64 μ moles/100 gm before hydrolysis and 85.20 μ moles/100 g after hydrolysis. γ-Aminobutyric acid increased about 10 μ moles/100 g after hydrolysis as shown in Table 1. Anserine-like small peak was seen and 1-methylhistidine was found only after hydrolysis in the order of 13.06 μ moles/100 g. A very small amount of 3-methylhistidine was detected in the same order before and after hydrolysis.

A small amount of hydroxylysine-like peak was found before hydrolysis and somewhat increased after hydrolysis.

Some unknown peptides were seen as follows: in Fraction I, after urea; in Fraction II, three peptides before aspartate and two peptides before and after glycine; in Fracsion III, several peptides before aspartate and two peptides before and after glutamate. One peptide in Fraction II which appeared after glycine in a fairly large amount might be cysteinylglycine. After hydrolysis of Fraction II, the increments of glycine and half cystine were almost in the same order (about 20 μ moles/100 g).

After hydrolysis of Fraction I, an unknown peak which had the same height as serine was seen just after serine.

In Fraction IV, glycine and glutamate were usually predominant in the other tissues, but aspartate, serine, threonine, and alanine were found in a fairly large amount in ox kidney.

IV. Ox Lung

DCEC and CMC were not detected in this tissue. The concentrations of free lysine, arginine, and glycine in lung were somewhat higher than in the other tissues. After hydrolysis of Fraction I, lysine and ethanolamine were increased remarkably, and an unknown peptide which immigrated before ornithine disappeared. The amounts of ß-alanine and γ-aminobutyric acid were in trace and not increased after hydrolysis, but hydroxylysine-like small peak appeared after hydrolysis. Carnosine was not seen and 1- and 3-methylhistidine were not detected before and after hydrolysis.

Glutathione content was moderate and some other unknown peptides were seen as follows: in Fraction II, four peptides before asparate and two peptides before and after glycine; in Fraction III, several peptides before aspartate.

A small amount of α-aminobutyric acid-like peak was seen in Fraction I before hydrolysis, and in Fraction II after hydrolysis.

In Fraction IV, aspartate, serine, threonine, glutamate, proline, glycine, and alanine were the major compounds.

In general, lung may be said to be a featureless tissue in the nitrogen pool.

DISCUSSION

DCEC and CMC have been found in animal urine[2], and they were found also in some ox tissues. Especially DCEC was distributed widely in the animal tissues. Studies on the physiological significance and the biosynthetic pathways of DCEC and CMC will be carried in future.

Although the following facts were not described in the results, a fairly large

amount of an unknown amino acid which overlapped on the ascending curve of aspartate was found in the hydrolysates of liver Fraction II and of bile Fraction I. Authentic erythro-ß-hydroxyglutamic acid just overlapped on aspartate but not on the unknown. Authentic threo-ß-hydroxyglutamic acid emerged faster than the unknown.

Some distinctive features of each tissue and fluid may be summarized as follows. Free lysine and arginine contents were low, and the contents of ornithine, ethanolamine, and glutathione were extremely high in liver. Some of these facts are suggestive of active urea cycle. The amino acid content of bile was very low, and glycine was a predominant constituent. The nitrogen pool of kidney contained all the amino acids moderately, but glutathione content was low. The latter may be due to a strong glutathionase activity of kidney. There are few reports on the nitrogen pool of lung. It was high in free arginine and lysine, and low in ornithine. In general, when histidine content was high, the contents of lysine and arginine were low and vice versa.

SUMMARY

The compositions of nitrogen pools of ox liver, bladder bile, kidney and lung were analyzed with an especial bearing on their minor components, and some distinctive features of these tissues were described.

DCEC and CMC were found in ox liver and kidney.

Liver was low in free arginine and lysine, but high in ornithine, ethanolamine, and glutathione.

Glycine was only a predominant amino acid in ox bile.

All amino acids were contained moderately in kidney, but glutathione content was low.

The concentrations of arginine and lysine were relatively high in lung.

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