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Abstract

Concentrations of ampholytes in the nitrogen pool of ox ocular tissues and nervous tissues were analyzed systematically by an automatic amino acid analyzer with a special reference to their minor components. DCEC was found in lens and also in nervous tissues. Ophthalmic acid was found in lens (highest), in retina (moderate), and in vitreous humor and spinal cord (trace). Glutathione content was extremely high in lens, and moderate in nervous tissues, retina and cornea. Carnosine content was moderate in cornea and in retina, but hemocarnosine may be rather high in nervous tissues. Anserine-like compound was found only in spinal cord, but free 1- and 3-methylhistidine were detected in most ocular tissues. Ethanolamine and γ-aminobutyric acid were high in retina and their concentrations were comparable to those of nervous tissues.

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STUDIES ON NITROGEN POOL OF ANIMAL TISSUES

I. OX OCULAR TISSUES

II. OX NERVOUS TISSUES

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Several papers have been presented on the free amino acid composition of animal tissues. Their analytical methods, however, differ from each other and there are very few descriptions on the unusual components.

In this laboratory, several cysteine conjugates have been found in the acidic amino acid fraction of animal urine as the 'minor components'. So the author has analyzed the nitrogen pool of some animal tissues with special emphasis cn their minor components.

This paper will report on the free amino acids and peptides of ox ocular tissues and nervous tissues.

MATERIALS AND METHODS

Thirty ox eye balls and 500 g each of ox brain and spinal cord were obtained fresh from a slaughter house. The eye-balls were divided into five parts. The wet weight of each ocular tissue collected was as follows: cornea, 24 g; aqueous humor, 42 ml; lens, 65g; vitreous body and humor, 480 g; retina, 15.5 g.

Except aqueous humor, each tissue was homogenized with two volumes of water, and the homogenate was deproteinized with trichloroacetic acid (TCA, final concentration: 8 %). The supernatant were collected by centrifugation. The precipitate was washed twice with the two volumes of 5 % TCA. The combined supernatant and washings were treated twice with ethyl ether in a separatory funnel to remove TCA. The water layer was concentrated to a proper volume under reduced pressure below 40°C. The pH of the concentrated solution was adjusted to weakly acidic and filtered. The filtrate was transferred on a column containing Diaion SK-1 (H-form of sulfonated cation exchanger, Mitsubishi Kasei Co. Ltd., Tokyo; mesh 100), and the column was washed with deionized water.

The effluent and washings containing N-covered ampholytes were combined and evaporated to dryness. The dried residue was hydrolyzed in 6N-HCl. The

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free amino acids in the hydrolysate (Fraction IV) were collected again by using Diaion SK-1 column. This fraction IV was composed mainly of glutamic acid and glycine.

The N-free ampholytes absorbed on Diaion SK column were eluted with 2N-ammonia. The ammonia eluate was dried under vacuum. The residue was dissolved in a proper volume of water and the solution was made weakly acidic with acetic acid and filtered. The filtrate was transterred on a column containing Amberlite CG-4B (acetate form, mesh 100—200), and the column washed with 0.2 M-acetic acid (volume ratio of resin : 0.2M-AcOH = 1 : 10). The effluent and washing were combined and dried under vacuum. The residue contains mainly basic and neutral ampholytes (Fraction I). The Amberlite column was then eluted with 10 volumes of 2M-acetic acid and the eluate was dried under vacuum. The residue contains mainly acidic ampholytes (Fraction II). The Amberlite column was further eluted with 5 volumes of 2N-hydrochloric acid and the eluate was dried under vacuum. The evaporation was repeated several times by adding water to remove hydrochloric acid. The residue contains mainly strong acidic ampholytes (Fraction III).

These fractions so obtained were analyzed on an automatic amino acid analyzer (Beckman Model 120-B) by the usual methods. Fraction I was analyzed on the column of 150 cm at 50°C for neutral ampholytes and of 50 cm column at 30°—50°C for basic ampholytes. Fractions II and III were analyzed on 150 cm column at 30°C.

If necessary, these fractions were hydrolyzed and the hydrolysates were again fractionated on Amberlite CG-4B column as described above.

RESULTS

The composition of free ampholytes before hydrolysis in ox tissues tested was summarized in Table I. The values were expressed in terms of μ moles per 100 g of wet weight or per 100 ml in the case of aqueous humor.

In general, a small amount of creatinine and most of taurine were contained in Fraction I of all tissues, and not negligible amount of cysteic acid was detected in Fractions II and III, but their amounts were not calculated.

I. Ocular Tissues

Some distinctive features of the ocular tissues were as follows.

Cornea : Carnosine concentration (around 4.02 μ moles/100 g) was the highest among ocular tissues and glutathione content was moderate. Free α-aminobutyric acid was found in the order of 2.2 μ moles/100 g, but ophthalmic acid was not detected. 1- and 3-methylhistidine were found in a small amount, but anserine was not detected. The concentrations of serine and valine were

Table 1

	Ocular Tissues					Nervous Tissues	
	Cornea	Aqueous Humor	Lens	Vitreous Humor	Retina	Brain	Spinal Cord
Aspartic Acid	3.77	0.38	1.46	1.76	28.15	225.27	95.56
Threonine	13.02	10.84	9.82	6.73	11.3	2.29	63.33
Serine	46.85	45.46	23.4	12.7	115.5	17.68	65.2
Glutamic Acid	56.35	48.36	113.86	9.42	350.2	747.65	324.33
Proline	9.85	6.10	9.48	0.34	12.05	0.87	2.60
Glycine	34.04	10.98	49.22	2.71	113.75	12.30	147.8
Alanine	51.35	68.22	56.24	8.14	64.10	12.0	47.1
Half Cystine	±	±	0.68	trace	11.30	23.01	10.42
Valinne	26.35	51.82	15.42	10.3	6.3	1.28	trace
Methionine	±	±	±	0.08	±	±	±
Isoleucine	9.85	17.90	7.40	4.30	8.65	0.61	3.8
Leucine	15.90	26.18	10.82	0.42	13.5	1.18	6.8
Tyrosine	7.65	13.90	7.24	3.40	5.65	0.36	3.46
Phenylanine	7.75	14.32	7.42	2.88	6.20	0.49	3.16
Histidine	7.6	13.46	3.48	0.70	8.38	1.05	2.62
Lysine	16.2	14.66	4.92	4.18	0.25	7.80	12.04
Arginine	15.1	15.46	5.50	4.10	7.45	5.80	3.75
Ornithine	3.7	8.48	2.40	1.97	1.80	2.81	2.09
γ-Aminobutyric Acid	0.7	0.20	trace	0.52	148.0	115.2	28.4
Ethanolamine	4.4	0.68	0.94	1.28	11.47	21.30	39.97
Glutathione	6.48	±	209.58	0.25	15.65	21.78	26.62

relatively high compared with those of the other amino acids.

Aqueous Humor : Relatively high concentration of serine and valine was also found in this humor. Ornithine concentration was the highest in the humor among occlar tissues. Two distinct peaks of methionine sulfoxide was found only in this part of eye, but free methionine was negligible. Almost the same amount of 1- and 3-methylhistidine was found in the order of around 1.5 μ moles per 100 ml, but anserine was not found and carnosine was in trace. Free α-aminobutyric acid was found in the Fraction I in the order of 2.1 μ moles per 100 ml, and ophthalmic acid and glutathione were found in a trace amount.

Lens : Lens has been known to contain S-(1, 2-dicarboxyethyl) gluathione (DCEG) and S-(1, 2-dicarboxyethyl)-L-cysteine (DCEC)[2], and they were found only in the Fraction III. The total amount of these conjugates (3.55 μ moles/100 g) was calculated as DCEC after hydrolysis of Fraction III. Some additional experiments revealed that ox lens contained 3.2—6.1 μ moles of DCEC/100 g after hydrolysis.

Extremely high concentration of glutathione was seen in Fractions II and III. Ophthalmic acid was found in a large amount in the Fraction II and in a small amount in Fraction III. The total amount of ophthalmic acid (49.83 μ moles/100 g) was calculated as α-aminobutyric acid after hydrolysis of Fractions II and III. A very small amount of free α-aminobutyric acid was detected in Fraction I.

Norophthalmic acid has been found in lens[3], but it was not detected in this experiment. Norophthalmic acid should be contained in Fraction II, but no remarkable increase of alanine was observed after hydrolysis of Fraction II.

Carnosine was found in the order of 2.2 μ moles per 100 g, and 1- and 3-methylhistidine were detected in trace before hydrolysis of Fraction I. After hydrolysis, ß-alanine increased, but 1- and 3-methylhistidine did not. A very small amount of hydroxylysine was detected after hydrolysis of Fraction I.

Vitreous Body and Humor : Vitreous body was homogenized together with humor. The concentration of ampholytes was fairly low compared with the other ocular tissues. But glutathione, ophthalmic acid, carnosine, α-aminobutyric acid, and 1- and 3-methylhistidine were all detected in a very small amount. Some unknown acidic peptides were seen in Fraction II.

Retina : Retina was collected by scratching the inner surface of eye-ball.

Extremely high concentration of γ-aminobutyric acid (GABA) was fonnd in retina. Its concentration was comparable to that of brain. The concentrations of ethanolamine and serine were the highest among ocular tissues. But cystathionine content was not attractive in this tissue.

Next to lens, the concentration of glutathione and ophthalmic acid (2.9 μ moles/100 g) were high in retina. A very small amount of hydroxylysine was detected after hydrolysis of Fraction I. Carnosine was in trace and 1- and 3-methylhistidine were not detected. ß-Alanine did not increase after hydrolysis of Fraction I.

II. Ox Nervous Tissues

Nervous tissues have been known to be quite high in glutamate metabolism, and the contents of glutamate and γ-aminobutyric acid were quite high as shown in Table 1. High ethanolamine content was also understandable because of the high phospholipid content of these tissues.

The concentrations of branched and aromatic amino acids were relatively low in nervous tissues compared with the other tissues.

Cystathionine content has been known to be quite high in primate nervous tissues, but it was low in ox : brain, 0.68 μ moles/100 g ; spinal cord, 15.18 μ moles/100 g.

Glutathione content was moderately high in these tissues, and carnosine-like

compound was found as follows: brain 8.2 μ moles/100 g; spinal cord, 11.76 μ moles/100 g. After hydrolysis, however, the increment of γ-aminobutyric acid was much higher than ß-alanine. So homocarnosine[8] content may be much higher than carnosine in nervous tissues.

Anserine-like compound was found only in spinal cord, but 1-methylhistidine was detected in a small amount both in brain and spinal cord after hydrolysis of Fraction I.

When Fraction III was hydrolyzed and fractionated on Amberlite column, DCEC was found as follows: brain 0.28 μ moles/100 gm; spinal cord, 0.21 μ moles/100 g.

DISCUSSION

Detailed analyses on the nitrogen pool of lens have been done by WALEY *et al*[2,3,4,5], but there are few systematic and accurate analyses of all ocular tissues. This paper will serve as useful perspectives on the nitrogen metabolism of ocular tissues.

Although there are many papers on the amino acid composition of nervous tissues, this is the first roport for the presence of DCEC in brain and spinal cord. DCEC has also been found in urine and kidney[6], but its concentration was the highest in lens, and DCEG and DCEC were found only in lens among ocular tissues. The concentrations of ophthalmic acid and glutathione were also extremely high in lens. So these peptides and DCEC might have a significant meaning in the physiology of lens. Ophthalmic acid was at first found in lens[4], but it was also contained fairly in a large amount in retina, a small amount in vitreous humor and spinal cord. Norophthalmic acid has been found in lens[2,5], but it was not detected in this experiment.

Carnosine has been found in all ocular[7] and nervous tissues[8]. Homocarnosine[8] might be rather high in nervous tissues, because the increment of γ-aminobutyric acid was much higher than that of β-alanine after hydrolysis of Fraction I.

Anserine was not detected, but a small amount of free 1- and 3-methylhistidine was detected in most ocular tissues Only 1-methylhistidine was detected in a small amount in nervous tissues after hydrolysis of Fraction I.

Retina was very similar to nervous tissues in the high contents of γ-aminobutyric acid and ethanolamine but not similar in cystathionine content.

Hydroxylysine phosphate has been found in lens[5], and a very small amount of hydroxylysine was seen in the hydrolysate of Fraction I in lens and also in retina.

Amino acid pattern of aqueous humor was quite similar to that of cornea in the high contents of serine, valine, lysine and arginine.

SUMMARY

Concentrations of ampholytes in the nitrogen pool of ox ocular tissues and nervous tissues were analyzed systematically by an automatic amino acid analyzer with a special reference to their minor components.

DCEC was found in lens and also in nervous tissues.

Ophthalmic acid was found in lens (highest), in retina (moderate), and in vitreous humor and spinal cord (trace).

Glutathione content was extremely high in lens, and moderate in nervous tissues, retina and cornea.

Carnosine content was moderate in cornea and in retina, but hemocarnosine may be rather high in nervous tissues.

Anserine-like compound was found only in spinal cord, but free 1- and 3-methylhistidine were detected in most ocular tissues.

Ethanolamine and γ-aminobutyric acid were high in retina and their concentrations were comparable to those of nervous tissues.

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