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오리고기중의 Adenosine Triphosphate Creatine Phosphotranseferase에 관한 연구(Ⅰ)

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초 록

오리를 시중에서 구입하여 도살하고 breast muscle을 취하여 Creatine Kinase를 추출하여 specific activity를 결정하여 76.32 units/㎎을 얻었으며, Thiol group은 6.2mol/M of enzyme을 갖고 있음을 알았다. 한편 Amino acid는 모든 필수아미노산이 함유되어 있음을 알았다.

The creatine kinase(ATP-creatine phosphotranseferase, E.C.2.7.3.2) catalyse the reaction:

Creation+ATP⇄Creatine phosphate+ADP+H^+ in the presence of divalent cations such as Mg^{2+} and Mn^{2+}. Creatine kinase is known to be intimately involved in normal muscle contraction and probably occurs in close physical association with the contractile elements. Serum creatine kinase activities are generally greatly elevated in the progressive degeneration of the skeletal muscles. The elevated enzyme activities are valuable for the diagnostic purpose(Ebashi, Toykura, Momoi & Sugita[1], 1959; Walton & Gardner-Medwin, 1969)[2].

The enzyme occures in many veterbrate tissue and muscle(Dawson & Fine, 1967)[3]. A number of investigations for creatine kinase have been made on the enzyme from various tissues of the rabbit(Simonarson, 1971)[4], but a few findings have been made on the frog, turtle, dogfish, chicks and Human beings(Thomson, Eveleigh & Miles, 1964[5], 1968; keto & Doherty, 1968[6]; Simonarson & Watts[7], 1972; Atherton, Laws & Thomson[8], 1970; Moreland, Kumudavalli & Watts, 1970)[9]. The enzyme contains 10 to 20% in the soluble sarcoplasm protein of the muscle(Kudy, Noda & Lardy[10], 1954; Czok & Buecher[11], 1960; Warren 1973)[12].

It is well known that creatine is associated with malnutrition and disintegration of muscular tissue and in fever. Therefore, large amounts are found in diseases of the muscles.

It is known that the enzyme has molecular weight of approximate from 80,000 to 86,000(Kuby, Nods & Lardy[10], 1954; Olson & Kuby[13], 1964; Eppenberger, Dawson &

Kaplan[14], 1967; Watts, Kumudavalli & Morelad[9], 1970; Park, 1980)[15], and consist of two very similar subunits(Dance & Watts[16], 1962; Park, 1980) each with one highly reactive and essential thiol group(Mahowald & Kudy, 1972[17], Watts, Rabin & Crook, 1961[18]; Eppenberger, 1967)[14].

Materials and Methods

All ducks were male and at least 2 months old. ATP(disodium salt) and ADP (trisodium salt) were obtained from Sigma Chemical Co. St. Louise, Mo, U.S.A.. Creatine, Tris-HCl buffer and DEAE-Cellulose DE-52 were obtained from Fisher Co. U.S.A.. Enzyme grade Guanidine Hydrochloride, 5,5′-dithiobis-(2-nitrobenzoic acid) and Dithioerythritol were obtained from Sigma Chemical Co. St. Louise, Mo, U.S.A.. All other chemical reagents were of analytical or equivalent grade or the purest grade obtainable.

1) Purification of the Enzyme.

Creatine kinase was prepared from Duck muscle by a procedure essentially the same as that of Eppenberger et al(1967). The procedure used for the purification of Duck muscle creatine kinase is summarized in the following Fig. 1.

Step 1. Duck muscle homogenate dilute 1:3 or 1:4(w/v) with 10m M-KCl, pH adjusted to 8.5. The KCl extract treated with ethanol(2 volums); stirred at 20℃ for 2 1/2h, then centrifuged at 15,000 gr for 10 min. and supernatant retained.

Step 2. 2M-$MgSO_4$ added to the suppernatant. Precipitation completed in 1/2 hr. and centrifuged at 15,000 gr for 30 minutes.

Step 3. Sediment extracted with 70mM-Magnesium acetate equal to 10% of the volume of initial extract. Dialysed exhaustively against the equilibrating buffer(5mM-tris-HCl buffer, pH 7.7, containing di-thioerythritol(80 *mg*/1).

Step 4. DEAE-cellulose chromatography, gradient 0.005—0.25M NaCl in 5mM-tris-HCl buffer, pH 7.7, containing dithioerythritol(80 *mg*/1). Elutes monitored for enzyme activity. This elute was monitored continuously at 254 nm.

Step 5. Enzymically active fraction pooled and precipitated with $(NH_4)_2SO_4$ (70% saturation). Dialysed against 10 mM-tris-HCl buffer, pH 7.7 containing dithioerythritol(80*mg*/1).

Stock purified enzyme stored at 2℃ in above solution.

Fig. 1. Purification of Duck breast muscle creatine kinases. Extractions were carried out at 2℃. Ducks were killed by cervical sectien and breast muscles were immediately frozen at −15℃. These were thawed put when required by keeping overnight in the cold-room at 4℃.

The presence of dithioerythritol was required to maintain enzyme activity. On occasions 2-mercaptoethanol was used instead.

2) Enzyme assay(Phosphate method).

The enzyme was assayed by the phosphate method of Kuby et al(1954) by following the conversion of creatine into creatine phosphate.

The standard assay mixture contained(final concentration) creatine(80 mM), magnesium acetate(0.1 M) and glycine(0.4M), and after addition of ATP(5mM) the reaction mixture was equilibrated at 30℃. The diluted enzyme was then added to start the reaction. Adenosine triphosphatase activity was determined by incubation with ATP in the absence of creatine.

$$\text{Sample creatine} \xrightarrow[\text{CPK, } 30℃]{CH_3COOMg} \text{Creatine phosphate}$$

Specific activities are expressed as units of enzyme activity per *mg*. of protein, the unit being as defined by Kuby et al(1954). The phosphate liberated was then measured colorimetrically at 660 nm.

3) Determination of the thiol group.

Thiol groups were determined by reaction with 5,5′-dithiobis(2-nitrobenzoic acid) in 10 mM-tris-HCl buffer, pH 8.0 both in the presence and in the absence of guanidine hydrochloride(6M). Number of thiol groups titrated by 5,5′-dithiobis-(2-nitrobenzoic acid) related to activity loss. When the reaction had gone to completion, as judged by following $E_4$12, a sample was assayed for residul enzyme activity. To prevent oxidation of thiol groups during dialysis enzyme solutions were dialyed against 10 mM-tris-HCl buffer, pH 8.0, containing dithioerythritol(1 *mg*/1), the diffusate being used as the reference solution in the spectromphotometer.

4) Amino acid ananysis.

Enzyme solutions were thoroughly dialysed against distilled water, pH 9.0, and portions were then frozen, dried and hydrolysed with 6.5M-HCl for 24 hrs. in vacuo at

105℃. After rapid removal of HCl in vacuo at approx. 30℃, samples for analysis were dissolved in 0.2M-sodium citrate buffer, pH 2.0. Amino acid compositions were determined with an automatic amino acid analyser, which operation conditions are as follows.

Table 1. Amino acid analysis conditions for operation

Sample number	Neutral & acidic amino acid		Basic amino acid	
	STD	sample	STD	sample
Injected sample size	0.25㎖	1.0㎖	0.25㎖	1.0㎖
Column size	0.6×133㎝		0.6×133㎝	
Resine	Dowe×108		Dowe×108	
Flow rate				
buffer	30㎖/hr		30㎖/hr	
color reagent	40㎖/hr		40㎖/hr	
Column Temp.	60℃		60℃	
Buffer soln.	Citrate		Citrate	

Results and Discussion

Details of typical preparations of creatine kinase from normal duck breast muscle are given in Table. 2,3. and Fig 1.

1)Creatine kinase

The specific activity of the Human enzyme, assayed by the procedure of Kuby et al. was about 25 units after the second electrophoresis purification followed by a final dialysis against 1 mM-dithioerythritol, pH 8.5. The monkey enzyme and a specific activity of 89 units, and the rabbit enzyme had 75 units. (Mahowald, Noltmann & Kuby, 1962). The dogfish enzyme had a specific activity of 13.1 units(Simonarson & Watts,

Table 2. Purification of creatine kinase from duck breast muscle

	Volume (㎖)	T-protein (㎎)	T-activity (units)	Specific activity (units/㎎)	Recovery (%)
Crude Extract	2500	52500	275500	5.24	100
Mg-acetate extract	380	2850	105000	36.84	38
Pooled column fraction	30	950	72500	76.32	25

* The values are corrected to 1 ㎏ wet weight of muscle.

1972) and the snake enzyme specific activity is 16 units(Park, 1980). The enzyme of the chicken have a specific activity of 84 and 70 units(Roy, Law & Thomson, 1970). The duck breast muscle creatine kinase has a specific activity of 76.32 units through this experiment. According to Hooton & Watts(1966), mouse enzyme had a specific activity of 204 units.

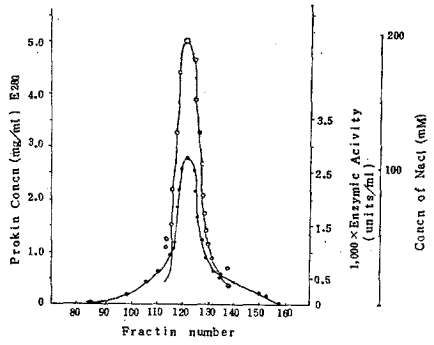

Fig 2. Chromatogramy of creatine kinase from duck breast muscle on DEAE-ellulose. 10 ml fractions were collected.

2) Thiol groups.

In enzymse from duck breast muscle approx. 2 thiol groups/molecule reacted in the absence of denaturant Table 3.

Table 3. Thiol groups in creatine kinase from duck breast muscle.

	Total thiol groups (mol/M. of enzyme)
Duck enzyme	6.2
Chicken (1)	4.8 — 7.11
Mouse (2)	5.5 — 6.8
Rabbit (3)	6.3 — 6.5
Human (4)	6.66

1. Roy, Law & Thomson (1970)
2. Hooton & Watts (1966)[19]
3. Mahowald, Noltman & Kuby (1962)
4. Jecobs, Okabe, Yue, Deutel, Zeiter, Palmchi & Kuby (1969)[20]

3) Amino acid compositions

The methods used are described in the text and the values found are compared with those from dogfish(Simonarson & Watts), Rabbit(Noltman et al.) and Chick (Eppenberger). The results are as following Table 4.

Table 4. Amino acid compositions of Duck meat breast muscle creatine kinase. The numbers of residues/mol have been corrected to nearest integral number.

Amino acid	Rabbit	Dogfish	Chick	Duck*
Amino acid	83	83	73	80
Threonine	34	33	29	30
Serine	41	32	32	30
Glutamic acid	74	73	83	75
Proline	37	36	41	39
Glycine	62	63	60	65
Alnanine	25	30	33	35
Valine	53	48	49	50
Methionine	19	20	20	23
Isoleucine	25	32	24	30
Leucine	70	70	71	70
Tyrosine	19	19	16	17
Phenylalanine	30	26	33	29
Histidine	33	36	34	38
Lysine	65	61	65	63
Arginine	34	38	40	35

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Studies on Adenosine Triphosphate Creatine Phospho-transeferase from Muscle of the Duck (Oriental Korean)

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>***Abstract***<

A relatively simple procedure was devised for the purification of Adenosine Triphosphate Creatine Phosphotranseferase(E. C. 2. 7. 3. 2.) from duck breast muscle extracts. The enzyme was purified and yield of close to 25%. The specific enzyme activity, thiol group and amino acids were determined.

The results are as follows:

1. Adenosine Triphosphate Creatine Phosphotranseferase from duck breast muscle extract has a specific activity of 76.32 units/*mg*.

2. The thiol group of the duck breast muscle creatine kinase was 6.2 mol/M. of enzyme.

3. All most essential amino acids are contained in this enzyme.