Adenosine 5'-Triphosphate-Arginine Phosphotransferase from Lobster Muscle

AMINO ACID COMPOSITION

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1. The amino acid composition of lobster-muscle arginine kinase is given and discussed briefly. 2. The results were used to calculate a partial specific volume of 0.735. 3. The results are compared with the amino acid composition of creatine kinase.

Arginine kinase (EC 2.7.3.3) from *Homarus vulgaris* (lobster) muscle, which catalyses the reaction $\text{ATP}^{4-} + \text{arginine}^+ \rightleftharpoons \text{ADP}^{3-} + \text{phospho-arginine}^- + \text{H}^+$, has been obtained in a high state

of purity by Virden, Watts & Baldwin (1965) and its molecular weight has been determined (Virden, Watts, Watts, Gammack & Raper, 1966). In this paper we report the amino acid composition of

Table 1. Amino acid composition of arginine kinase

For most amino acids the estimate was the mean of two or three determinations for hydrolysis times of 22, 42 and 71 hr. with the native enzyme. For serine and threonine the values were obtained by extrapolation to zero time. The estimate for valine was based on 42 and 71 hr. values; that for isoleucine was based on the 71 hr. values. Estimates for proline, carboxymethylcysteine and half-cystine were obtained from analyses of the alkylated enzyme. Tryptophan was estimated from the tyrosine/tryptophan ratio (Virden *et al.* 1965). The deviations given are the average values of absolute deviations from the mean.

	Estimate from 22, 42 and 71 hr. hydrolyses 10 × (μmoles/0.395 mg. of protein)	Amino acid residues (μg.)	No. of residues/ 37 000 g. of protein	Nearest whole number of residues
Aspartate	3.113 ± 0.112	35.80	31.29	31
Threonine	1.830	18.48	18.40	18
Serine	1.830	15.92	18.40	18
Glutamate	3.851 ± 0.120	49.67	38.71	39
Proline	1.265 ± 0.048	11.26	12.71	13
Glycine	2.673 ± 0.086	15.24	26.87	27
Alanine	2.346 ± 0.105	16.66	23.58	24
Valine	1.801 ± 0.012	17.83	18.10	18
Cystine (half)	0.023 ± 0.004	0.23	0.23	0
Methionine	0.747 ± 0.016	9.79	7.51	8
Isoleucine	1.721 ± 0.002	19.45	17.30	17
Leucine	3.095 ± 0.070	34.97	31.11	31
Tyrosine	1.014 ± 0.013	16.53	10.19	10
Phenylalanine	1.778 ± 0.013	26.14	17.87	18
Ammonia	2.141 ± 0.198	— 0.15*	15.32*	15*
Lysine	2.805 ± 0.040	35.90	28.19	28
Histidine	0.820 ± 0.012	11.23	8.24	8
Arginine	1.560 ± 0.041	24.34	15.68	16
Tryptophan	0.195	3.63	1.96	2
Carboxymethylcysteine	0.506 ± 0.010	5.16	5.09	5
Total		368.06		
		(93.9% recovery)		

* Amide residues, corrected for hydrolysis of tryptophan, threonine and serine.

arginine kinase as a further step in the characterization of this enzyme.

MATERIALS AND METHODS

Arginine kinase was prepared from lobster muscle by the procedure of Virden *et al.* (1965) and accorded with their criteria of purity. Two different preparations were used. Protein concentration was determined by the biuret procedure (Gornall, Bardawill & David, 1949), calibrated with a nitrogen determination of arginine kinase by the micro-Kjeldahl procedure assuming a 16.7% nitrogen content. The nitrogen content is based on the amino acid composition assuming 100% recovery of nitrogen.

The analysis was carried out by the modified method of Moore, Spackman & Stein (1958), by using a Technicon Autoanalyzer with either the standard 20 hr. run or with the modified procedure of Hacket, Mathias & Pennington (1965), which yields a complete amino acid analysis of a protein hydrolysate in 10 hr. The enzyme was prepared for hydrolysis either by exhaustive dialysis against distilled water or by precipitation with 0.1 N-HCl (AnalaR, redistilled) and thorough washing of the precipitate with distilled water.

The latter procedure was used because arginine kinase treated with iodoacetamide was precipitated upon dialysis. Alkylation of arginine kinase by iodoacetamide was carried out, and was monitored with a silver-silver iodide electrode, as described by Virden & Watts (1966). The composition of the reaction mixtures is given in Table 2. Hydrolysis of the freeze-dried protein was carried out for 22, 42 or 71 hr. in evacuated sealed ampoules at 110° in the presence of constant-boiling HCl. After hydrolysis the HCl was removed by rotary film evaporation and the residue dissolved in 0.1 N-HCl. Samples were applied to the column together with 0.1 μ mole of norleucine as internal standard.

RESULTS AND DISCUSSION

The results are summarized in Table 1. The sum of the weights of the amino acid residues corresponds to 93.9% recovery. The amino acids that were determined with lower degrees of precision were proline, glycine, glutamic acid, aspartic acid and alanine, with average deviations of 3–5%, and amide nitrogen 9%. Comparison with standards

Table 2. Comparison of amino acid compositions of native and alkylated arginine kinase

The native enzyme was exhaustively dialysed against distilled water. For alkylation of the denatured enzyme the reaction mixture contained: *p*-phenolsulphonate buffer, pH 8.22 and *I* 0.05; KI, 1 μ M; sodium lauryl sulphate, 1% (w/v); iodoacetamide, 1 mM; arginine kinase, 8.2 mg.; total vol., 4 ml. For partial alkylation the reaction mixture contained: *p*-phenolsulphonate buffer, pH 8.0 and *I* 0.025; KI, 1 μ M; Tween 80, 0.02% (v/v); iodoacetamide, 4 mM; arginine kinase, 2 mg.; total vol., 8 ml. Alkylation was followed by measuring I^- ion liberated in the reaction with a silver-silver iodide electrode (Virden & Watts, 1966). The data for the denatured enzyme and its control are derived from analyses of hydrolysates at 22, 42 and 71 hr.; those for the partially alkylated enzyme and its control are from analyses of 22 hr. hydrolysates only.

Residue	Residues/37000 g. of protein			
	Native enzyme	Denatured alkylated enzyme	Native enzyme	Partially alkylated enzyme
Carboxymethyl-cysteine	0.01*	5.09 $\pm$ 0.10	—	3.94
Aspartate	31.28 $\pm$ 1.13	30.48 $\pm$ 0.77	36.72	31.46
Threonine	18.39	17.57	18.32	19.40
Serine	18.39	18.10	17.30	21.30
Glutamate	38.69 $\pm$ 1.20	41.63 $\pm$ 2.13	41.89	39.90
Proline	—	12.72 $\pm$ 0.48	15.04	15.33
Glycine	26.86 $\pm$ 0.86	25.96 $\pm$ 0.87	27.08	28.51
Alanine	23.57 $\pm$ 1.03	23.40 $\pm$ 0.75	21.32	20.88
Valine	18.10 $\pm$ 0.10	17.09 $\pm$ 0.35	18.81	17.84
Cystine (half)	—	0.23 $\pm$ 0.04	2.04	1.46
Methionine	7.50 $\pm$ 0.15	7.05 $\pm$ 0.15	7.01	6.28
Isoleucine	17.29 $\pm$ 0.02	16.58 $\pm$ 0.25	18.32	17.42
Leucine	31.10 $\pm$ 0.69	31.10	32.01	29.58
Tyrosine	10.18 $\pm$ 0.13	10.03 $\pm$ 0.42	9.79	9.05
Phenylalanine	17.86 $\pm$ 0.12	17.41 $\pm$ 0.41	16.66	16.92
Amide	15.32 $\pm$ 1.41	17.89 $\pm$ 2.17	12.04	19.82
Lysine	28.19 $\pm$ 0.40	29.20 $\pm$ 0.71	30.47	27.46
Histidine	8.24 $\pm$ 0.11	8.27 $\pm$ 0.26	8.40	8.57
Arginine	15.68 $\pm$ 0.40	15.47 $\pm$ 0.79	15.38	14.63

* Methionine sulphoxide is not resolved from carboxymethylcysteine.

suggests that these errors are inherent in the analytical procedure. Proline was determined only in the alkylated enzyme since in the native enzyme cysteine was incompletely oxidized to cystine and the remaining cysteine interfered with the proline peak (A. P. Mathias & N. B. Hacket, personal communication). Threonine and serine degraded with increase in hydrolysis time and estimates were obtained by extrapolation to zero time assuming the degradation to be a first-order process (Noltmann, Mahowald & Kuby, 1962). Valine and isoleucine recovery increased with hydrolysis time so that values from the longer hydrolysis time were preferred. On this basis the best estimate of the nearest whole number of amino acid residues per molecule of enzyme is also given in Table 1, assuming a molecular weight 37 000 (Virden *et al.* 1966).

Table 2 compares the amino acid compositions of the normal enzyme and enzyme fully alkylated with iodoacetamide in the presence of 1% (w/v) sodium lauryl sulphate. Negligible amounts of methionine sulfoxide, which runs in the same position as carboxymethylcysteine, could be detected in the control. The data suggest that this might be increased to 6% of the total methionine in the alkylated enzyme but this would only slightly affect the value for carboxymethylcysteine. There was no evidence that any other amino acid was affected to a significant extent.

The partial specific volume of the protein was calculated from the weight percentages of the

amino acids by using the residue specific volumes given by Cohn & Edsall (1943). It is of interest that the calculated value 0.735 ml./g. is the same as that found for creatine kinase from rabbit muscle by Noltmann *et al.* (1962), which was in good agreement with the value found by other methods.

A comparison of the amino acid compositions of arginine kinase and rabbit creatine kinase on a mole/100 g. of protein basis is given in Table 3. Interesting features of the two compositions are that the total of aspartate, asparagine, glutamate and glutamine is almost the same for both enzymes although the amide nitrogen is probably much less in arginine kinase. Tyrosine, lysine and arginine are similar in amount but there is about 45% more histidine in creatine kinase. Of the neutral amino acids creatine kinase has more proline, which might have important structural implications, and more valine but less alanine, isoleucine and phenylalanine. As a rough approximation, if creatine kinase (mol.wt. 81000) is composed of two identical sub-units (Dance & Watts, 1962; Thomson, Eveleigh & Miles, 1964; Burger, Richterich & Aebi, 1964) nine of 18 amino acids differ in composition between a molecule of arginine kinase and a sub-unit of creatine kinase by not more than two residues. This suggests that the general shapes of arginine kinase and a sub-unit of creatine kinase may be very similar. If the two proteins are indeed homologous (Watts, 1965) these differences perhaps reflect part of the evolutionary changes associated with the alteration of substrate specificity and association to form a dimeric molecule.

Table 3. Comparison of the amino acid compositions of arginine kinase and creatine kinase per 100 g. of protein

Residue	Arginine kinase $10^3 \times$ (no. of residues)	Creatine kinase $10^3 \times$ (no. of residues)	$10^3 \times$ no. of residues different
Aspartate plus asparagine	84	102	18
Threonine	49	42	-7
Serine	49	51	2
Glutamate plus glutamine	105	91	-14
Proline	35	46	11
Glycine	73	77	4
Alanine	65	31	-34
Valine	49	65	16
Methionine	22	23	1
Isoleucine	46	31	-15
Leucine	84	86	2
Tyrosine	27	23	-4
Phenylalanine	49	37	-12
Lysine	76	80	4
Histidine	22	41	19
Arginine	43	42	-1
Tryptophan	5	9	4
Cystine (half)	14	10	-4

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