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Identification of α-(β-alanyl)-lysine in rabbit muscle

Subsequent to our recent demonstration of the occurrence of α-(γ-aminobutyryl)-lysine[1] and γ-aminobutyrylhistidine (homocarnosine)[2] in rabbit brain and the fact that β-alanylhistidine (carnosine) has long been known to be present in muscle, we have speculated on the presence of α-(β-alanyl)-lysine in muscle. This speculation has been verified in this study.

3 kg of rabbit skeletal muscle was chopped into small pieces, homogenized with 3 l of 10 % trichloroacetic acid and diluted with 4 l of 5 % trichloroacetic acid. After standing overnight at room temperature the residue was filtered by suction and the filtrate was passed through a 6 cm × 11 cm column of Amberlite IR-120 (100–200 mesh, H^+ form). The basic amino acids were eluted with 3 l of 2 M ammonia in 50 % ethanol. The eluate was evaporated to dryness, dissolved in 200 ml of water and applied to a 6 cm × 11 cm column of Amberlite CG-50 (200–400 mesh, ammonium form). Histidine, carnosine and related compounds are not retained on the column. After washing the column with 3 l of water, basic compounds such as ornithine, lysine, arginine and unknown basic compounds were eluted with 3 l of the ammoniacal ethanol. A band corresponding to α-(β-alanyl)-lysine was detected on a high-voltage electrophoretogram of an aliquot of this fraction under the conditions described in Table I.

TABLE I

PAPER CHROMATOGRAPHY AND HIGH-VOLTAGE ELECTROPHORESIS DATA OF β-ALANINE, LYSINE AND THEIR DIPEPTIDE

Toyo-Roshi No. 51 paper which is of similar quality to Whatman No. 1 was used. The high-voltage electrophoresis migration is the distance travelled towards the cathode after 40 min of electrophoresis with a potential gradient of 70 V/cm in pyridine–acetic acid–water (33:17:950, by vol., pH 5.3). The compounds were located by staining with ninhydrin.

	R_F values		*High-voltage electrophoresis migration (cm)*
	Pyridine–acetone–3 M ammonia (50:30:25, by vol.)	*Isopropanol–formic acid–water (8:1:1, by vol.)*	
β-Alanine	0.45	0.55	5.3
Lysine	0.43	0.17	18.5
β-Alanyl-α-lysine	0.55	0.19	16.4

Further purification of this compound was effected by ion-exchange chromatography on a 1.2 cm × 21 cm column of Amberlite IR-120 (200–400 mesh, ammonium form). Gradient elution was used with a mixing flask containing 150 ml of water and with 0.5 M ammonia in a reservoir. α-(β-Alanyl)-lysine emerged in the 146–163-ml fraction slightly contaminated with lysine. The contaminating lysine was removed by paper electrophoretic separation; the portion of electrophoretogram

corresponding to α-(β-alanyl)-lysine was cut out, and the compound extracted 3 times with 0.1 M acetic acid. The extract was evaporated to dryness and used for identification experiments.

Upon acid hydrolysis of an aliquot of the fraction in 6 M HCl at 105° for 16 h, the compound was split into β-alanine and lysine. Estimation of these amino acids in an aliquot of hydrolysate using an amino-acid analyzer revealed that they were equimolar (β-alanine:lysine, 1:1.03). Determination of the N-terminal amino acid of the peptide by the dinitrofluorobenzene method[3] indicated that it was α-(β-alanyl)-lysine.

To confirm the presence of α-(β-alanyl)-lysine in muscle the dipeptide was synthesized. α-(Carbobenzoxy-β-alanyl)-N^{ε}-carbobenzoxy-L-lysine (m.p., 148–149°), was prepared with a yield of 64% in a manner similar to that described earlier for the synthesis of α-carbobenzoxy-γ-aminobutyryl-N^{ε}-carbobenzoxy-L-lysine[1]. Catalytic hydrogenation of the intermediate product in 30 ml of 50% aq. methanol, containing a few drops of acetic acid in the presence of palladium black, yielded an oily material. This was dissolved in 15 ml of water and acidified to pH 2.0 by the addition of *p*-hydroxyazobenzenesulfonic acid. It was refrigerated overnight. Reddish needles (0.87 g) crystallized (yield, 87%; m.p., 216–218.5°, decomp.). Elementary analysis of the product was C, 49.77%; H, 5.06%; N, 12.09% (analytical calculation for α-(β-alanyl)-L-lysine 2 *p*-hydroxyazobenzenesulfonate monohydrate, $C_{33}H_{41}O_{12}N_7S_2$, C, 50.01%; H, 5.22%; N, 12.38%). The behavior of the purified dipeptide from skeletal muscle compared well with that of the authentic peptide in paper chromatography and high-voltage electrophoresis (Table I).

GULEWITSCH AND AMIRADZIBI[4] isolated carnosine from muscle and its structure was elucidated by SIFFERD AND DU VIGNEAUD[5]. Its high concentration in muscle drew attention to its physiological importance in muscle. The effect of the peptide on the metabolic system in muscle has been reported as being caused by its buffering action[6] or its ability to chelate metal impurities[7]. The occurrence of α-(β-alanyl)-lysine can well be expected in muscle because its precursors, β-alanine and lysine, and carnosine synthetase which has a broad substrate specificity, exist in muscle. In fact, KALYANKER AND MEISTER[8] have shown a peptide with the properties of β-alanyllysine in the muscle of chicks fed diets containing 2% lysine. However, they could not find it in chicks fed commercial feed. A large difference between the concentrations of the dipeptide and carnosine is interesting; whereas the activity of carnosine synthetase for lysine is 60% of that for histidine[8] and the concentration of histidine in muscle is 60% that of lysine[9], the concentration of carnosine (about 16 mmoles/kg muscle) is much higher than that of α-(β-alanyl)-lysine (20 μmoles/kg) which was calculated from the amounts of the constituent amino acids in the hydrolysate of the purified material. Many factors must be considered, such as storage and degradation mechanisms. At this point it may be important to note that α-(β-alanyl)-lysine is not hydrolyzed by carnosinase but by a different peptidase which splits peptides containing lysine, arginine and ornithine at their carboxyl terminals. The enzymatic study will be reported separately.

Many carnosine derivatives such as anserine (β-alanyl-1-methylhistidine)[11,12], balenine (β-alanyl-3-methylhistidine)[13] and ophidine (β-alanyl-2-methylhistidine)[14,15] have been reported in muscle. Recently, the structure of the last compound was found

to be β-alanyl-3-methylhistidine by nuclear magnetic resonance and *N*-methyl analysis[16]. α-(β-Alanyl)-lysine is thus added to the above list of β-alanyl dipeptides in muscle.

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