S-MYOTROPHIN, A NOVEL MUSCLE CELL GROWTH FACTOR IN PORCINE SKELETAL MUSCLE

Tatsumi Ito[1], Toshiya Hayashi[1], Kentaro Asahi[1], Nobuyuki Tsuchida[1], Keisuke Takeshita[1] and Shunichiro Kawabata[2].

[1]Laboratry of Chemistry and Technology of Animal Products, Faculty of Agriculture, Kyusyu University 49-06, and [2]Department of Biology, Faculty of Science, Kyusyu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812, JAPAN.

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INTRODUCTION (Backgrounds and Objectives)

It is known that the growth of skeletal muscle cells is promoted by a few growth factors, such as growth hormone, insulin-like growth factor-1 (IGF-1) and IGF-2. Sen et al. (1990) have purified a novel growth factor, myotrophin, from hypertrophied cardiac muscle of spontaneously hypertensive rats. Myotrophin is a water-soluble protein having its moleculer weight of 12kDa, and less amount of this factor has been found even in cardiac muscle of normal rats. However, myotrophin hasn't been found in any mammalian skeletal muscle, so far. Recentry, we found that the distribution of a water-soluble factor in skeletal muscle having almost the same moleculer weight as that of myotrophin is decreased by the denervation of motor nerves, although specific activity of the factor of denerved muscles is similar to that of control muscles. This result indicates that there is a signalling substance responsible for the signal from nerves to muscle cells in skeletal muscle and the signalling substance has a similar molecular size to that of cardiac myotrophin.

The objectives of the present study were to purify a signalling substance, having growth promoting activity of primary cultures of skeletal muscle cells, from porcine skeletal muscle and to characterize some properties of the substance.

MATERIALS AND METHODS

Purification of a signal substance

Purification of a signal substance of skeletal muscle was made fundamentally according to the method of Sen et al. (1990) with some modifications (Fig. 1). CM-cellulofine chromatography was effective to eliminate remaining myoglobin in the preparation of the signal substance. Second and third cycles of reverse phase liquid chromatography on Vydac C_{18} were essential steps to obtain a single elution peak in the chromatography. Resulting purified substance was named as s-myotrophin (s atands for skeletal).

Primary culture

Dispersed muscle cells free from fibroblast was prepared from thigh muscle by collagenase and dispase treatment and by the following filtration and differential cell adhesion. Growth promoting activity of crude and purified s-myotrophin was examined in DME medium containing L-glutamine, 5% fetal bovine serum, transferrin (1mg/ml), fetuin (10mg/ml), hydrocortizon (2.5μg/ml). Dispersed muscle cells (10^6cells/dish) in the medium in gelatin-coated plastic dishes were incubated at 37°C for 2hr after the addition of ^{3}H-leucine (272.2 $\times 10^6$ dpm/n mol) and crude/purified s-myotrophin to the medium. The incorporation of ^{3}H-leucine into the muscle cells was counted with a scintillation counter.

SDS-polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis was performed according to the method of Laemmli (1970). Gels were stained with a silver-staining kit (Silver stain Kanto II).

Amino acid analysis

S-myotrophin was hydrolysed in 6N HCl for 22hr in vacuo. Amino acid composition of resulting hydrolysate was analyzed with a amino acid analyzer (Pico-Tag System, Waters).

Digestion of the substance with proteases and peptide seqencing

S-myotrophin was digested with lysyl-endpetidase, trypsin, chymotrypsin and thermolysin and the resulting peptides, if any, were subjected to partial sequencing (ABI,473).

RESULTS AND DISCUSSION

A fraction of water-soluble substances of porcine skeletal muscle sedimentable between 30-80% saturation of ammonium sulfate has a growth promoting activity of muscle cells. This fraction contains a number of substances having widely distributed molecular weights. Sephadex G-75 gel filtration was able to fractionate active fractions in promoting the growth of muscle cells in rough-and -ready manner. The active fractions were distributed in a shoulder area of the chromatogram of the 30-80% ammonium sulfate-saturated fraction. SDS-polyacrylamide gel electrophoretogram of the active fraction showed a number of high- low molecular weight's substances, and a band having the molecular weight of 12kDa was visible in the electrophoretogram (Data not shown). Rechromatography of the eluate of Sephadex G-75 gel filtration was effective in eliminating high molecular weight's substances, resulting in the increase in the specific activity of the active fraction (Table1). CM-cellolofine chromatography of the active eluate of the rechromatogram of Sephadex G-75 gel filtration was originally designed to eliminate slightly basic substances. As described in MATERIALS AND METHODS, remaining myoglobin in the eluate partially co-eluated from Sephadex G-75 gel column was successfully eliminated by this chromatographic procedure. Likewise, FPLC on Mono-Q column was designed to separate acidic substances. Specific activity of active fraction of the eluates of Mono-Q indicates the effectiveness of this step to purify a active substance. Finally, a single peak of the active substance having strong activity in promoting the growth of muscle cells was obtained by three cycles of reverse phase of HPLC on Vydac C_{18} (Fig.2). The yield of s-myotrophin was approximately 2.5μg from 500g porcine muscle. SDS-polyacrylamide gel electrophoresis cleary showed that this peak has only one band having the molecular weight of 12kDa (Data not shown). Since the 12kDa band was visible in every active fractions after silver-staining, the substance visible at the same migrating distance as that of cytochrome C could be a substance having reducing ability. Acid-hydrolyzed s-myotrophin gave unique ninhydrin positive substances (Data not shown). The purified s-myotrophin was highly resistant against the proteolysis, i.e., lysyl-end peptidase, trypsin, chymotrypsin and thermolysin couldn't hydrolyze s-myotrophin. Since it has been found that cardiac myotrophin has its molecular weight of 12kDa and it is accessible to trypsin digestion, the extreme resistance of s-myotrophin against proteolysis indicates that s-myotrophin is a novel signal substances, having unique molecular properties, responsible for the growth of postnatal muscle cells.

CONCLUSION

A novel muscle cell growth factor was isolated from porcine skeletal muscle. The molecular weight of the factor was estimated to be 12kDa from the mobility on SDS-polyacrylamide gel. The molecular weight of s-myotrophin was almost the same as that of cardiac myotrophin. This factor was highly resistant against the attack of proteases such as lysyl-endpeptidase, trypsin, chymotrypsin and thermolysin. Difference in the acceptability of proteolysis indicates that the factor is different from cardiac muscle myotrophin, so that the name of the growth factor, s (skeletal)-myotrophin, was named after myotrophin of cardiac muscle.

REFERENCES

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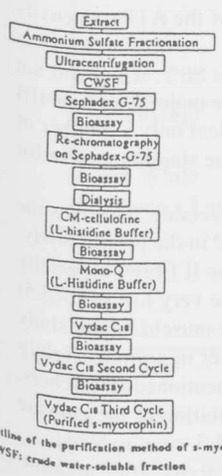

Fig.1 Outline of the purification method of s-myotrophin
CWSF: crude water-soluble fraction

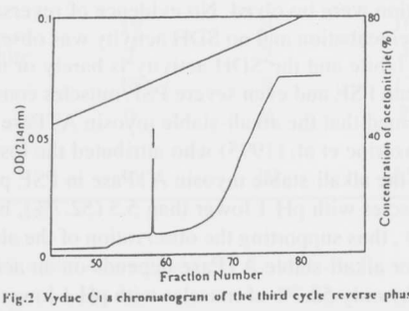

Fig.2 Vydac C_{18} chromatogram of the third cycle reverse phase HPLC

Table 1. Specific activity of s-myotrophin at each purification step

Purification Steps	Specific Activity
	activation(%)/mg protein
$(NH_4)_2SO_4$ precipitation	70
Gel filtration on Sephadex G-75 column	180
Rechromatography on Sephadex G-75 column	330
Ion exchange chromatography on CM cellulofine column	410
Ion exchange chromatography on Mono-Q column	790
Reverse-phase chromatography on Vydac C_{18} column	1,600
Rechromatography on Vydac C_{18} column second cycle	11,700
Rechromatography on Vydac C_{18} column third cycle	61,100