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Isolation and structural characterization of porcine coupling factor 6 from intestinal tissues

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A polypeptide purified from an extract of thermostable, porcine intestinal peptides was found to correspond to coupling factor 6, previously known as a component of the mitochondrial oxidative phosphorylation system. The intestinal presence of this peptide offers a new source for preparation of the component in large quantities, and possibly suggests further functions of the polypeptide. Amino acid sequence analysis of this porcine form reveals it to be identical to the bovine form, except for two replacements, at position 62 (Thr in the porcine, Phe/Thr in the bovine form), and position 70 (Ala/Val). The extensive conservation suggests strict structural constraints on the functional properties of the polypeptide.

Coupling factor 6; Amino acid sequence; (Porcine intestine)

1. INTRODUCTION

Coupling factor 6, a component of the complex for phosphorylation-coupled oxidation in mitochondria, was originally purified from bovine heart [1] and the 76-residue amino acid sequence of the protein from that source has been determined [2]. We have independently isolated what appears to be the same heat-stable protein, but have obtained it with completely different procedures and from a different source, porcine intestinal material. Sequence analysis shows that the porcine intestinal coupling factor 6-like protein also consists of 76 residues in a structure with two substitutions relative to the bovine factor.

We report here on the isolation and amino acid sequence of the porcine intestinal coupling factor 6-like protein, identifying its presence in intestinal extracts, establishing the strict structural conservation of the peptide between the species, and suggesting wider peptide functions and origins than previously anticipated.

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2. MATERIALS AND METHODS

2.1. *Isolation of porcine intestinal coupling factor 6-like protein*

The starting material is a concentrate of thermostable, porcine intestinal peptides [3]. Of this, 200 g was dissolved in 1.6 l water containing 0.5% (v/v) thiodiglycol after which 7.2 l isopropanol was added at room temperature to produce a precipitate (F0). To the filtrate, an additional 7.2 l isopropanol, now precooled to −20°C, was added. After 24 h at −20°C, the precipitate (F1) was collected by suction filtration, washed with isopropanol and ether, and dried under reduced pressure. The yield was about 16.6 g (dried powder) from 200 g concentrate. 6 g of the white precipitate was chromatographed on Sephadex

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G-25 fine (Pharmacia) in 0.2 M acetic acid as shown in fig.1A, and 1.58 g of the material corresponding to the coupling factor 6-like protein (fraction 3) was obtained. 500 mg of this material was chromatographed on a CM-cellulose (Whatman CM22) column (2.5 × 32 cm) which had been equilibrated with 0.01 M ammonium bicarbonate, pH 8. Elution was performed by stepwise increases of the ammonium bicarbonate concentration from 0.01 M to 0.02 M, 0.04 M, 0.1 M and finally 0.2 M. Fractions containing the coupling factor 6-like protein (the 0.01 M buffer eluate) were combined and lyophilized. The weight of the lyophilized material was about 200 mg.

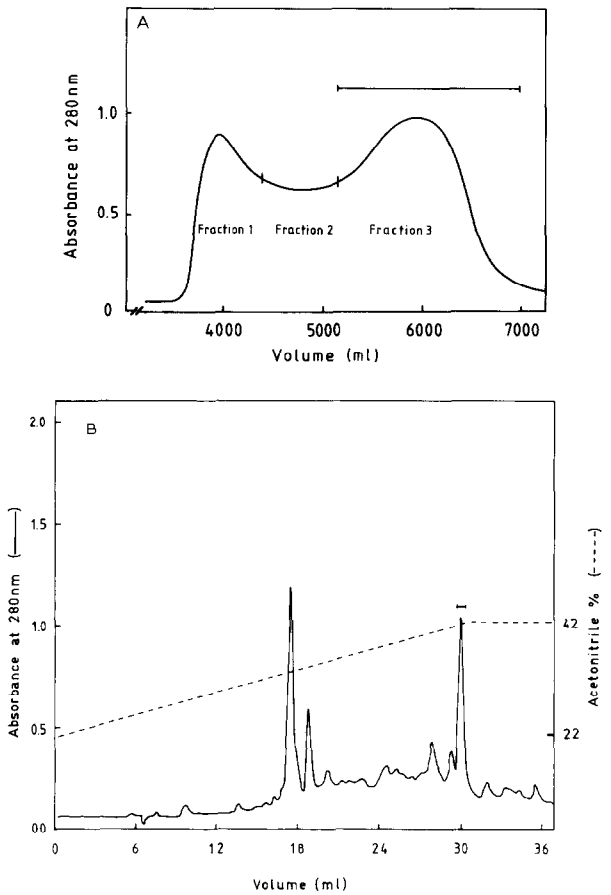

Fig.1. Purification of the porcine intestinal coupling factor 6-like protein. (A) Chromatography of the isopropanol fractionated peptide material (6 g; F1, cf. text) on Sephadex G-25 fine (10 × 100 cm) in 0.2 M acetic acid. The fraction with the coupling factor 6-like protein is indicated by the bar. (B) Final purification by high-performance liquid chromatography on Ultropac TSK ODS 120T (10 μm; 7.8 × 300 mm). 1.0 mg from the CM-cellulose chromatography step (cf. text) was applied, and the material was eluted with a gradient of acetonitrile (22–42% in 40 min as indicated by the dashed line; flow: 1.5 ml/min) in 0.1% trifluoroacetic acid.

Final purification (fig.1B) was by high-performance liquid chromatography in an instrument (Waters Associates) equipped with a TSK-ODS 120T (10 μm) column (7.8 × 300 mm), and utilizing a gradient (40 min at a flow of 1.5 ml/min) from 22 to 42% acetonitrile in 0.1% trifluoroacetic acid.

2.2. *Analytical methods*

Porcine coupling factor 6-like protein (~400 μg) was cleaved with CNBr (0.2 g/ml) in 70% formic acid at room temperature for 24 h. Fragments were separated by reverse-phase high-performance liquid chromatography on Vydac (4.6 × 25 cm) with 0.1% trifluoroacetic acid and an acetonitrile

Table 1

Compositional data for the porcine coupling factor 6-like protein and its CNBr fragments (CBI-III)

Residue	Intact protein	CBI	CBII	CBIII
Asn	10.1 (10)	6.0 (6)	1.1 (1)	3.1 (3)
Thr	4.1 (4)	2.1 (2)		1.7 (2)
Ser	2.4 (2)	1.4 (1)		1.4 (1)
Glx	13.0 (13)	9.0 (9)		3.8 (4)
Pro	5.9 (6)	2.8 (3)		2.6 (3)
Gly	4.4 (4)	3.4 (3)	1.4 (1)	
Ala	3.3 (3)	1.1 (1)	1.2 (1)	1.2 (1)
Val	4.2 (4)	3.4 (3)		1.4 (1)
Met	2.0 (2)	0.5 (1)	0.5 (1)	
Ile	1.3 (1)	0.9 (1)		
Leu	5.4 (5)	5.0 (5)		
Tyr	3.0 (3)	1.7 (2)	0.8 (1)	
Phe	5.7 (6)	2.0 (2)		3.8 (4)
Lys	9.1 (9)	5.8 (6)	0.9 (1)	1.8 (2)
Arg	4.3 (4)	4.1 (4)		
Sum	76	49	6	21

Values are molar ratios after acid hydrolysis and, within parentheses, the integers deduced from the sum of the sequence analysis

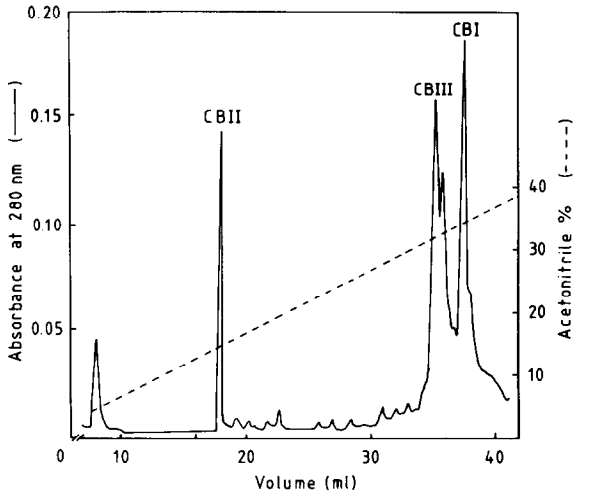

Fig.2. Separation of the CNBr fragments. A CNBr fragment mixture (468 μg) was applied to a Vydac column (4.6 × 25 cm) and the peptides were eluted with a gradient of acetonitrile (0–60%) in 0.1% trifluoroacetic acid at a flow of 1.0 ml/min. CBI, CBII and CBIII indicate the three CNBr-fragments in fig.3.

gradient of 0–60% in 60 min; flow 1 ml/min. Acid hydrolysis was performed in evacuated tubes at 110°C for 20 h with 6 M HCl containing 0.5% phenol, and amino acid compositions were determined on a Beckman 121M instrument. N-terminal amino acids were analyzed by the dimethylaminoazobenzene isothiocyanate method [5]. Liquid-phase sequencer degradations were performed in a Beckman 890D sequencer, using a 0.1 M quadrol peptide program in the presence of glycine-pretreated polybrene [6].

3. RESULTS

3.1. *Isolation of porcine coupling factor 6-like protein*

The isolation procedure for porcine coupling factor 6-like protein from intestines involved 6 main steps (cf. section 2). After the final step of reverse-phase high-performance liquid chromatography (fig.1B) a homogeneous material was obtained in a yield of 15 mg of pure protein per 100 kg intestine.

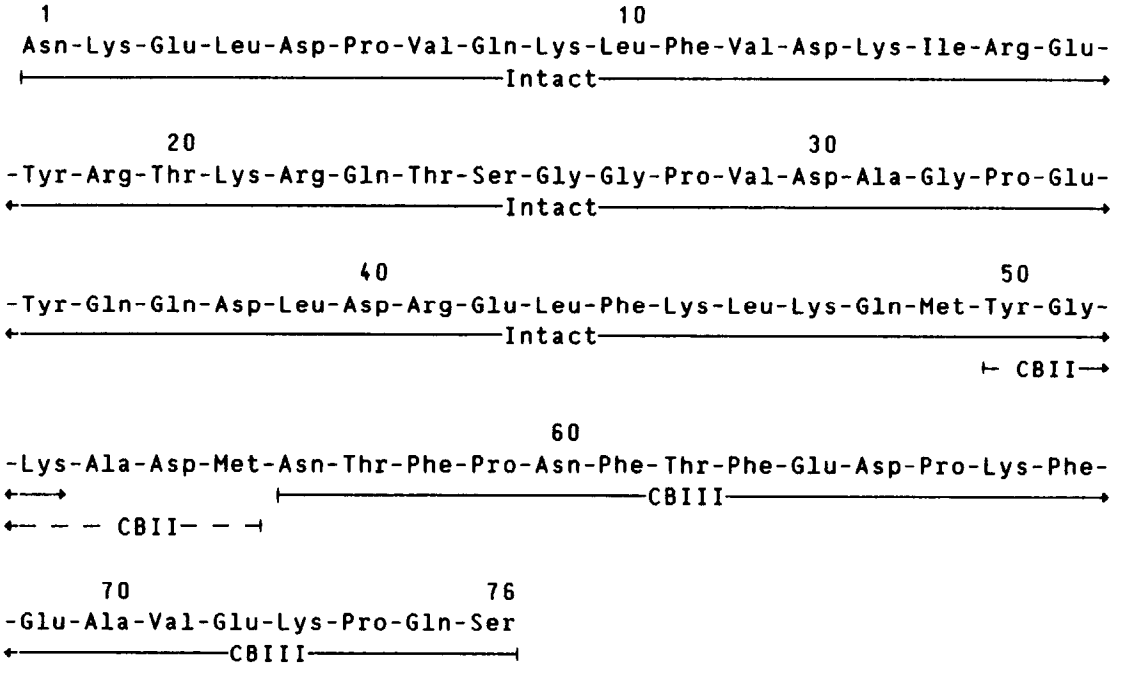

Fig.3. Amino acid sequence of porcine intestinal coupling factor 6-like protein, and positions of CNBr fragments analyzed. Solid lines indicate those segments passed by sequencer-assisted Edman degradations, dashed lines those passed by manual degradations. 'Intact' denotes degradation of the intact peptide. Fragments CBI (positions 1–49), II and III were separated as shown in fig.2.

3.2. *Structural analysis of porcine coupling factor 6-like protein*

The intact porcine coupling factor 6-like protein was found to have a free N-terminus (Asn) by manual sequence analysis, and was therefore susceptible to direct sequencer degradation, which could be interpreted for 52 cycles, as shown in fig.3. The amino acid composition (table 1) revealed two residues of methionine in the peptide. Therefore, another batch (~400 μg) was cleaved with CNBr, and three segments (CBI, CBII, and CBIII, cf. fig.2) were separated by high-performance liquid chromatography. The amino acid composition of the three segments are given in table 1.

Before analysis, the nature of the intestinal polypeptide was unknown, but upon comparison of the 52-residue sequence with structurally known proteins, the N-terminal part of the porcine intestinal polypeptide was found to be completely identical to the corresponding amino acid sequence of bovine heart mitochondrial coupling factor 6 [1,2]. According to the composition, two amino acid substitutions in the C-terminal segment (CBIII) exist, and they were established at positions 62 and 70 (Thr and Ala in the porcine peptide versus Phe/Thr and Val, respectively, in the bovine form) by liquid-phase sequencer degradation of CBIII to give the primary structure of the porcine peptide (fig.3).

4. DISCUSSION

So far, the factor 6 protein coupled to oxidative phosphorylation has been purified to a high degree of homogeneity only from bovine heart [1,2]. It was now unexpectedly detected also in porcine intestine. Usually, heart tissue is used for extraction of mitochondrial proteins but we purified a high yield of the coupling factor 6-like protein (15 mg from 100 kg tissue) from porcine intestine. This source might be valuable for production of sufficient material for structure-function relationships of this and possibly other mitochondrial proteins. The results highlight the importance of the gut as an organ involved in many biochemical processes. Possibly, the abundance of the coupling factor might suggest that it has additional biological functions. In any event, the new source provides large quantities of the material. Interestingly, the amino acid sequence reveals only two substitutions in 76 residues (Thr/Phe at position 62 and Ala/Val at position 70) in relation to the bovine coupling 6 factor. This demonstrates that coupling factor 6 is a highly conserved protein, suggesting strict structure-function relationships for this molecule.

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