Different isozymes of cytochrome *c* oxidase are expressed in bovine smooth muscle and skeletal or heart muscle

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Cytochrome *c* oxidase (COX) was isolated from bovine smooth muscle (rumen), and compared with the enzyme from bovine liver, heart and skeletal muscle. A new isozyme of COX was found to be expressed in smooth muscle, which differs from the isozyme in liver and heart or skeletal muscle. SDS-PAGE as well as N-terminal amino acid sequencing of separated subunits from gel bands revealed the expression of the liver isoforms for subunits VIa and VIII and of the heart isoform for subunits VIIa in COX from smooth muscle.

Smooth muscle; Cytochrome *c* oxidase; Isozyme; N-terminal amino acid sequence; Tissue specificity

1. INTRODUCTION

The protein components of the contractile system in vertebrates are tissue- and developmental-specifically expressed. In smooth muscle-specific isoforms of myosin heavy chain [1,2], myosin light chain kinase [3,4] and actin [5] have been characterized, which differ from those in skeletal muscle. The different isoforms of actin and myosin constitute different ATPases, varying in the amount and dynamics of ATP utilization. Correspondingly the mitochondrial ATP-generating activity, in particular cytochrome *c* oxidase (COX), the bottleneck of oxidative phosphorylation [6], should also be expressed and regulated in a tissue-specific manner. Tissue-specific isozymes of COX have been identified, based on two isoforms for 3 of the 10 nuclear coded subunits [7]. The COX enzyme complexes from bovine liver and heart both contain 13 subunits, but have different isoforms of subunits VIa, VIIa and VIII [8]. For subunit VIa from rat [9,10] and VIII from bovine [11] the liver- and heart-type isoforms of the cDNAs have been characterized.

Based on 2 isoforms for each of the 3 subunits VIa, VIIa and VIII, 8 isozymes of COX are theoretically possible if the holoenzyme consists of 13 different subunits in stoichiometric amounts [7]. In rat heart and brown fat tissue a COX isoform was found composed of the liver-type of subunit VIa and the heart-type of subunit VIII [12]. Here we describe a new COX isoform in bovine smooth muscle which differs from the isoforms expressed in bovine heart or skeletal muscle and in liver.

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2. MATERIALS AND METHODS

2.1. *Isolation of mitochondria*

Smooth muscle mitochondria were isolated from bovine rumen. From 5 kg of rumen 1 kg of smooth muscle tissue was prepared by removal of the adjacent fat and connective tissue. Mitochondria were prepared by a modified procedure according to Smith [12]. The diced tissue was passed through a mechanical meat grinder and homogenized for 15 s in a Waring blender in the medium: 0.1 M mannit, 0.1 M KCl, 0.02 M Tris-Cl, pH 7.4, 0.07 M sucrose, 2 mM EDTA, 1 mM NaN_3 and 50 units/ml heparin. After centrifugation of the mitochondria, actinomyosin and connective tissue were removed by washing and centrifugation first at 2000 rpm (Beckman, rotor JA10) and second at 4000 rpm. Mitochondria were collected at 8500 rpm. The heme a content was 0.6 nmol/mg protein. The visible spectrum was very similar to that of bovine liver mitochondria. Mitochondria from bovine liver and heart, diaphragm or skeletal muscle were isolated by published standard procedures.

2.2. *Isolation of cytochrome c oxidase*

COX was isolated by use of nonionic detergents (Triton X-114 and Triton X-100) as previously described [13]. The ratio of absorbance 280/240 nm was 2.8. The spectrum was indistinguishable from that of COX from other tissues.

2.3. *SDS-PAGE*

The isolated enzymes were separated by SDS-PAGE either by the method of Kadenbach et al. [14] or by the method of Schägger and von Jagow [15]. The two methods differ in the migration rate of the small subunits and in the lack of urea in the latter system, which blocks N-terminal amino acids.

2.4. *N-terminal amino acid sequencing*

For N-terminal amino acid sequencing the COX subunits were separated by the system of Schägger and von Jagow [15], using 10% acrylamide and 0.3% bisacrylamide in the spacer gel and 16.5% acrylamide and 1% bisacrylamide in the separation gel, electrotransferred to glass-fiber sheets, stained with Coomassie blue R250 and excised as described previously [16]. The protein bands were sequenced in a gas phase sequencer (470 A, Applied Biosystems) as described by Eckerskorn et al. [17] and further detailed in [16].

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3. RESULTS

The separation of COX from bovine heart, smooth muscle (rumen), liver, skeletal muscle and diaphragm by an urea containing SDS-PAGE separation system [14] is presented in Fig. 1. Differences in apparent molecular weights are found for subunits VIa and VIII. Subunit VIa from liver and smooth muscle runs faster than that of heart and skeletal muscle, including diaphragm. Also subunit VIII from liver and smooth muscle runs faster than that of heart and skeletal muscle, but an additional band corresponding to heart muscle subunit VIII is found in smooth muscle, which amounts to about half of the liver-type subunit VIII. No differences in the apparent molecular weights are found for the other 11 subunits of the COX from different tissues.

A different picture is obtained, if the enzymes are separated by the urea-free SDS-PAGE system [15] as shown in Fig. 2. A change of the relative position of subunit Vb, VIc and VIIc is seen, as compared to Fig. 1. Subunits VIab and c are not well separated, but subunits VIII from liver and smooth muscle again run faster than subunit VIII from the other tissues. A second band, which runs at the position of heart-type subunit VIII, is hardly seen in smooth muscle COX. With this separation system, however, a different apparent molecular weight is obtained for subunit VIIa from liver, as compared to COX from the other tissues. Thus the subunit composition of COX from smooth muscle appears to differ from that of all other investigated tissues.

In order to prove this differnce, subunits VIa, VIIa and VIII of smooth muscle COX were characterized by N-terminal amino acid sequencing after separation by a modified urea-free SDS-PAGE, as shown in Table I. The N-terminal amino acid sequences of smooth muscle subunit VIa and of the lower band of subunit VIII are identical to the liver-type subunits, whereas that of subunit VIIa is identical to the corresponding heart-type subunit VIIa. The presence of a small amount of heart-type subunit VIII in smooth muscle (upper band in Figs 1 and 2, lanes 2), indicates a further COX isoform composed of liver-type subunit VIa and heart-type subunits VIIa and VIII. These data do not rule out the possibility that the amino acid sequences of subunits VIa, VIIa and VIII from smooth muscle are different from both, the liver-type and the heart-type, respectively, because the complete amino acid sequence has not

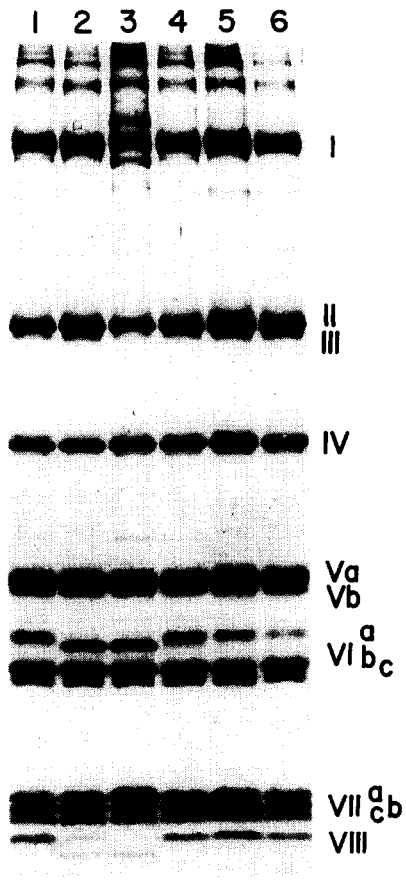

Fig. 1. Separation of isolated cytochrome *c* oxidase from different bovine tissues by SDS-PAGE according to the method of Kadenbach et al. [14]. The separation gel contained 18.75% acrylamide and 0.5% bisacrylamide and had a pH of 8.45. Lanes: (1) heart; (2) smooth muscle; (3) liver; (4) diaphragm; (5) skeletal muscle; (6) heart.

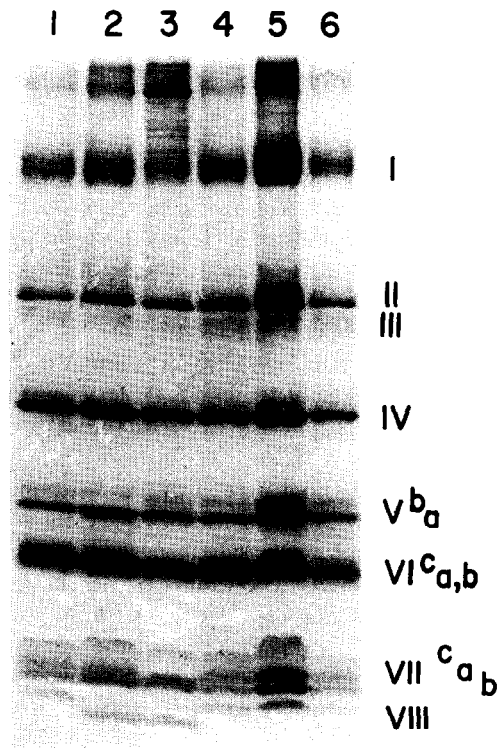

Fig. 2. Separation of isolated cytochrome *c* oxidase from different bovine tissues by SDS-PAGE according to the method of Schägger and von Jagow [15]. The spacer gel contained 10% acrylamide and 0.5% bisacrylamide, the separation gel 16.5% acrylamide and 0.5% bisacrylamide. Lanes: (1) heart; (2) smooth muscle; (3) liver; (4) diaphragm; (5) skeletal muscle; (6) heart.

Table I

N-Terminal amino acid sequences of subunits VIa, VIIa and VIII of cytochrome *c* oxidase from bovine liver, heart and smooth muscle (rumen)

Subunit	Tissue	N-terminal sequence
VIa	Liver	SSGAHGEE
	Heart	ASAAKGDH
	Smooth muscle	SSGAHG
VIIa	Liver	FENKVPEK
	Heart	FENRVAEK
	Smooth muscle	FENRVA
VIII	Liver	IHSKPPRE
	Heart	ITAKPAKT
	Smooth muscle	
	upper band	ITAKPA
	lower band	IHSKPP

The N-terminal amino acid sequences of COX from bovine liver and heart were taken from [18].

Table II

Tissue-specific expression of COX isozymes in different species

		Liver	Heart	Skeletal muscle	Smooth muscle	Brown fat
Rat	VIa	L [9,16]	L+M [9,16]	M [9]	-	L [16]
	VIIa	L [26]	-	-	-	-
	VIII	L [16]	M [16]	-	-	M [16]
Bovine	VIa	L [8]	M [8]	M*	L*	-
	VIIa	L [8]	M [8]	M*	M*	-
	VIII	L [8,11]	M [8,11]	M*	L+M*	-
Human	VIa	-	L+M [24]	-	-	-
	VIIa	-	-	M [21]	-	-
	VIII	L [25]	L [23,25]	-	-	-

The table indicates the expression of the liver-type (L) or heart-type (M) of subunits VIa, VIIa and VIII. The possible occurrence of isoforms for the other 7 nuclear coded subunits is unknown. *This study. The numbers in square brackets are reference numbers

been determined. So far, however, no third isoform of COX subunits VIa, VIIa and VIII has been found.

4. DISCUSSION

The above data demonstrate the expression of different isozymes of COX inbovine smooth muscle, heart and liver. The physiological meaning of the tissue-specific expression of COX isozymes is yet unknown, but different kinetic properties have been described for COX from bovine liver and heart [19,20]. The presence of the 'heart-type' subunit VIIa in all muscle tissues (heart-, skeletal-, diaphragm- and smooth-muscle), could indicate the involvement of this subunit in a muscle-specific regulation of energy supply. In COX of human muscle both, the liver-type and the heart-type of subunit VIIa, have been found by amino acid sequencing of the HPLC-separated subunits [21]. However, since the liver-type occurred only at about 10%, it could not be ruled out that it was derived from endothelial cells lining the blood vessels in skeletal muscle.

The other two subunits which occur in isoforms, subunits VIa and VIII, are not muscle-specifically expressed. In COX of rat heart about 1/3 of subunit VIa is of the liver-type, whereas subunit VIII is exclusively of the heart-type [16]. In cold-adapted rats COX from brown fat tissue contains the liver-type of subunit VIa and the heart-type of subunit VIII [16].

A species-specific variation of the tissue-specific expression of subunits VIa and VIII was found. Whereas in bovine [18], pig [13], rat [16] and chicken [22] exclusively the liver-type in liver and the heart-type in heart of subunit VIII are found, in human heart only the liver-type of subunit VIII was detected [23]. In bovine heart only the heart-type of subunit VIa was described [18], but in rat [16] and human heart [24] both, the liver-type and the heart-type of subunit VIa were found. Table II represents a summary of the presently known isozymes of COX in mammalian tissues.

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REFERENCES

[1] Sartore, S., DeMarzo, N., Borrione, A.C., Zanellato, A.M.C., Saggin, L., Fabbri, L. and Schiaffino, S. (1989) Eur. J. Biochem. 179, 79–85.
[2] Kuro-O, M., Nagai, R., Tzuchimochi, H., Katoh, H., Yazaki, Y., Ohkubo, A. and Takaku, F. (1989) J. Biol. Chem. 264, 18272–18275.
[3] Lukas, T.J., Burgess, W.H., Predergast, F.G., Lan, W. and Waterson, D.M. (1986) Biochemistry 25, 1458–1464.
[4] Ikebe, M., Maruta, S. and Reardon, S. (1989) J. Biol. Chem. 264, 6967–6971.
[5] Strauch, A.R. and Reeser, J.C. (1989) J. Biol. Chem. 264, 8345–8355.
[6] Erecinska, M. and Wilson, D.F. (1982) J. Membr. Biol. 70, 1–14.
[7] Kadenbach, B., Kuhn-Nentwig, U. and Büge, U. (1987) Curr. Top. Bioenerg. 15, 113–161.
[8] Yanamura, W., Zhang, Y.-Z., Takamiya, S. and Capaldi, R.A. (1988) Biochemistry 27, 4909–4914.
[9] Schlerf, A., Droste, M., Winter, M. and Kadenbach, B. (1988) EMBO J. 7, 2387–2391.
[10] Kadenbach, B., Hüther, F.-J., Büge, U., Schlerf, A. and Johnson, M. (1989) in: Molecular Basis of Membrane Associated Disease (Azzi, A., Drahota, Z., Jaz, J. and Papa, S. eds) pp. 216–227, Springer-Verlag, Berlin.
[11] Ligtowlers, R., Ewart, G., Zhang, Y.-Z., Calavetta, L. and Capaldi, R.A. (1990) J. Biol. Chem. 265, 2677–2681.
[12] Smith, A.L. (1967) Methods Enzymol. 10, 81–86.
[13] Kadenbach, B., Stroh, A., Ungibauer, M., Kuhn-Nentwig, L., Büge, U. and Jarausch, J. (1986) Methods Enzymol. 126, 32–45.
[14] Kadenbach, B., Jarausch, J., Hartmann, R. and Merle, P. (1983) Anal. Biochem. 129, 517–521.

[15] Schägger, H. and von Jagow, G. (1987) Anal. Biochem. 166, 368-379.
[16] Kadenbach, B., Stroh, A., Becker, A., Eckerskorn, C. and Lottspeich, F. (1990) Biochim. Biophys. Acta 1015, 368-372.
[17] Eckerskorn, C., Mewes, W., Goretzki, H. and Lottspeich, F. (1988) Eur. J. Biochem. 176, 509-519.
[18] Yanamura, W., Zhang, Y.-Z., Takamiya, S. and Capaldi, R.A. (1988) Biochemistry 27, 4909-4914.
[19] Merle, P. and Kadenbach, B. (1982) Eur. J. Biochem. 125, 239-244.
[20] Büge, U. and Kadenbach, B. (1986) Eur. J. Biochem. 161, 383-390.
[21] Van Beeumen, J.J., Van Kuilenburg, A.B.P., Van Bun, S., Van den Bogert, C., Tager, J.M. and Muijsers, A.O. (1990) FEBS Lett. 263, 213-216.
[22] Merle, P. and Kadenbach, B. (1980) Hoppe-Seyler's Z. Physiol. Chem. 361, 1257-1259.
[23] Van Kuilenburg, A.B.P., Muijsers, A.O., Demol, H., Dekker, H.L. and Van Beeumen, J.J. (1988) FEBS Lett. 240, 127-132.
[24] Fabrizi, G.M. and Schon, E.A. (1990) (unpublished results).
[25] Rizzuto, R., Nakase, H., Darras, B., Francke, U., Fabrizi, G.M., Mengel, T., Walsh, F., Kadenbach, B., DiMauro, S. and Schon, E.A. (1989) J. Biol. Chem. 264, 10595-10600.
[26] Kadenbach, B., Schlerf, A., Mengel, T., Hengst, L., Cao, X., Suske, G., Eckerskorn, C. and Lottspeich, F. (1989) in: Organelles in Eucaryotic Cells; Molecular Structure and Interactions (Tager, J.M., Azzi, A., Papa, S. and Guerrieri, F. eds) pp. 143-156, Plenum Press, New York.