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Amino acid sequence surrounding the lipoic acid cofactor of bovine kidney 2-oxoglutarate dehydrogenase complex

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The 2-oxoglutarate dehydrogenase complex was succinylated using 2-oxo[5-^{14}C]glutarate in the presence of *N*-ethylmaleimide to label the lipoic acid cofactor of the transuccinylase (E2) component. Following peptic digestion, ^{14}C-lipoate-containing peptides were purified and subjected to automated Edman degradation and amino acid analysis. The amino acid sequence surrounding the lipoyllysine residue is reported.

2-Oxoglutarate dehydrogenase complex; Lipoic acid; Succinylation; Primary structure

1. INTRODUCTION

The 2-oxoglutarate dehydrogenase complex is one of three multi-enzyme 2-oxoacid dehydrogenases present in mammalian mitochondria [1]. The complex catalyses the oxidative decarboxylation of 2-oxoglutarate to succinyl CoA, a key reaction of the tricarboxylic acid cycle. The activity of OGDC is regulated by allosteric mechanisms and is subject to acute hormonal control, primarily via alterations in the intra-mitochondrial levels of free Ca^{2+} [2].

OGDC is composed of multiple copies of three component enzymes. A thiamine pyrophosphate-dependent 2-oxoglutarate dehydrogenase (E1) and an FAD-linked lipoamide dehydrogenase (E3) are each bound non-covalently to a central, symmetrical core consisting of 24 succinyl transferase (E2) polypeptides [3]. E2 has an essential cofactor, lipoic acid, which is covalently bound to a flexible region of the E2 polypeptide, allowing interaction with the active sites of the individual components of the complex [3,4].

Although the genes encoding the constituent polypeptides of OGDC from *E. coli* have been cloned and sequenced and the protein sequence deduced [5,6], no sequence data is available for the mammalian complex. The amino acid sequence surrounding the lipoyllysine site of the mammalian PDC has been determined [7] and here we report the primary structure surrounding the lipoic acid attachment site on E2 of OGDC from bovine kidney.

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Abbreviations: OGDC, 2-oxoglutarate dehydrogenase complex; PDC, pyruvate dehydrogenase complex

2. MATERIALS AND METHODS

Hydroxyapatite (HTP) was from BioRad, pepsin from Boehringer and 2-oxo-[5-^{14}C]glutaric acid from Amersham International.

OGDC was purified from bovine kidney cortex, using essentially the method described in [8], except that the gel filtration step was omitted. Instead, contaminating PDC was removed by adsorption chromatography. OGDC was applied to an hydroxyapatite column (1.6 × 6 cm)

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equilibrated in 10 mM potassium phosphate, 1 mM dithiothreitol, pH 7.3, which was then washed extensively with 150 mM potassium phosphate buffer (500 ml) to remove PDC. OGDC was eluted using 500 mM potassium phosphate buffer and further purified and concentrated by ultracentrifugation at 144000 × g for 2.5 h. Activity of OGDC was determined by monitoring the production of NADH spectrophotometrically at 340 nm [9].

Peptic digestion of succinylated OGDC and purification of lipoic acid-containing peptides were carried out as in [7], high-voltage electrophoresis being performed at pH 1.9 in 7% (v/v) formic acid and HPLC using TSK DEAE 3SW ion-exchange and Vydac C_{18} reverse-phase columns. Automated amino acid sequencing was carried out on an Applied Biosystems 477 pulsed-liquid-phase sequencer with on-line PTH analyser. Amino acid compositions were determined using an Applied Biosystems 420A derivatiser equipped with on-line 130A analyser.

3. RESULTS AND DISCUSSION

Incubation of homogeneous OGDC with 2-oxo-[5-^{14}C]glutarate resulted in the reductive acylation of the lipoic acid cofactor of the E2 (transsuccinylase) component of the complex. This intermediate was stabilised by treatment with *N*-ethylmaleimide, which caused substrate-induced inactivation of the complex (fig.1). No significant loss of OGDC activity was observed in incubations lacking 2-oxoglutarate or *N*-ethylmaleimide (not shown). Maximum incorporation of succinyl groups was approx. 2.7 nmol/mg OGDC, corresponding to 0.33 mol/mol of E2 polypeptide. This low stoichiometry is consistent with previous observations [10] but has yet to be fully explained. Similar substoichiometric labelling of OGDC using 3-methyl-[2-^{14}C]oxobutyrate has also been reported [11].

High-voltage electrophoresis and autoradiography of a peptic digest of ^{14}C-succinylated OGDC yielded a single radioactive band of mobility approx. 0.24 relative to that of serine. The radioactive material was then subjected to ion-exchange HPLC, the column being equilibrated in 30 mM imidazole/HCl, pH 6.0, and peptides

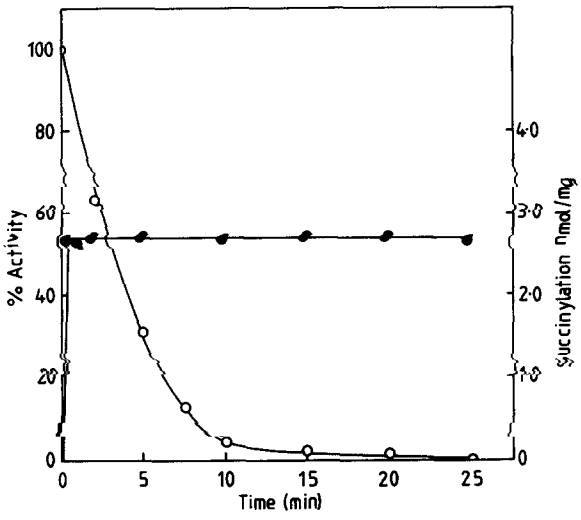

Fig.1. Succinylation of OGDC with 2-oxo-[5-^{14}C]glutarate in the presence of *N*-ethylmaleimide. OGDC (2 mg/ml) was incubated at 4°C in 0.5 ml sodium phosphate buffer, pH 7.4, containing 0.4 mM thiamine pyrophosphate, 2 mM $MgCl_2$, 0.5 mM *N*-ethylmaleimide and 0.2 mM 2-oxo-[5-^{14}C]glutarate (4.8×10^4 cpm/nmol). At the indicated times, aliquots were removed for determination of enzyme activity (○) and protein-bound radioactivity (●) [9].

eluted with a linear gradient (0–400 mM) of NaCl in the same buffer. This resolved the radioactivity into a major (A) and a minor (B) peak, comprising 75 and 15% of the recovered counts respectively. Peptides A and B were further purified by reverse-phase HPLC [7], each eluting from the reverse-phase columns as a single major peak of radioactivity (not shown). However the yield from the first column was low, as the majority (60%) of the applied radioactivity was unretarded by the column. Analysis of this material by high-voltage electrophoresis indicated that it was acidic in nature and likely to be succinate derived from the succinylated lipoic acid under the acidic conditions of the chromatography.

The results of automated sequence determination of peptides A and B are shown in table 1. No residue was detected at positions 5 and 2 respectively in peptides A and B. However, amino acid analysis indicated the presence of a single lysine residue in each peptide (table 2), which was not seen at any position during the sequencer runs. Furthermore, as observed previously for the lipoyl

Table 1

Amino acid sequence of OGDC lipoyl-peptides

	1				5				10				15
A:	Ile-	Glu-	Thr-	Asp-	Lys[a]-	Thr-	Ser-	Val-	Gln-	Val-	Pro-	Ser-	Pro-	Ala-	Asn-	Gly
B:					Asp-	Lys[a]-	Thr-	Ser-	Val-	Gln-	Val-	Pro-	Ser-	Pro-	Ala-	Asn-	Gly

[a] Denotes lipoyllysine residue

Table 2

Amino acid composition of OGDC lipoyl-peptides

Peptide	A	B
Asx	2.34 (2)	1.93 (2)
Glx	1.76 (2)	1.02 (1)
Ser	1.83 (2)	1.87 (2)
Gly	1.05 (2)	1.6 (1)
Thr	1.8 (2)	1.11 (1)
Ala	0.99 (2)	1.05 (1)
Pro	1.94 (2)	1.93 (2)
Val	2.05 (2)	1.58 (2)
Ile	0.92 (1)	0 (0)
Lys	1.13 (1)	1.23 (1)
Total	16	13

Peptides A (600 pmol) and B (75 pmol) were hydrolysed in the vapour phase with 6 M HCl under nitrogen atmosphere at 110°C for 24 h. Amino acids are listed in their order of elution from the reverse-phase column

peptides derived from PDC [7], the lysine residue to which the lipoic acid is attached is not identified by the sequencer. On this basis it is concluded that lipoyllysine is the residue at position 5 and 2 in peptides A and B, respectively. No significant radioactivity was recovered in any fraction from the sequencer run, again presumably reflecting instability under acid conditions of the succinyl group attached to the lipoic acid moiety.

The sequence around the lipoate residue in OGDC shows considerable homology with the equivalent region in 2-oxoacid dehydrogenase complexes from several different sources and with the H-protein of the glycine-cleavage enzyme system, which also utilises lipoic acid as a co-factor in its catalytic mechanism (table 3). There is at least 50% identity with the corresponding regions from the 2-oxoacid dehydrogenases and 31% identity with the H-protein. The homology between the 2-oxoacid dehydrogenase sequences is particularly

Table 3

Amino acid sequences flanking lipoyllysine residues

Source	Enzyme	Sequence	% identity
Bovine kidney[a]	OGDC E2	Ile-Glu-Thr-Asp-Lys[b]-Thr-Ser-Val-Gln-Val-Pro-Ser-Pro-Ala-Asn-Gly	
E. coli [6]	OGDC E2	Ile-Glu-Thr-Asp-Lys[b]-Val-Val-Leu-Glu-Val-Pro-Ala-Ser-Ala-Asp-Gly	56
E. coli [12]	PDC E2	Val-Glu-Gly-Asp-Lys[b]-Ala-Ser-Met-Glu-Val-Pro-Ala-Pro-Phe-Ala-Gly (Ser / Gln)	56
Bovine heart [7]	PDC E2	Val-Glu-Thr-Asp-Lys[b]-Ala-Thr-Val-Gly-Phe (Ile)	50
Chicken liver [13]	Glycine-cleavage H-protein	Leu-Glu-Ser-Val-Lys[b]-Ala-Ala-Ser-Glu-Leu-Tyr-Ser-Pro-Leu-Thr-Gly	31

[a] This work

[b] Lipoyllysine residue

Residues identical to those in the bovine kidney OGDC E2 are underlined

striking in the region immediate to the N-terminal side of the lipoyllysine residue, indicating that this may be a recognition site for the enzyme responsible for attachment of the lipoic acid onto the polypeptide chain. The finding of a unique lipoate-containing sequence for OGDC is consistent with the presence of a single lipoate-containing domain in each E2 polypeptide. The low stoichiometry of succinylation may be due to incomplete modification, possibly due to the lack of coupling of each lipoic acid to an active site on E1, or due to the apparent instability of the succinyl group on the lipoic acid. Alternatively it is possible that each potential site on E2 is not occupied by a lipoic acid residue. In this regard it is of interest that it has been reported that E2 contains less than a full complement of lipoic acid [10].

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