STRUCTURE OF THE PROSTHETIC GROUPS OF CITRATE LYASE AND CITRAMALATE LYASE

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1. Introduction

Citrate lyase and citramalate lyase are enzyme complexes which are very similar in structure and mechanism of action [1–4]. The acyl carrier protein of citrate lyase [5] contains a covalently-bound prosthetic group, which is composed from 4′-phosphopantetheine and a substituted adenylic acid yielding a coenzyme A-like structure [6,7]. The two compounds were thought to be linked to a substituted isomeric dephospho-CoA [6,7]. Chemical analyses of the acyl carrier-protein from citramalate lyase indicated a similar composition to that from citrate lyase. However, a higher amount of phosphate than reported earlier [6,7] was found with a different analytical procedure. This led to a re-evaluation of our previous results.

It will be shown in this paper that the two lyases have a common prosthetic group, which is covalently-bound dephospho-CoA. In citrate lyase the linkage between dephospho-CoA and the protein occurs through ribose-5-phosphate. This is bound in phosphodiester linkage to serine and glycosidically to the 2′- or 3′-hydroxyl group of ribose of dephospho-CoA. The binding of dephospho-CoA to citramalate lyase is probably the same, since its acyl carrier-protein contains one mole each of phosphate and sugar in addition to the components of dephospho-CoA. The results are in agreement with a proposal made recently for the structure of the prosthetic group of citrate lyase [8].

2. Materials and methods

Materials and methods were those described previously [7]. Molar concentrations of pure citramalate lyase acyl carrier protein solutions (pH 8.0) (manuscript in preparation) were calculated from $\epsilon_{260} = 15.8\ mM^{-1}cm^{-1}$.

Identification of carboxymethyl dephospho-CoA was performed by thin-layer chromatography on cellulose plates in isobutyric acid/NH_3/H_2O, 57/4/39, or butan-1-ol/acetone/acetic acid/10% NH_3/H_2O 35/15/15/7.5/22.5, or on polyethyleneimine cellulose plates developed with 1 M NaCl, or by DEAE-cellulose chromatography [9].

3. Results and discussion

3.1. *Chemical composition of citramalate lyase acyl carrier protein*

Citramalate lyase and citrate lyase are very similar in structure and catalytical functions. One could expect therefore that the prosthetic group of citramalate lyase had a coenzyme A-like structure as in citrate lyase. The presence of adenine in citramalate lyase acyl carrier-protein was confirmed by quantitative ultraviolet spectroscopy and by thin-layer and ion-exchange chromatography (see below). The spectrum (320–250 nm) of an acyl carrier protein solution was identical to that of an equimolar solution of adenosine, tyrosine and phenylalanine (1:1:1) (table 2) yielding $\epsilon_{260} = 15.8\ mM^{-1}cm^{-1}$.

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Table 1
Adenine, phosphate and ribose content of acyl carrier-proteins and of the prosthetic group from citrate lyase

Sample	A Adenine (nmol)	B Phosphate (nmol)	C Ribose (nmol)	Ratio A:B:C
Acyl carrier-protein of citrate lyase	4.4	13.8	9.0	1:3.1:2
Prosthetic group of citrate lyase	4.6	13.5	10.1	1:2.9:2.2
Acyl carrier-protein of citramalate lyase	4.2	11.9	8.7	1:2.8:2.1

The adenine content was calculated from ϵ_{260}. Organic phosphate was determined by the method of Chen et al. [10]. The ashing procedure was that of Ames and Dubin [11]. Ribose was determined by the orcinol method [12] using ribose as a standard.

Table 2
Amino acid composition of the acyl carrier-protein from citramalate lyase

Amino acid residue	Citramalate lyase acyl carrier-protein			
	Residues/mol		Residues/mol (assumed)	Citrate lyase acyl carrier-protein residues/mol [6]
	24 h hydrolysis	72 h hydrolysis		
Cysteine	0	0	0	1
Aspartic	9.65	9.40	9	5
Threonine	2.89	2.78	3	3
Serine	1.81	1.57	2	3
Glutamic	13.46	12.87	13	17
Proline	2.10	1.96	2	2
Glycine	5.63	6.00	6	6
Alanine	7.73	7.94	8	13
Valine	8.18	7.60	8	9
Methionine	2.46	3.00	3	3
Isoleucine	7.13	7.73	7	5
Leucine	8.07	7.86	8	9
Tyrosine	0.96	0.96	1	0
Phenylalanine	0.97	1.02	1	1
Lysine	11.15	10.64	11	3
Histidine	0	0	0	0
Tryptophan	–	–	0	1
Arginine	2.06	2.06	2	4
β-Alanine	0.93	0.49	1	1
Cysteamine	0.91	0.94	1	1

Carboxymethylated samples were hydrolysed in 6 M HCl at 110°C. Cysteine and cysteamine were determined as the carboxymethyl derivatives. The absence of tryptophan was estimated from the absorption spectrum.

The results of phosphate and sugar determinations (table 1) indicate the presence of two ribose and three phosphate residues/adenine residue in citramalate lyase acyl carrier-protein. The ribose content in the acyl carrier-proteins of citramalate lyase and citrate lyase is the same. However, only two phosphate residues have previously been found in the latter protein and in the prosthetic group isolated thereof [6,7]. Reinvestigation of the phosphate content by use of a different ashing procedure indicated the presence of three phosphate residues also in citrate lyase acyl carrier-protein and in its isolated prosthetic group (table 1). It was shown that the ashing procedure used in our initial studies yielded low values under our experimental conditions. Thus, the results of phosphate determinations reported previously [6,7] are erroneous. The present data are in agreement with those of Robinson et al. [8].

The amino acid composition of the acyl carrier-proteins of citramalate lyase and citrate lyase is similar (table 2). Thus the total number of 6 different α-amino acids is the same and the total number of 5 other α-amino acids differs by only one. However, a remarkable difference consists in a higher lysine content of citramalate lyase acyl carrier protein if compared with that of citrate lyase.

3.2. *Structure of the prosthetic groups of citrate lyase and citramalate lyase*

The chemical composition of citramalate lyase acyl carrier protein suggested that its prosthetic group had a coenzyme A-like structure as in citrate lyase. Part of the prosthetic group was identified as dephospho-CoA as follows. Cysteamine-*S* [2-^{14}C] carboxymethyl citramalate lyase (7 mg, 1.9×10^6 counts/min) and carrier carboxymethyl dephospho-CoA (0.6 μmol) were heated in 1.0 ml, 0.1 M HCl, for 8 min at 100°C. This treatment led to the liberation of [^{14}C]-carboxymethyl dephospho-CoA in a yield of about 40% of the total radioactivity. The product was copurified with the carrier by paper electrophoresis (4% HCOOH) followed by thin-layer chromatography (butan-1-ol/acetic acid/H_2O, 5/3/2). There was exact coincidence of adenine and radioactivity in ion-exchange and thin-layer chromatography and in paper electrophoresis of the purified substance (see Materials and methods). Likewise mild acid hydrolysis of a peptide from citrate lyase carrying the [^{14}C]carboxymethylated prosthetic group led to the liberation of [^{14}C]carboxymethyl dephospho-CoA. This was identified by chromatography, component analyses and degradation with phosphodiesterase I yielding carboxymethyl-4′-phosphopantetheine and 5′-AMP. This enzyme preparation likewise cleaved dephospho-CoA to AMP and 4′-phosphopantetheine.

The peptide fragment from which dephospho-CoA had been liberated by acid hydrolysis was isolated by ion-exchange chromatography. Besides four amino acid residues (aspartic (1) serine (2) and glutamic (1)), the peptide contained the residual components of the prosthetic group, i.e., one phosphate and one sugar residue. The phosphate residue was not hydrolysed by alkaline phosphatase, but after alkaline hydrolysis of the peptide (50 mM NaOH, 65°C, 30 min) the same treatment led to a quantitative liberation of phosphate. Additionally, the alkaline hydrolysis led to the loss of one of the two serine residues. These results are in agreement with a phosphodiester-linkage between serine and the second sugar residue (ribose-5-phosphate [8]) of the prosthetic group. In summary the results lead to conclusions which are drawn in the introduction.

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