TUNGSTEN, A COMPONENT OF ACTIVE FORMATE DEHYDROGENASE FROM *CLOSTRIDIUM THERMOACETICUM*

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1. Introduction

Formate dehydrogenase from *Clostridium thermoaceticum* catalyzes the reaction: $CO_2 + NADPH \rightleftharpoons HCOO^- + NADP^+$. The activity of the enzyme in growing cells is enhanced when selenite and molybdate are added together to the growth medium [1]. Tungstate replaces and is better than molybdate, and ^{75}Se-selenite is incorporated into the protein fraction with the formate dehydrogenase activity [1]. It was concluded that the formate dehydrogenase may be a metalloenzyme containing both selenium and tungsten or molybdenum. In this communication we report that ^{185}W-tungsten is incorporated into the active enzyme fraction. In addition, a second protein fraction with a mol. wt of about 60 000 was found labelled with radioactive tungsten.

2. Materials and methods

C. thermoaceticum was grown on 1.85 l medium described earlier [2] containing 0.036 mM ^{185}W-sodium tungstate (1 mCi). Growth was at 58°C for 40 hr and the cell yield was 27 g wet weight. Preparation of cell-free extract, assay of formate dehydrogenase at 45°C using NADP as electron acceptor, and protein were determined as described earlier [3]. Radioactivity was assayed with a Liquid Scintillation Spectrometer using a premixed scintillation cocktail (Unisolve). The counting efficiency was 80%.

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3. Isolation of formate dehydrogenase

The formate dehydrogenase from *C. thermoaceticum*, which is extremly sensitive to oxygen, was isolated under anaerobic conditions. The previously described triethanolamine-maleate, pH 7.5, was used throughout the purification [1,3] which is outlined in table 1 using 13.5 g wet weight of cells. The centrifuged French-press extract, 30 ml, was applied to a Sepharose 6B column (5 by 100 cm) using 0.05 M buffer as solvent. Fractions of 6.8 ml were collected. An elution profile is shown in fig.1. The formate dehydrogenase eluted after fraction 147 in a sharp peak which was followed by a long tail of weak activity. The place of elution corresponded to a mol. wt of about 290 000. The column was standardized using Blue dextran, ferritin (mol. wt 540 000), catalase (mol. wt 240 000), aldolase (mol. wt 158 000) and vitamin B_{12}. Three peaks containing ^{185}W were eluted, of which one coincided with the formate dehydrogenase. Fractions 150 to 165 containing formate dehydrogenase activity were pooled and applied to a DEAE-cellulose column (10 by 100 mm) on which both the formate dehydrogenase and the ^{185}W were adsorbed. The activities were eluted using a linear gradient from 120 ml 0.05 M and 120 ml 0.3 M buffer. Fractions of 11 ml were collected. The formate dehydrogenase eluted in fractions 7 to 12. The ^{185}W activity was eluted in 2 overlapping peaks, the first and smaller just ahead of the formate dehydrogenase, and the second coincided exactly with the enzyme activity. The fractions with the enzyme were combined and diluted with an equal volume of oxygen-free water. This solution was applied to a second DEAE-cellulose column (10 by 100 mm) on which again both activi-

Table 1
Isolation of formate dehydrogenase from cells of *C. thermoaceticum* grown in the presence of ^{185}W-sodium tungstate.

Step		Protein mg	Enzyme units	^{185}W cpm × 10^{-3}	units/mg	cpm/unit	cpm/mg
I	French-press extract	683.1	310.8	8388	0.455	—	—
II	Sepharose 6B column	118	141	57	1.193	406	484
III	DEAE-I gradient column						
	Fractions 7–12	8.21	68.6	25.4	8.36	370	3089
	After dilution	8.21	38.3	25.4	4.67	663	3089
IV	DEAE-II column						
	Fraction 3	3.84	25.3	20.0	6.58	790	5193
V	Bio-Gel A-0.5m column	4.08	5.91	6.0	1.45	1016	1471

ties were adsorbed. They were eluted using 0.25 M buffer in a fraction of 4 ml which moved with the front of the buffer. The so concentrated enzyme was applied to a Bio-Gel A-0.5m, 100–200 mesh column (2.5 × 90 cm), using 0.05 M buffer as solvent. Fractions of 6.8 ml were collected and enzyme and ^{185}W activities were eluted together in fractions 26 to 34.

As shown in table 1 the enzyme lost activity at the dilution step before application to the second DEAE-column, and on the Bio-Gel A-0.5m column. Such inactivation is often observed [1,3]. Therefore, the above procedure was modified. Only the most active fractions from the Sepharose 6B column containing a total of 32 mg of protein, 57 enzyme units, and 38 400 cpm were combined and applied to the DEAE-cellulose gradient column. Fraction 9 from this column was directly applied to the Bio-Gel A-0.5m column. The formate dehydrogenase and the ^{185}W were eluted at a constant ratio in one single peak as shown in fig.2. The enzyme activity (units mg^{-1}) was over 25.

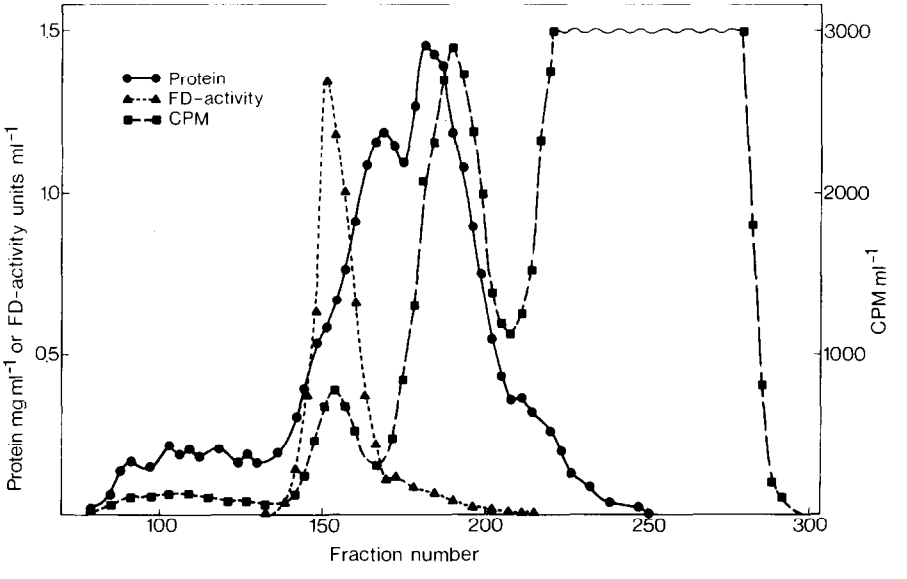

Fig.1. Elution from Sepharose 6B column. Step II of table 1

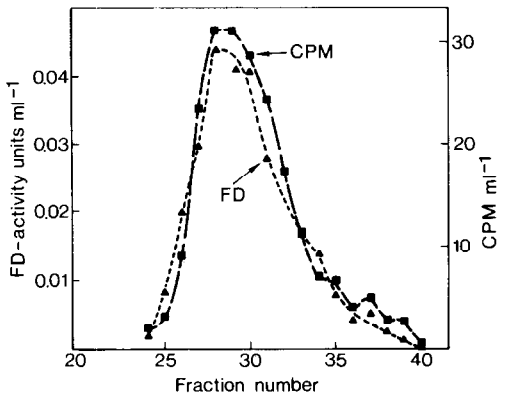

Fig.2. Elution profile from Bio-Gel A-0.5m column of fraction 9 from the DEAE-cellulose gradient column containing protein. 0.3 mg; formate dehydrogenase, 7 units; and 2700 cpm.

4. Results and discussion

During growth ^{185}W-tungstate was not concentrated by cells of *C. thermoaceticum*. Thus the growth medium had the same radioactivity before and after the fermentation (1.018×10^6 cpm ml^{-1}). The cells after harvest had an activity of 8.3×10^5 cpm g^{-1}. The cell-free extract from 13.5 g of cells contained 8.4×10^6 cpm. Most of the radioactivity eluted in the low-molecular weight fraction of the Sepharose 6B column (fig.1). However, two protein fractions were labeled with ^{185}W. The most radioactive of these contained 3.8×10^5 cpm and had a mol. wt of about 60 000. This fraction had very little formate dehydrogenase activity. Precipitation of the protein with ammonium sulfate (55% saturation) also precipitated all radioactivity. Denaturation of the protein with ethanol (70%) and trichloracetic acid (20%) resulted in partial release of the ^{185}W from the protein. The fraction has not yet been further characterized.

The formate dehydrogenase fraction from the Sepharose 6 B column contained a total of 7.5×10^4 cpm. The formate dehydrogenase peak was not symmetrical indicating heterogeneity which is in agreement with earlier results [3]. The long tail of low formate dehydrogenase activity indicates that the enzyme may dissociate into subunits which retain some enzyme activity. During chromatography on Sepharose 6B, DEAE-cellulose, and Bio-Gel A-0.5m, the main fractions with formate dehydrogenase activity contained ^{185}W in a constant ratio to the formate dehydrogenase activity. The formate dehydrogenase and the ^{185}W coprecipitated with ammonium sulfate (55% saturation), but denaturation with trichloroacetic acid and ethanol released most of the ^{185}W from the protein. These results, together with earlier findings [1], are evidence that tungsten is a component of the formate dehydrogenase from *C. thermoaceticum* and is required for the enzyme activity.

Addition of tungstate or molybdate together with selenite to the growth medium increased also the formate dehydrogenase activity in *Clostridium formicoaceticum* [4] and tungstate promotes the growth of *Methanococcus vannielii* [5]. With *C. formicoaceticum* again tungstate is better than molybdate. This is in contrast to the formate dehydrogenase activity in *Escherichia coli* which is increased by addition of selenite and molybdate to the growth medium, but not by tungstate [6,7]. A negative effect of tungstate on molybdo-enzymes has been reported [8–17]. It apparently involves the formation of an inactive tungsto-protein.

It is of course premature to discuss the role of tungsten in the formate dehydrogenase of *C. thermoaceticum*, but it is of interest that CO_2 is inserted at room temperature into hexakis (dimethylaminato) tungsten to form a tungsten-CO_2-N complex (18). The physiological role of formate dehydrogenase in *C. thermoaceticum* is to reduce CO_2 to formate [19].

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