Electron microscopy of the hydrogenase from the hydrogen-oxidizing bacterium *Alcaligenes eutrophus* Z1

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The quaternary structure of the NAD-dependent hydrogenase from the hydrogen-oxidizing bacterium *Alcaligenes eutrophus* Z1 has been studied by electron microscopy. A model of the hydrogenase molecule with two small subunits arranged between two large ones in linear fashion is proposed. The electron microscopy studies are in accordance with the previously proposed linear electron transport chain of the hydrogenase and distribution of the substrate binding sites within the enzyme molecule.

Hydrogenase *Enzyme structure* *Electron microscopy*

1. INTRODUCTION

The NAD-dependent soluble hydrogenase (EC 1.12.1.2) from hydrogen-oxidizing bacteria has a complex quaternary structure. The enzyme preparations were isolated from two strains of *Alcaligenes eutrophus*, H16 [1] and Z1 [2,3], and from *Nocardia opaca* 1b [4]. All isolated enzymes were composed of 4 non-identical subunits – two large ones of about 60 kDa and two small ones of about 30 kDa. In the case of the hydrogenase from *A. eutrophus* Z1 the molecular masses of the subunits were 65, 55, 29 and 26 kDa [5].

The NADH-dependent hydrogenase contains a number of prosthetic groups including nickel, FMN, Fe_4-S_4 and Fe_2-S_2 clusters [6,7]. These enzymes have a broad substrate specificity and catalyse both the hydrogenase and NADH-dehydrogenase activities with a number of electron acceptors [1,2]. In our previous works we have demonstrated the relations between the quaternary structure of the enzyme from *A. eutrophus* Z1 and its catalytic activities [8,9], and the existence of several spatially independent substrate binding sites in the hydrogenase molecule [10]. On the basis of these studies a model of the electron transport chain of the hydrogenase was proposed [10].

The purpose of the present work was to visualise the arrangement of the subunits in the molecule of hydrogenase from *A. eutrophus* Z1 with the help of electron microscopy.

2. MATERIALS AND METHODS

Bacterial cells were destroyed by sonication. The hydrogenase was purified by double hydrophobic chromatography on phenyl-Sepharose and gel filtration on Toyopearl HW-55F as described in [3].

Judging by polyacrylamide gel electrophoresis, the purity of the enzyme preparations was not less than 95%. For electron microscopy studies hydrogenase solutions of 0.1 mg/ml in 0.05 M potassium phosphate buffer (pH 7.8) were used. A drop of protein solution was placed on a thin carbon grid and stained with a 2% aqueous solution of phosphotungstic acid. Alternatively, some of the preparations were stained with 1% uranyl acetate. The grids were examined in a Philips

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EM-400 electron microscope at a magnification of 50000 and accelerating voltage of 80 kV.

3. RESULTS AND DISCUSSION

The micrographs of the hydrogenase preparations (fig.1) revealed elongated particles of length 13 ± 1 nm and maximal width 4.5 ± 1 nm. It is possible to distinguish several types of particle images, which apparently result from rotation of the hydrogenase molecule around its longitudinal axis on the supporting film. The first type is represented by particles in which two small morphological units are located between the two large triangular ones (fig.2a). Particles of the second type have the form of an 'arrow' (fig.2b). Particles composed of two large morphological units linked by a smaller one comprise the third type of image obtained (fig.2c). Finally, particles of the fourth type are composed of two large triangle-shaped morphological units connected by a narrow protein link which is usually inclined to the longitudinal axis (fig.2d).

All the images observed can be accounted for by a model of the hydrogenase molecule according to which the two small enzyme subunits are located between the two large ones. The dimensions of the morphological units visible on the micrographs are in accordance with the molecular masses of the

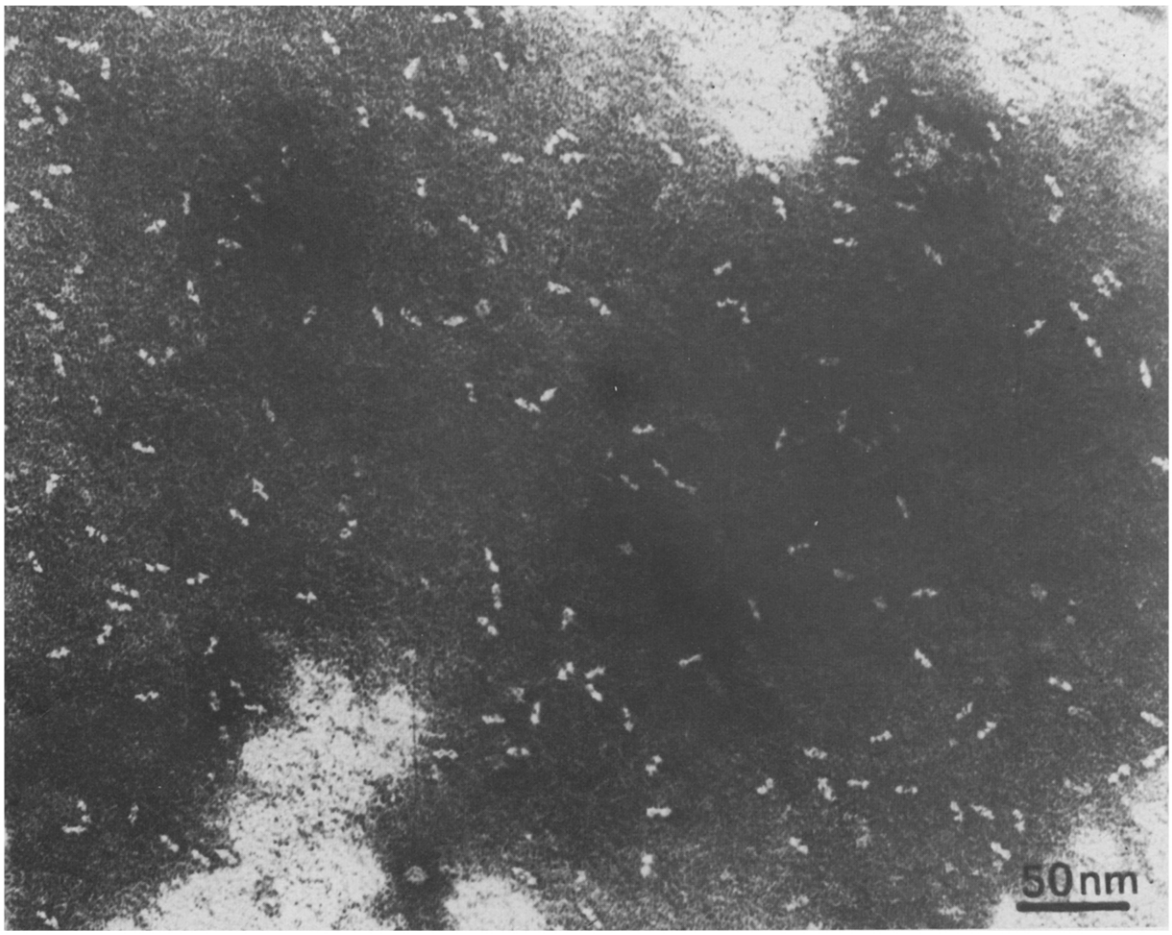

Fig.1. General view of hydrogenase preparations from the bacterium *A. ?utr hus* Z1 negatively stained with phosphotungstic acid solution.

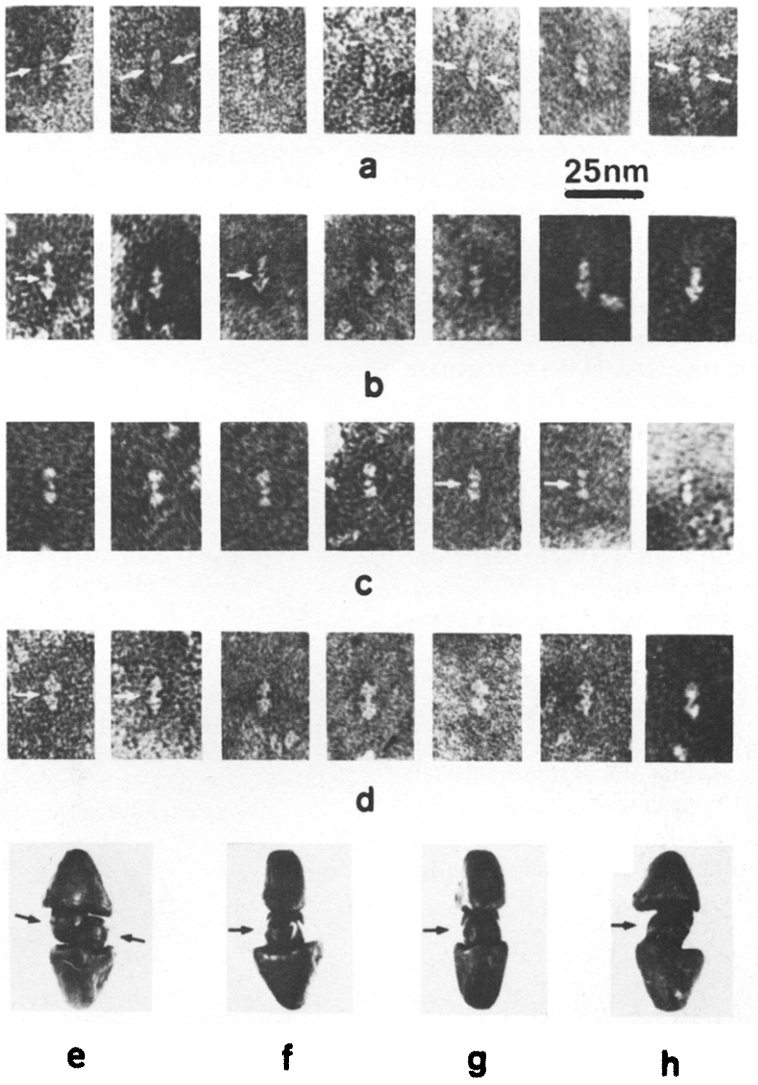

Fig.2. Types of images of the hydrogenase molecule. Small morphological units are indicated by arrows. Images of: (a) the first type ($\theta = 0°$); (b) second type ($\theta \approx 45°$); (c) third type ($\theta = 90°$); (d) fourth type ($\theta \approx 30°$). (e–h) Different projections of the enzyme model, corresponding to types a–d, respectively.

small and large hydrogenase subunits estimated by various biochemical methods [5,8]. Thus it is possible to identify these morphological units with the subunits of the enzyme molecule.

The best fit between the images of the hydrogenase molecule on the micrographs and its model is obtained assuming that both large enzyme subunits are similar in shape and twisted against each other around a longitudinal axis at an angle of about 45°. The hydrogenase molecule thus may be approximately characterized by a two-fold rotational axis which is perpendicular to the longitudinal direction and situated between the two small enzyme subunits. The view along this two-fold axis (fig.2e) corresponds to particles of the first type. Starting from this position rotation of the model around the longitudinal axis at an angle $\theta \approx 45°$ produces particles of the arrow type. The dumb-bell-shaped particles may be obtained at $\theta \approx 30°$.

Two major features of the structure of the hydrogenase molecule become evident from elec-

tron microscopy studies of the enzyme: (i) the linear arrangement of the subunits comprising the enzyme molecule; and, (ii) the presence of two spatially isolated fragments in the hydrogenase each of which is composed of one large and one small enzyme subunit. Both structural features are in accordance with the previous studies of the enzyme [8]. The presence of the two spatially independent 'heterodimers' in the hydrogenase molecule validates our earlier conclusion that the NAD-dependent hydrogenase might be composed of two simpler enzymes, NADH-dehydrogenase and conventional hydrogenase, linked together [8]. The two structural fragments of the enzyme molecule may be ascribed to these two catalytic fragments: the 'NADH-dehydrogenase' part of the enzyme molecule comprising FMN and some of the Fe-S clusters and the 'hydrogenase' part comprising Ni and the remainder of the Fe-S clusters. A similar model was also recently suggested for the hydrogenase from *N. opaca* [11].

The linear arrangement of protein subunits in the hydrogenase molecule assumes that the electron transport chain of the enzyme is also linear with the hydrogen-activating site (presumably Ni) and coenzyme-binding site (FMN) situated at opposite ends of the protein and with the Fe-S clusters comprising the electron transport bridge between them.

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