Properties of the quinoprotein aldehyde dehydrogenase from '*Acetobacter rancens*'

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The membrane-bound aldehyde dehydrogenase from '*Acetobacter rancens*' CCM 1774, solubilized from the membrane fraction by treatment with surfactants and subsequently purified to homogeneity, was characterized with respect to the M_r of the dimeric enzyme (145000), pH optimum (5.1), pI value (5.3), substrate specificity towards straight-chain aldehydes, substrate inhibition and the effects of various inhibitors and ions. Methanol extracts of the membrane fraction and of the purified enzyme showed properties identical with pyrroloquinoline quinone as revealed by spectrophotometric methods and reactivity with apoquinoprotein glucose dehydrogenase. Pyrroloquinoline quinone could not be liberated by EDTA treatment. The enzyme activity of an apoenzyme could be partly restored by addition of membrane extracts containing pyrroloquinoline quinone adducts. The solubilized, purified quinoprotein aldehyde dehydrogenase and the membrane-bound enzyme differed in substrate binding, substrate inhibition, pH optimum and linkage to a *c*-type cytochrome which acts as electron acceptor in membrane fractions. Since double-reciprocal plots of initial reaction rates with various concentrations of aldehyde or electron acceptor showed intersecting lines, and different inhibition patterns were obtained for the two forms of the enzyme, a ping-pong kinetic behaviour with the two reactants was excluded. The kinetic mechanism was suggested to be changed after solubilization from a random-order type to a compulsory-order type, and is thus atypical for quinoproteins reported so far.

Introduction

'*Acetobacter rancens*' CCM 1774 can use n-alkanes as a source of carbon and energy (Hommel & Kleber, 1984a). Hexadecane is predominantly oxidized via monoterminial attack to palmitic acid. Aldehydes are considered to be metabolic intermediates in microbial alkane utilization, being oxidized to fatty acids by aldehyde dehydrogenase. For most enzymes involved in hydrocarbon metabolism pyridine-nucleotide-dependency and induction by growth substrate have been reported (reviewed by Sorger & Aurich, 1978).

'*A. rancens*' possesses two constitutive aldehyde dehydrogenases: a cytoplasmic NADP⁺-dependent enzyme that is highly specific towards short-chain aldehydes (Hommel *et al.*, 1988) and a membrane-bound dye-linked enzyme. Their relative specific activity is influenced by the carbon source, e.g. glucose or n-hexadecane, with the

activity of the membrane-bound enzyme being enhanced to a greater extent by hydrocarbons (Hommel & Kleber, 1984b).

The membrane-bound aldehyde dehydrogenase was purified by Hommel & Kleber (1984c). This hydrophobic enzyme was very unstable and could be stabilized only slightly in the presence of high surfactant concentrations and by addition of benzaldehyde (Hommel *et al.*, 1985).

Ameyama & Adachi (1982) characterized the dye-linked aldehyde dehydrogenase from acetic acid bacteria; this enzyme is a quinoprotein, a novel class of dehydrogenases that possess pyrroloquinoline quinone as prosthetic group (Duine *et al.*, 1987).

The present paper describes the chemical and kinetic properties of the purified membrane-bound aldehyde dehydrogenase from '*A. rancens*'.

Methods

Organism and culture conditions. '*A. rancens*' CCM 1774, obtained from the Czechoslovak Collection of Micro-organisms, Brno, Czechoslovakia, was used throughout this study. [Inverted commas indicate

Abbreviations: PQQ, pyrroloquinoline quinone, [2,7,9-tricarboxy-1H-pyrrolo(2,3-f)quinoline-4,5-dione]; PMS, phenazine methosulphate; pCMB, *p*-chloromercuribenzoate; DCPIP, 2,6-dichlorophenol-indophenol; AldDH(acc), quinoprotein aldehyde dehydrogenase (aldehyde:pyrroloquinoline quinone oxidoreductase; EC 1.2.99.3).

that the organism is not in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980), has not been validly published since 1 January 1980, and therefore does not have nomenclatural standing.] Culture conditions were as described by Hommel & Kleber (1984b).

Preparation of crude extracts, solubilization and purification. All procedures were done at 0 to 4 °C. Sodium/potassium phosphate buffer (PB) was used throughout. Crude extracts were obtained by ultrasonication and membranes by differential centrifugation (Hommel & Kleber, 1984b). Membranes in PB (10 mM, pH 6.1) were solubilized by two consecutive detergent treatments: (i) with a Brij 35:protein ratio of 0.8 mg mg⁻¹; after centrifugation at 130000 g for 1 h at 4 °C the precipitate was dissolved in PB and solubilized (ii) at a Triton X-100:protein ratio of 4 mg mg⁻¹ at a protein concentration of 2.5 mg ml⁻¹. The centrifugation was repeated and benzaldehyde (final concentration 2.5 mM) was added to the supernatant to lessen the otherwise rapid inactivation.

The supernatant was applied to a DEAE-Sephacrose CL-6B column (2.6 × 7.6 cm) previously equilibrated with PB (10 mM, pH 6.1 containing 5%, w/v, Triton X-100 and 2.5 mM-benzaldehyde) and eluted with a linear gradient of 10 to 150 mM-PB at a flow rate of 16 ml cm⁻² h⁻¹. Active fractions appearing at a conductivity of 5.2 mS were collected and concentrated by ultrafiltration (XM 50, Amicon). The concentrated enzyme was applied to a Sepharose 4B column (4 × 28 cm) equilibrated with 1 mM-PB and eluted with the same buffer (2.5 ml cm⁻² h⁻¹). Active fractions were applied to a hydroxyapatite-cellulose column (2.6 × 10 cm) and eluted (10 ml cm⁻² h⁻¹) by a discontinuous gradient of 1 to 150 mM-PB. Active fractions eluting at 6.2 mS were concentrated by ultrafiltration as above.

Cytochrome *c*-thiol-Sepharose. Glutathione was linked to CNBr-activated Sepharose (Cuatrecasas & Anfinsen, 1971) as described by Brocklehurst *et al.* (1974). The thiol-Sepharose was washed with NaHCO₃ buffer (0.1 M, pH 8.3, containing 0.5 M-NaCl) and sodium acetate buffer (0.1 M, pH 4.0, containing 0.5 M-NaCl) and finally suspended in PB (0.1 M, pH 6.1). The packed column was equilibrated with PB (10 mM, pH 6.1). Cytochrome *c* (horse heart) (1.7 mg per g of gel) was circulated for 15 h. After washing with 3 column vols of PB the circulation of cytochrome *c* was repeated. The cytochrome *c*-thiol-Sepharose column was washed and equilibrated with PB (pH 6.1, containing 5%, w/v, Triton X-100 and 2.5 mM-benzaldehyde). Samples were applied to the column, washed with 5 column vols of buffer and eluted by stepwise increase of ionic strength of the buffer (0.01, 0.1, 1 M). The flow rate was 20 ml cm⁻² h⁻¹. Under these conditions the aldehyde dehydrogenase was eluted specifically at 0.55 mS conductivity. No further purification was obtained.

Lysozyme treatment. Bacteria grown on n-hexadecane (0.5 g wet wt) were suspended in 10 ml PB (0.1 M, pH 7.5) and treated with lysozyme (2 mg) for 30 min at room temperature followed by disintegration of spheroplasts and protoplasts with glass beads in the presence of RNAase and DNAase (1 mg each). Membranes were sedimented at 130000 g_{av} for 60 min at 4 °C, and the pellet was resuspended in 50 mM-PB (pH 6.1) containing sucrose (10%, w/v) and resedimented as above. This centrifugation was repeated twice. The final membrane pellet was suspended in buffer containing 25% (w/v) sucrose. Of this suspension 0.35 ml, containing 3 mg protein, was layered on top of a discontinuous sucrose gradient (4.2 ml, 30 to 50%, w/v, sucrose) and centrifuged for 7 h at 80000 g_{av}. The gradient was then fractionated and analysed.

Measurement of enzyme activities. All tests were done at 25 °C. The oxidation of aldehydes by aldehyde dehydrogenase was measured as reported by Hommel & Kleber (1984b). NADH dehydrogenase and NAD⁺ oxidase were estimated according to Matsushita *et al.* (1978). Dye-linked membrane-bound glucose and alcohol dehydrogenases were assayed spectrophotometrically at 600 nm by following the

oxidation of DCPIP. The assay mixture consisted of DCPIP (40 µM), Tris/HCl (20 mM, pH 7.6), PMS (0.4 µM), and glucose (16 mM) or ethanol (66 mM), respectively.

Determination of *M_r*. The *M_r* of the homogeneous native enzyme was determined (i) by electrophoresis using the method of Hedrick & Smith (1968) with acrylamide concentrations from 4–11% (w/v) in the presence of Triton X-100 (1%, w/v) with BSA oligomers as *M_r* standards, and (ii) by gel-filtration on a Sephadex G-200 superfine column (0.9 × 60 cm) in PB (0.1 M, pH 7.5, containing 1%, w/v Triton X-100) as described by Andrews (1964) with myoglobin, pepsin, ovalbumin, BSA, and lactate dehydrogenase as *M_r* standards. The *M_r* of the subunits was determined by SDS-PAGE using the system of Weber & Osborn (1969) with BSA, ovalbumin, pepsin, chymotrypsin A (bovine), trypsin, cytochrome *c* (horse heart) and RNAase as standards.

Staining for enzyme activity in gels was done as in Hommel & Kleber (1984b). Protein was stained with Coomassie brilliant blue G 250.

Determination of isoelectric point. Isoelectric focussing in polyacrylamide gels was done according to Vestenberg & Svensson (1966). After electrophoresis for 7 h at 250 V, activity staining was done as above and the pI value was estimated from the pH gradient of reference rods and from the following standards: amyglucosidase, glucose oxidase, human serum albumin, insulin and cytochrome *c*.

Extraction of prosthetic group and generation of apoenzyme. Membrane suspensions or enzyme fractions concentrated by ultrafiltration using a XM-50 membrane were extracted by addition of methanol (Ameyama *et al.*, 1981). The methanol extract was evaporated to dryness, dissolved in PB (10 mM, pH 6.1) and extracted five times with chloroform to remove surfactants. The buffer containing the prosthetic group was designated methanol extract.

The remaining membrane fractions and proteins treated with methanol showed no enzyme activity and were considered to be apoenzyme.

Analytical methods. Protein was determined according to Dully & Grieve (1975) with BSA as standard. 3-Deoxy-D-manno-2-octulosonic acid was determined as described by Osborn *et al.* (1972). The concentration of aldehydes was estimated enzymically (Bergmeyer, 1962).

Absorption spectra were recorded with a Perkin-Elmer M-336 spectrophotometer (*d* = 1 cm) at 25 °C. Fluorescence spectra of the prosthetic group were measured with a MPF-4 spectrofluorimeter (*d* = 0.5 cm) in 10 mM-PB (pH 6.1, 25 °C); excitation and emission wavelengths were 370 and 480 nm, respectively.

Kinetic parameters. The apparent *K_m* (*K_m^{app}*) and *V* values were determined by measuring the initial velocities in the presence of seven non-saturating concentrations of the first substrate, at a fixed saturating concentration of the second substrate. The Michaelis and inhibitor constants (*K_{ald}*, *K_{iald}* for the aldehydes and *K_{ic}*, *K_{ifc}* for ferricyanide, respectively) were determined by the two-substrate method. In kinetic experiments each point represents the mean of at least five independent measurements. Standard deviations were less than 5%. Linear correlations were fitted by regression. Results were used with 0.98 ≤ *r* ≤ 1.

Chemicals. All chemicals were of reagent-grade quality or better. Aldehydes were distilled under nitrogen prior to use. Chloral was redistilled immediately before application. The proteins and enzymes were obtained from Serva, Boehringer and AWD (Dresden, GDR).

PQQ and apoquinoprotein glucose dehydrogenase were gifts of Professor J. A. Duine, Delft University, The Netherlands.

Results

Localization

Outer and cytoplasmic membranes were separated by density gradient centrifugation of membrane fractions of bacteria grown on n-hexadecane. Aldehyde dehydrogenase, alcohol dehydrogenase and glucose dehydrogenase showed maxima similar to those of the marker enzymes of cytoplasmic membranes, NADH dehydrogenase and NADH oxidase (Matsushita *et al.*, 1978). The outer membrane marker 3-deoxy-D-manno-2-octulosonic acid exhibited only low aldehyde dehydrogenase activities (less than 10% of those of the cytoplasmic membrane) and no NADH oxidase and NADH dehydrogenase activities. Aldehyde dehydrogenase was purified 2.7-fold. No enzyme activity was measurable at the top of gradient. Using cells grown on glucose or on other water-soluble substrates separation of membranes failed.

Purification, M_r and isoelectric point

Membrane-bound aldehyde dehydrogenase from '*A. rancens*' was purified 173-fold (Table 1). The specific activity of the final enzyme preparation was about 2000 nkat (mg protein)⁻¹. The activity of the purified enzyme was reduced to 50% after 4 d in the presence of the stabilizing agents Triton X-100 and benzaldehyde (Hommel *et al.*, 1985). Therefore, the purification procedure was also accompanied by a decrease in enzyme activity, which resulted in low yields.

SDS-PAGE revealed two aldehyde dehydrogenase subunits, with M_r values of 66000 and 78000 (mean of five determinations). In some estimations a minor band (less than 5% of the total protein) of M_r 48000 was detected. It was thus concluded that native aldehyde dehydrogenase is composed of two dissimilar subunits. Electrophoresis under non-denaturing conditions in the presence of Triton X-100 gave an apparent M_r of 70000 (mean of seven determinations) for the active

enzyme. This indicates dissociation of the enzyme into its subunits in the presence of Triton X-100. The M_r , determined by gel-filtration on Sephadex G-200 superfine in the presence of Triton X-100 was 145000 (± 7000).

The pI of the active enzyme in the presence of Triton X-100 was 5.3.

Investigation of the prosthetic group

Absorption spectra and fluorescence studies of the purified enzyme and the methanol extracts revealed the presence of PQQ by comparison with literature values (Ameyama *et al.*, 1981; Anthony & Zatman, 1967; Dekker *et al.*, 1982) and with authentic PQQ which was treated in the same way as the methanol extracts.

Reconstitution experiments were done with the apoenzyme generated by methanol extraction. After incubation of the apoenzyme with buffer containing PQQ enzyme activity was partly restored. Optimal restoration (about 50%) of activity was obtained after preincubation for 60 min in the presence of Mg²⁺. The concentration-dependent reactivation exhibited saturation kinetics which could be linearized by double reciprocal plots. Shorter incubation periods also yielded good results (10% of initial activity). Activity could also be partly restored by addition of extracts containing PQQ.

Similar activation plots were obtained for glucose dehydrogenase apoquinoprotein with methanol extracts of the quinoprotein aldehyde dehydrogenase [AldDH(acc)].

Attempts to obtain apoenzyme by dialysis following the protocol of Duine *et al.* (1979) failed.

Cytochrome binding

The difference spectrum of the membrane fraction showed cytochrome reduction during incubation with either ethanal or glucose (Fig. 1). The peaks with

Table 1. Purification of membrane-bound aldehyde dehydrogenase from '*A. rancens*'

Step	Protein (mg)	Total activity (nkat)	Specific activity [nkat (mg protein) ⁻¹]	Yield (%)	Purification (-fold)
Crude extract			(5.3)		
Membrane fraction	321	3600	11.2	100	1
Solubilization	18.7	4070	217	113	19.4
DEAE-Sepharose	5.4	2700	500	75	45
Sepharose 4B	1.1	677	615	19	55
Hydroxyapatite	0.08	155	1938	4.3	173

Fig. 1. Difference spectra of membrane fractions of '*A. rancens*' CCM 1774 after incubation with ethanal (a) and glucose (b). Membrane fractions were prepared as described in Methods. AldDH(acc) activity was 5.2 nkat (mg protein)⁻¹ and glucose dehydrogenase activity was 3.7 nkat (mg protein)⁻¹; final protein concentrations were 1 mg ml⁻¹. The membrane fractions were incubated for 5 min in the same buffers described for the kinetic test without artificial electron acceptors. Membrane fractions containing no substrates served as a control (c).

absorption maxima at 553 and 554 nm and at 523 and 528 nm can be assigned respectively as α and β absorption peaks characteristic of reduced *c*-type cytochrome, e.g. cytochrome *c*-553 (Ameyama *et al.*, 1987). Appropriate difference spectra were not obtained with the purified enzyme. Both the purified and the membrane-bound enzyme were able to transfer electrons to horse heart cytochrome *c*. Furthermore, the purified enzyme bound to cytochrome *c*-thiol-Sepharose.

Effect of pH on enzyme activity

Aldehyde oxidation catalysed by purified and membrane-bound AldDH(acc) was optimal at pH 5.1 for the former and at pH 4.7 to 5.7 for the latter. No reduction reaction could be observed at any pH with straight-chain aldehydes ranging from ethanal to dodecanal. The pK values estimated according to Alberty & Massay (1954) and Dixon (1953), and by calculation from the half-height of peak width were 4.1 and 6.1, and 3.9 and 6.6, for the purified and membrane-bound enzyme, respectively. The nature of the buffer seemed not to affect the initial activity, neither did the use of aldehydes of different chain-length.

Fig. 2. Dependence of pK_m^{app} (a) and V^{app} (b) of the purified [$\circ$, mkat (mg protein)⁻¹] and membrane-bound [$\bullet$, nkat (mg protein)⁻¹] AldDH(acc) on the chain-length of substrates.

Effects of salts and inhibitors on enzyme activity

The effect of selected salts and inhibitors on both aldehyde dehydrogenases was examined by incubating them with each of the compounds at 25 °C for 3 min prior to assaying enzyme activity. The results were compared to reference assays in the absence of the effectors. Both forms of AldDH(acc) were activated 1.5-fold by 0.05 mM-MgSO₄, -MnSO₄ and (to a minor extent) -CaSO₄. At a concentration of 5 mM, MgSO₄ and MnSO₄ activated 3.1- and 2.3-fold, respectively. This activation was reversed by 5 mM-EDTA, whereas EDTA itself had no effect on either enzyme form. A 1.9-fold activation of both aldehyde dehydrogenases was obtained with 5 mM-NH₄Cl. Salts of univalent cations (e.g. KCN and NaN₃) had no effect on the membrane-bound enzyme. However, there was a slight activation of the purified enzyme by KCN and NaN₃. Thiol reagents (*p*CMB, AgNO₃, Hg₂Cl₂, NaAsO₂) and iodoacetic acid (all 0.05 mM) were potent irreversible inhibitors of both enzyme forms.

Kinetic properties

For the purified AldDH(acc) substrate inhibition became apparent at concentrations of aldehydes above $5 \times K_m^{\text{app}}$ (ethanal), $1 \times K_m^{\text{app}}$ (propanal, butanal, pentanal) and $3 \times K_m^{\text{app}}$ (other aldehydes), while the membrane-bound enzyme was not affected by any substrate at concentrations up to $5 \times K_m^{\text{app}}$ (data not shown). Below these concentrations the initial velocity followed Michaelis-Menten kinetics. Plots of the apparent pK_m values versus the number of carbon atoms in the aldehydes gave a straight line from ethanal to decanal with the membrane-bound AldDH(acc) and to dodecanal with the purified enzyme (Fig. 2a). With the solubilized enzyme the highest V was measured with

Table 2. Comparison of kinetic constants and derived quotients of kinetic constants

The constants shown are for the oxidation reactions of the membrane-bound and the purified AldDH(acc) with the substrates ethanal, hexanal and octanal under the conditions described in Methods.

Aldehyde	Kinetic constants (mM)				Kinetic quotients		
	K_{ald}	K_{fc}	K_{iald}	K_{ifc}	$\frac{K_{ald} K_{ifc}}{K_{fc} K_{iald}}^*$	$\frac{\varphi_1 \varphi_2}{\varphi_{12} \varphi_0}^\dagger$	V [nkat (mg protein) ⁻¹]
(a) Membrane-bound enzyme							
Ethanal	1.96	0.221	2.19	0.269	1.10	0.89	3.05
Hexanal	0.064	0.420	0.036	0.345	1.44	0.82	1.54
Octanal	0.028	0.348	0.025	0.409	1.29	0.83	1.49
(b) Purified enzyme							
Ethanal	0.913	0.652	1.060	0.763	1.00	1.00	3,410
Hexanal	0.134	0.446	0.147	0.521	1.06	1.09	3,258

* Quotient was calculated according to Cleland (1970).

† Quotient was calculated according to Dalziel (1957).

butanal (Fig. 2b). The K_m^{app} values for ferricyanide of both enzymes were constant irrespective of the aldehydes tested: 0.362 ± 0.018 mM [membrane-bound AldDH(acc)] and 0.552 ± 0.023 mM [purified AldDH(acc)].

Kinetic constants (Table 2) were estimated from replots of slopes and intercepts of double reciprocal plots with intersecting lines on the abscissa, which exclude ping-pong mechanisms. The kinetic constants suggested rapid equilibrium conditions, since $K_{iald} \approx K_{ald}$ and $K_{ifc} \approx K_{fc}$. The quotients calculated according to Dalziel (1957) and Cleland (1970) for the purified enzyme gave a value of 1, typical of a random-order mechanism. Quotients calculated for the membrane-bound enzyme were < 1 (according to Dalziel, 1957) and > 1 (according to Cleland, 1970), which exclude such a mechanism for this enzyme.

For further characterization of the reaction mechanism inhibition studies were done: the two products, and the substrate analogues acetic acid, butanoic acid, hexanoic acid and decanoic acid esters, did not inhibit the enzyme reaction (data not shown). No inhibitory analogue was found for ferricyanide.

The use of chloral hydrate, a substrate analogue that acts as a competitive inhibitor of ethanal (cf. Table 3) and of other aldehydes (data not shown) allowed discrimination between random-order and other sequential reaction mechanisms (Fromm, 1975). The inhibition patterns for the membrane-bound AldDH(acc) (Table 3) were consistent with a compulsory-order mechanism, in which the aldehyde binds to the enzyme first (Fromm, 1975). In intact membranes electron transfer to ferricyanide should occur, as already demonstrated, via *c*-type cytochrome, which is obviously impossible for the

Table 3. Inhibition of AldDH(acc) by chloral hydrate

Substrate	Inhibition by chloral hydrate	
	Membrane-bound enzyme	Purified enzyme
Ethanal	Competitive	Competitive
Ferricyanide	Nonlinear*	Noncompetitive
K_i (mM)	13 (30)†	20 (15)†
Mol inhibitor bound per mol active centre†	1.21	0.94

* Analysis by the method of Baici (1981) showed partially noncompetitive inhibition.

† Estimated according to Chou & Talalay (1981).

purified enzyme. Results of inhibition studies with the purified enzyme agreed with a random-order mechanism.

Discussion

Sucrose density-gradient centrifugation after lysozyme treatment revealed AldDH(acc) to be located in the cytoplasmic membrane. This location would allow electron transfer via a *c*-type cytochrome to the respiratory chain with oxygen acting as terminal acceptor (Hommel & Kleber, 1984b). Aldehyde dehydrogenase in '*A. rancens*' is a hydrophobic integral membrane protein, as shown by its solubilization at a Triton X-100:protein

ratio of 4, its instability in the solubilized form and its specific lipid requirement for phosphatidylglycerol (Hommel *et al.*, 1985).

The M_r of AldDH(acc), a dimeric enzyme with two dissimilar units, is 145 000, which is in the range reported by Ameyama & Adachi (1982) and Adachi *et al.* (1980). By comparing the M_r obtained under denaturing and nondenaturing conditions it may be suggested that the monomer of M_r 70 000 (in the presence of Triton X-100) is the enzymically active subunit; this is in agreement with Fukaya *et al.* (1989) who cloned the gene encoding the M_r 75 000 subunit from *Acetobacter polyoxogenes*. The M_r of the minor band agrees with that (48 000) of cytochrome *c*-553 (Ameyama *et al.*, 1987). However, there is no evidence for its presence in the purified enzyme.

Spectrophotometric investigations of methanol extracts showed differences from highly purified PQQ; these are due to formation of reversible adducts with, e.g., methanol and water, which react with PQQ (Duine *et al.*, 1987). The reconstitution experiments are in accordance with the proposed classification of aldehyde dehydrogenases of acetic acid bacteria as quinoproteins (Ameyama & Adachi, 1982). In reconstitution experiments with extracts from different enzyme preparations, saturation kinetics were obtained in each case. Reconstitution was strongly stimulated by Mg^{2+} , as was that of the glucose dehydrogenase apoquinoprotein. This agrees with the stimulation of enzyme activity by Mg^{2+} , which has been suggested to promote and stabilize binding of PQQ (Adachi *et al.*, 1980; Duine *et al.*, 1987). In contrast to other microbial quinoenzymes (e.g. Dokter *et al.*, 1986), PQQ could not be liberated from the AldDH(acc) by EDTA treatment or dialysis.

The solubilization and purification procedure resulted in alterations in pH optimum without influencing the pK values, and in changes of substrate inhibition. From the dependence of pK_{app}^{25} on the chain-length of aldehydes, it can be concluded that substrate binding by both enzymes was strongly influenced by hydrophobic interactions (Schöpp & Aurich, 1973), as described for the aldehyde dehydrogenase from *Acinetobacter calcoaceticus* (Bergmann, 1981).

The kinetic properties indicate that the quinoprotein aldehyde dehydrogenase from '*A. rancens*' is quite different from other quinoprotein dehydrogenases described so far. The proposed kinetic mechanisms, i.e. compulsory-order for the membrane-bound and random-order for the purified enzyme, are not in accordance with investigations on purified quinoproteins, e.g. quinoprotein glucose dehydrogenase from *Acinetobacter calcoaceticus* (Geiger & Görsch, 1986; Dokter *et al.*, 1986) and quinoprotein methanol dehydrogenase from *Hyphomicrobium* X (Duine & Frank, 1980) for which ping-pong mechanisms have been elucidated. Furthermore, the

inhibition experiments do not agree with a covalent coupling of substrate to PQQ as shown for methanol dehydrogenase (Duine *et al.*, 1987); suicide inhibition by chloral hydrate did not occur, nor was enzyme activity diminished after prolonged incubation with substrates. The results confirm the direct transfer of reduction equivalents from substrate to PQQ, generating PQQH₂ and product.

Quinoproteins described so far have been studied in a highly purified form and appropriate conclusions have been drawn with respect to *in vivo* reactions (Duine *et al.*, 1987). Most microbial PQQ enzymes are membrane-bound or at least associated with membrane structures (Ameyama *et al.*, 1987; Duine *et al.*, 1986). The specific lipid requirements may influence the behaviour of the purified enzyme.

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