FEBS Letters 510 (2002) 196–200 FEBS 25641

A novel bifunctional molybdo-enzyme catalyzing both decarboxylation of indolepyruvate and oxidation of indoleacetaldehyde from a thermoacidophilic archaeon, *Sulfolobus* sp. strain 7

Takayoshi Wakagi[a,*], Eriko Fukuda[a], Yoko Ogawa[a], Hiroyasu Kino[a], Hiroshi Matsuzawa[b]

[a] *Department of Biotechnology, The University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan*
[b] *Department of Bioscience and Biotechnology, Aomori University, 2-3-1 Kohbata, Aomori 030-0943, Japan*

Received 24 September 2001; revised 30 November 2001; accepted 30 November 2001

First published online 11 December 2001

Edited by Judit Ovádi

Abstract An enzyme, which catalyzes both decarboxylation of indolepyruvate and subsequent oxidation of indoleacetaldehyde into indoleacetate, was purified from a thermoacidophilic archaeon, *Sulfolobus* sp. strain 7. The enzyme showed a M_r of 280 kDa on gel filtration and was composed of three subunits (a, 89; b, 30; and c, 19 kDa), possibly in a stoichiometry of 2:2:2. Mo and Fe were detected. Thiamine pyrophosphate was absent. Biotin was suggested to bind to the b-subunit. The first step, the decarboxylation reaction, was specific for 2-oxoacids with an aromatic group, while in the second reaction, various aldehydes including glyceraldehyde, which is a glycolytic intermediate in the organism, were oxidized.

Key words: Indolepyruvate; Indolealdehyde; Decarboxylase; Oxidoreductase; Archaeon

1. Introduction

Sulfolobus sp. strain 7 is a thermoacidophilic archaeon for which the optimal growth conditions are 75°C and pH 3 [1,2]. One of the general archaeal metabolic features is the use of 2-oxoacid:ferredoxin oxidoreductase (OOR) for the coenzyme A- and thiamine pyrophosphate (TPP)-dependent oxidative decarboxylation of 2-oxoacids [1,3,4]. OORs specific to pyruvate, 2-oxo(α-keto)glutarate, 2-oxoisovalerate [5], and indolepyruvate [6] are abbreviated as POR, KOR, VOR, and IOR, respectively [7]. In *Sulfolobus* sp. strain 7, we have identified an enzyme catalyzing the oxidation of both α-ketoglutarate and pyruvate, but neither indolepyruvate (IPyA) nor isovalerate [1]. For further understanding of this enzyme and 2-oxoacid metabolisms in *Sulfolobus*, we tried to obtain IOR and VOR from the same organism, using the conventional artificial electron acceptor methylviologen (MV) instead of ferredoxin for the assaying of this class of enzyme. An enzyme, IPyA:MV oxidoreductase, designated IMOR, was purified to electrophoretic homogeneity and characterized. The enzyme reaction resembles that of IOR in the utilization of IPyA and reduction of MV. However, to our surprise, the enzyme showed no structural or functional features of IOR so far reported, as it turned out that the enzyme was independent of both CoA and ferredoxin. In this paper, we describe the purification and partial characterization of the enzyme, which revealed that it is a biotin- and molybdenum-containing bifunctional enzyme catalyzing both the decarboxylation of IPyA and the oxidation of indole-3-acetaldehyde (IAAld) into indole-3-acetate (IAA). The specificity for other 2-oxoacids and aldehydes, as well as other electron acceptors, was examined.

*Corresponding author. Fax: (81)-3-5841 5152.
E-mail address: atwakag@mail.ecc.u-tokyo.ac.jp (T. Wakagi).

Abbreviations: OOR, 2-oxoacid:ferredoxin oxidoreductase; POR, pyruvate:ferredoxin oxidoreductase; KOR, 2-oxoglutarate(α-ketoglutarate):ferredoxin oxidoreductase; VOR, isovalerate:ferredoxin oxidoreductase; IOR, indolepyruvate:ferredoxin oxidoreductase; IMOR, indolepyruvate:methylviologen oxidoreductase; IAA, indole-3-acetic acid; IPyA, indolepyruvic acid, indole-3-pyruvic acid; IAAld, indole-3-acetaldehyde; DCPIP, dichlorophenolindophenol; MV, methylviologen; TPP, thiamine pyrophosphate

2. Materials and methods

2.1. Reagents

IPyA, IAAld, IAA, DL-glyceraldehyde and other 2-oxoacids were from Sigma Aldrich. DL-Glyceraldehyde-3-phosphate was purchased from Sigma Aldrich as a diethylacetal monobarium salt derivative and treated to obtain a pure material according to the manufacturer's manual. Benzaldehyde and indole-3-aldehyde were from Wako Pure Chemicals. The media for chromatography were from Amersham-Pharmacia and Toso.

2.2. Archaeal strain and purification of the enzyme

The archaeon used in this study was *Sulfolobus* sp. strain 7. The organism was aerobically grown at 75°C and pH 3 on nutrient broth as described previously [1], and then the cells were collected and stored frozen until use. Frozen cells (120 g wet weight) were thawed and resuspended in 50 mM Tris–HCl, pH 7.5 (designated buffer I), containing 0.1 mM phenylmethylsulfonyl fluoride and a trace amount of DNase, disrupted by ultrasonic treatment, and then centrifuged. The supernatant (cytosol) was applied to a column of DEAE-Sephacel (4×20 cm). The proteins were eluted from the column with a gradient of 0–0.5 M NaCl (1 l×2) in buffer I. The active fractions were collected, and made to 1.6 M with solid ammonium sulfate. The suspension was applied to a butyl-Toyopearl 650S column (3×12 cm) pre-equilibrated with 1.6 M ammonium sulfate in buffer I. The active fractions were eluted from the column with a linear gradient of 1.6–0 M ammonium sulfate in buffer I (total 1 l). The active fractions were eluted at 0.7–0.5 M ammonium sulfate. The fractions were dialyzed against buffer I to remove ammonium sulfate. The dialysate was applied to a Mono Q HR 10/10 column connected to an FPLC system (Pharmacia), and eluted with a linear gradient of 0–0.5 M NaCl in buffer I (total 50 ml). The active fractions were combined and concentrated with Centricon 30 (Amicon) to 50 μl, and then applied onto a Superdex 200 HR 10/30 column pre-equilibrated with 50 mM sodium phosphate, pH 7.5, and 150 mM NaCl. The proteins were eluted with the same buffer. The enzyme fractions were collected and dia-

0014-5793/02/$22.00
PII: S0014-5793(01)03259-8

lyzed against 50 mM sodium phosphate, pH 7.5. The enzyme was stored frozen at −80°C until use in the following experiments.

2.3. The assay methods for enzyme activities

Oxidoreductase activity was measured using various 2-oxoacids and aldehydes as electron donors, and an electron acceptor such as MV, ferredoxin/metronidazole, dichlorophenolindophenol (DCPIP), NAD (all from Sigma-Aldrich), or oxygen. IMOR activity was measured at 70°C in an assay mixture (0.5 ml) composed of 100 mM sodium phosphate, pH 8.0, 1 mM CoA, 5 mM $MgCl_2$, 2 mM IPyA (Sigma), 0.1 mM MV (Sigma), and enzyme in a sealed microcuvette. Oxygen was purged from the sealed cuvette by circulating nitrogen gas over the surface of the assay mixture. The absorbance at 600 nm was monitored. The amount of product was calculated using an extinction coefficient of $\varepsilon_{600nm} = 13\ mM^{-1}\ cm^{-1}$ [8] divided by 2 because the oxidation of IPyA releases 2 electrons/mol. One unit was defined as 1μmol product/mg IMOR. When indicated, IPyA was replaced with another substrate. The electron transport activity of ferredoxin was measured in a reaction mixture (500 μl) comprising 100 mM sodium phosphate, pH 7.0, 2 mM substrate, 0.1 mM metronidazole (Sigma), 1 μM *Sulfolobus* ferredoxin [2], 1 mM CoA, 5 mM $MgCl_2$, and enzyme at 70°C. Oxygen was removed from the assay mixture as described above. The reaction was followed as the reduction of metronidazole at A_{320} ($\varepsilon_{320nm} = 9.3\ mM^{-1}\ cm^{-1}$ [9]). The reaction with DCPIP as the electron acceptor was measured aerobically at 50°C in an assay mixture (0.5 ml) composed of 100 mM sodium phosphate, pH 7.0, 5 mM electron donor, 0.2 mM DCPIP, and enzyme. The decrease in absorbance at 600 nm was monitored. One unit was defined as reduction of 1 μmol DCPIP/min, using $\varepsilon_{600nm} = 19.1\ mM^{-1}\ cm^{-1}$ [10].

2.4. Protein determination

Protein was determined by the method involving a bicinchoninic acid assay kit (Pierce), with bovine serum albumin (BSA) as the standard.

2.5. Spectral analyses

Absorption spectra were recorded with a JASCO V-560 spectrophotometer. Circular dichroism (CD) spectra were recorded with a JASCO J750 spectropolarimeter equipped with cylindrical cell of 0.1 or 1 cm light path and a protein concentration of 0.021 or 1.07 mg/ml, respectively, in 10 mM sodium phosphate, pH 7.0. All the spectra were measured at room temperature.

2.6. Molecular weight estimation

The molecular mass of the native enzyme was estimated by Superdex 200 HR 10/30 column chromatography using standard proteins for molecular weight estimation from Sigma, i.e. thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), albumin (66 kDa), and carbonic anhydrase (29 kDa).

2.7. Amino acid sequence analysis

SDS–PAGE was carried out in 12% (w/v) polyacrylamide gels. One of the gels was stained with Coomassie brilliant blue (CBB) to determined the molecular masses of the subunits. The other gel was transblotted onto a polyvinylidene fluoride (PVDF) membrane (Millipore), which was then stained with CBB to visualize the protein bands. The bands were then cut out for amino acid sequence analysis with an Applied Biosystem Model 370A automated gas phase protein sequencer.

2.8. Analyses of biotin and TPP

IMOR (12 μg) was subjected to SDS–PAGE as described above. After the run, the gel was electrophoretically transblotted onto a PVDF membrane in buffer comprising 25 mM Tris–HCl, pH 8.3, 190 mM glycine, 10% (v/v) methanol, and 0.1% SDS. The membrane was washed with 20 mM Tris–HCl, pH 7.5, and 0.15 M NaCl (designated TBS), incubated with 3% gelatin in TBS for 2 h, and then rinsed with 0.2% Tween 20 (Sigma) in TBS (named TTBS). The membrane was incubated with 10 ml of 0.01 mg/ml peroxidase-labeled avidin (Sigma) in 1% (w/v) BSA and 0.02% (w/v) sodium azide in TTBS for 2 h at room temperature, and then washed with TTBS. The membrane was washed with TTBS, incubated with 5 ml of an ECL Western blotting system solution (Amersham Life Science) in a plastic bag, and then kept in the dark in close contact with an X-ray film (Fuji Film). Light emission from the reaction product was recorded by the film.

TPP was analyzed by conversion of the sample to a fluorescent thiochrome derivative by means of alkaline-ferricyanide treatment [11]. IMOR (114 μg, 0.41 nmol) was mixed with 10% (w/v) trichloroacetic acid to remove protein, and the supernatant was washed with diethylether and then analyzed directly or further separated by C_{18} reverse phase HPLC [12]. The detection limit of the fluorescence was about 5 pmol TPP.

2.9. Metal contents

Molybdenum and other metals, as indicated, were determined with an SPQ9000 plasma quadruple mass analyzer. Fe was determined with a Seiko SPS 1200VR inductively coupled plasma spectrometer. Standard solutions of metals were purchased from Wako Pure Chemicals. The enzyme was hydrolyzed at 90°C overnight and then dissolved at a concentration of 0.03–0.1 mg protein/ml in 0.1 N nitric acid.

2.10. Analysis of the reaction product by HPLC

A reaction mixture comprising 100 mM sodium phosphate, pH 7.0, 2 mM IAAld, 2 mM MV, and enzyme was anaerobically incubated at 80°C. After appropriate times, 50 μl aliquots were withdrawn and passed through a Minisart RC4 filter (Sartorius). After filtration, a 10 μl aliquot was analyzed by C_{18} reverse phase HPLC with isocratic elution with a mixture of methanol:water:acetic acid (33:62:5). The flow rate was 0.5 ml/min. The absorbance at 280 nm of the eluate was monitored. Standard IAAld and IAA showed retention times of 2.0 and 3.0 min, respectively.

3. Results and discussion

3.1. Purification of IMOR from Sulfolobus sp. strain 7

A crude extract of *Sulfolobus* sp. strain 7 showed the ability to oxidize IPyA with MV as an electron acceptor, which at first we regarded as the activity of IOR, because an artificial electron acceptor, MV, is usually used in place of ferredoxin in the 2-oxoacid:ferredoxin oxidoreductase assay [1,4]. The activity in the crude extract (cytosol) was not stable, probably due to protease digestion, so the crude extract was immediately applied to a DEAE- Sephacel column. The active fraction eluted from DEAE-Sephacel was tested for stability. There was no difference in residual activity after 10 days at 4°C between aerobic storage (as prepared) and anaerobic storage (replacement of air with N_2 in a sealed vial), suggesting

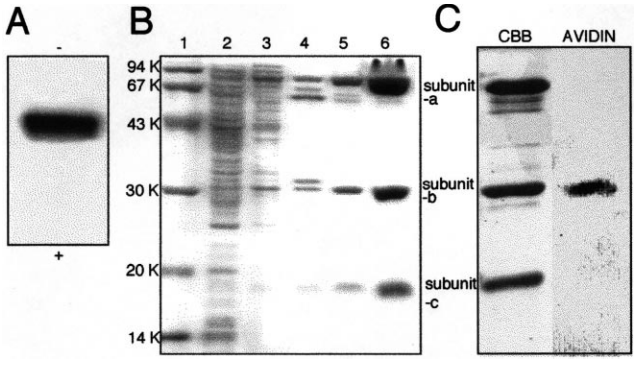

Fig. 1. Native PAGE (A) and SDS–PAGE (B and C) of IMOR. About 5 μg of the purified enzyme was subjected to native PAGE (A). Samples for SDS–PAGE (B): molecular weight standards (lane 1), the crude extract (lane 2), DEAE-Sephacel fraction (lane 3), butyl-Toyopearl fraction (lane 4), Mono Q fraction (lane 5), and Superdex S200 fraction (lane 6). Samples were visualized with CBB. Two 5 μg aliquots of the enzyme were subjected to SDS–PAGE in separate gels; one was stained with CBB (C, left lane), and the other was blotted onto a PVDF membrane. The membrane was incubated with peroxidase-labeled avidin, followed by visualization (C, right lane) as described in Section 2.

that IMOR is not sensitive to oxygen. A typical purification gave a final sample with specific IMOR activity of 8.6 U/mg, with 103-fold purification from the cytosol of the archaeon through four step chromatographies: DEAE-Sephacel, butyl-Toyopearl, Mono Q, and Superdex 200 HR. The recovery of activity was 3.5%, and the amount of the finally purified IMOR corresponded to 0.036% of the cytosol protein. The last step of the purification was gel filtration, and the chromatogram showed a sharp single peak corresponding to a molecular weight of 280 000 (data not shown). Native PAGE of the purified protein in the absence of SDS gave a single band (Fig. 1A), indicating the preparation was homogeneous. SDS–PAGE gave three polypeptide bands (a, 89 kDa; b, 30 kDa; and c, 19 kDa, see Fig. 1B). These results suggest that the enzyme is an $a_2b_2c_2$ hexamer. The absorption spectrum (Fig. 2A) showed maxima at 278, 341, 428 and 462 nm. The CD spectra showed characteristic features in the near UV and visible regions (Fig. 2B–D). Troughs at 378 and 550 nm, and a peak at 433 nm with a shoulder at 470 nm were found in a visible region (Fig. 2D).

3.2. Subunit composition, N-terminal sequence, and homology to aldehyde oxidase

Upon SDS–PAGE, the protein gave three bands corresponding to molecular weights of 89 000, 30 000 and 19 000, which were designated subunits a, b, and c, respectively. The N-terminal amino acid sequences of subunits a, b, and c were determined, and homologous sequences are shown in Table 1. Judging from the N-terminal sequences of the three subunits, the present IMOR is a similar protein to a putative CO dehydrogenase from *Sulfolobus solfataricus* P2 [13], and aldehyde oxidoreductase from *Sulfolobus acidocaldarius* [14]. The N-terminal sequence of subunit c was identical with that of an iron–sulfur flavoprotein from the same organism, which we reported previously [15]. Moreover, comparison of the subunit compositions (Fig. 1), absorption spectra (Fig. 2A), and CD spectra (Fig. 2D) revealed that these proteins are identical to each other.

The *S. solfataricus* P2 CO dehydrogenase large subunit exhibits homology with that of *Desulfovibrio gigas* aldehyde oxidase, which is a homodimeric enzyme composed of two 907 residue subunits that each contain a molybdenum coordinated by a single molybdopterin cytosine dinucleotide, and two different [2Fe–2S] clusters [16]. Recently, *S. acidocaldarius* aldehyde oxidoreductase, designated SaAO, was suggested to contain FAD, molybdopterin modified with GMP, and two different types of [2Fe–2S] clusters [14]. On the other hand, cofactors of the iron–sulfur flavoprotein from *Sulfolobus* sp. strain 7 contain at least two FMNs and two [2Fe–2S] clusters per $a_2b_2c_2$ molecule [15]. The present IMOR exhibits aldehyde oxidoreductase activity like SaAO, but its specificity for aldehydes is broader (Table 2). The metal contents, 0.8 mol Mo

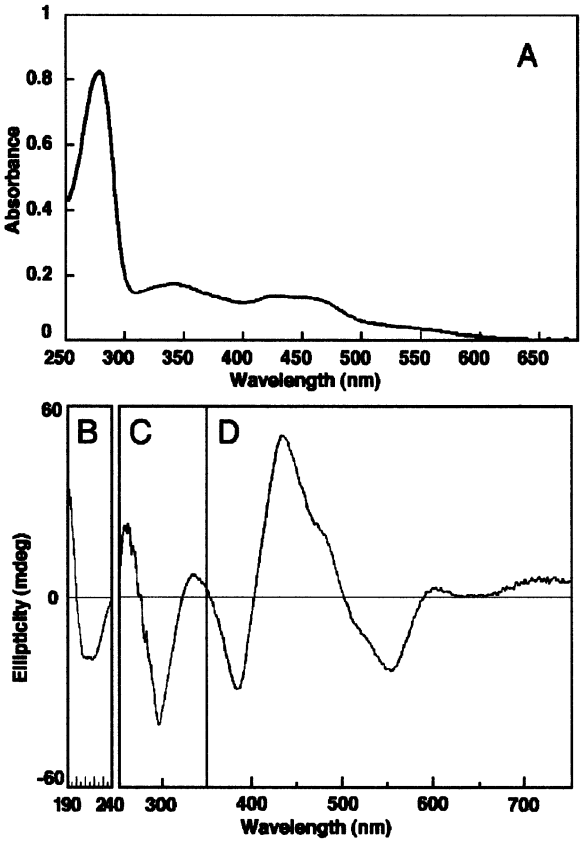

Fig. 2. Absorption spectrum (A) and CD spectra (B–D) of IMOR from *Sulfolobus* sp. strain 7. The protein concentration was 0.83 mg/ml (A), 0.021 mg/ml (B, 0.1 cm light path), or 1.07 mg/ml (C and D, 1 cm light path).

Table 1
N-terminal amino acid sequences of the three subunits, a, b and c, of IMOR from *Sulfolobus* sp. strain 7 and homologous proteins

Protein source	Sequence	Function[a]	Size
Sulfolobus sp. strain 7 a	1 **MRYVGQAVKRLYDDKFVTG** 19	IMOR	89 kDa
S. solfataricus P2 gi|3815444	1 **MYVGQRVKRKEDLKLITG** 18	(CO dehydrogenase)[b]	729 aa[b]
S. acidocaldarius α	1 **MYVGQRVKRKEDLKLITG** 18	aldehyde oxidoreductase[c]	80.5 kDa
Sulfolobus sp. strain 7 b	1 **MFPKQFGYYRPSSLADA** 17	IMOR	32 kDa
S. solfataricus P2 gi|3815948	1 **MYPPDFTYVRVSSSEEA** 17	(CO dehydrogenase)[b]	286 aa[b]
Amycolatopsis methanolica P80472	1 **MIPAQFTYRRVSSVDEA** 17	aldehyde dehydrogenase[d]	35 kDa
S. acidocaldarius β	1 **MYPFEFSYVRAESLQEA** 17	aldehyde oxidoreductase[c]	32 kDa
Sulfolobus sp. strain 7 c	1 **MLVRPGEKVKIRVKVN** 16	IMOR	19 kDa
Sulfolobus sp. strain 7 ISFP[e]	1 **MLVRPGEKVKIRVKVN** 16		19 kDa
S. solfataricus P2 gi|3815949	1 **MLVRPGEKVKIRVKVN** 16	(CO dehydrogenase)[b]	142 aa[b]
S. acidocaldarius γ	1 **LVVKKGEGVKVRVRRN** 16	aldehyde oxidoreductase[c]	19.5 kDa[c]

[a]Parentheses indicate that the function was deduced from the homology.
[b]From the genome database [13].
[c][14].
[d]The b-chain (sp\|P80472\|DHAB_AMYME), found on a homology search with SwissProt.
[e]Iron–sulfur flavoprotein of unknown function [15].

and 8.7 mol Fe per mol, of IMOR are in good agreement with those of *D. gigas* aldehyde oxidase (2 Mo and 8 Fe atoms) [16], except that the molybdenum content is about half.

The present IMOR aldehyde oxidase is likely to have pterin but is different from several archaeal pterin-dependent aldehyde oxidases that use ferredoxin as an electron acceptor [17–19].

3.3. Optimum temperature and pH for the reaction, and thermostability

The activities at 50, 60, 70, 80, 90, and 100°C were 0.05, 2.5, 7, 11, 32, and 47 U/mg, respectively. The relative activities at pH 6, 7, 8, 9, and 10 were 15, 30, 100, 86, and 55%, respectively. The optimum pH of the enzyme was around pH 8. Activity was scarcely lost on incubation of the enzyme at 70°C for 30 min at a concentration of 0.1 mg/ml in 0.1 M sodium phosphate, pH 7.0. However, 22 and 97% inactivation was observed at 90 and 100°C, respectively.

3.4. Cofactors and other electron acceptors

As shown above, IPyA showed the ability to reduce MV enzymatically. Other electron acceptors were tested. A zinc-containing ferredoxin from the same organism [2] was examined by using metronidazole, which accepts electrons from reduced ferredoxin. However, metronidazole was not reduced even in the presence of 0.25 mM coenzyme A and/or 0.1 mM TPP (data not shown; significant reduction of metronidazole was detected in a parallel experiment where the IMOR and IPyA were replaced with OOR and pyruvate, respectively). This indicates that the enzyme is not IOR.

The results of thiochrome analysis and C_{18} reverse phase HPLC showed that IMOR contained no TPP, whereas 1.9 mol FMN/mol enzyme was detected (data not shown). The ability of each subunit to bind avidin was examined. As shown in Fig. 1C, avidin reacted with subunit b, suggesting that subunit b binds biotin. Although non-specific binding of avidin to a certain peptide is possible [20], and biotin-dependent decarboxylation of a 2-oxoacid is unusual, we tentatively assume that biotin bound to subunit b is responsible for the decarboxylation reaction.

3.5. Reaction product from IAAld

Analysis of the products of the reaction mixture containing IAAld and MV was carried out by C_{18} reverse phase HPLC. The results are shown in Fig. 3. The concomitant decrease in IAAld (2.0 min retention) and increase in IAA (3.0 min retention) are clearly shown. This indicates that the IMOR catalyzes the oxidation of IAAld into IAA.

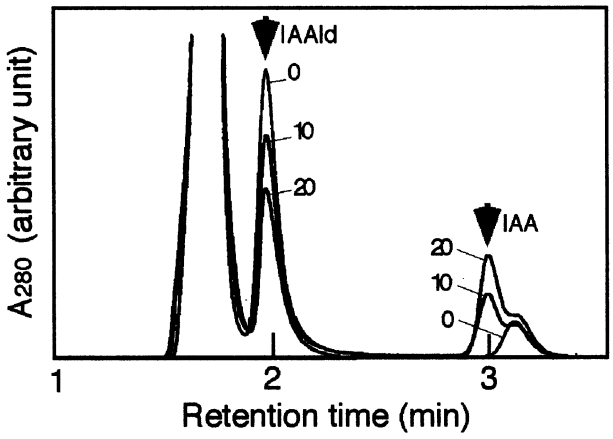

Fig. 3. HPLC analysis of IMOR reaction products. Samples containing 5 mM IAAld, 5 mM MV, and enzyme were anaerobically incubated at 80°C for 0, 10, and 20 min. The reaction mixture was filtered and then injected into a C_{18} reverse phase HPLC column. The positions of standard IAAld and IAA are indicated by arrows.

3.6. Dependence of the reaction on other electron acceptors

DCPIP was reduced at rates of 1.16 and 1.10 U/mg of IMOR for DL-glyceraldehyde and DL-glyceraldehyde-3-phosphate (5 mM each), respectively, at 50°C. Other substrates such as IPyA and IAAld were not suitable for measuring the activity because both reduced DCPIP non-enzymatically even at 40°C. Neither NAD, NADP, FMN nor FAD was enzymatically reduced with IpyA or IAAld in 50 mM sodium phosphate, pH 8.0, at 40 or 80°C.

3.7. Substrate specificity with MV as the electron acceptor

Based on the sequence homology, we assumed that the IMOR activity could be accompanied by aldehyde oxidase activity. Table 2 summarizes the kinetic parameters of substrate:MV oxidoreductase at 80°C, with not only 2-oxoacids but also several aldehydes as substrates. Among the 2-oxoacids tested, IPyA was the best substrate, while phenylpyruvate reacted slowly, and other 2-oxoacids with non-aromatic side chains did not react at all. As expected, aldehydes were good substrates. IAAld was one of the best substrates in terms of both the K_m and k_{cat} values. Phenylacetaldehyde and benzaldehyde reacted with similar V_{max} values to that for IAAld,

Table 2
Substrate specificity of IMOR

Substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} mM^{-1})
2-Oxoacids:			
Indole-3-pyruvate (IPyA)	0.788	45.3	57.5
Phenylpyruvate	1.66	13.1	7.88
Pyruvate	–	0	0
2-Oxoglutarate	–	0	0
2-Oxoisovalerate	–	0	0
Aldehydes:			
Indole-3-acetaldehyde (IAAld)	0.00903	50.0	5530
Phenylacetaldehyde	0.105	32.8	312
Benzaldehyde	0.395	35.8	90.6
Glyceraldehyde	0.0420	97.6	2320
Glyceraldehyde-3-phosphate	–	0	0
Indole-3-aldehyde	–	0	0

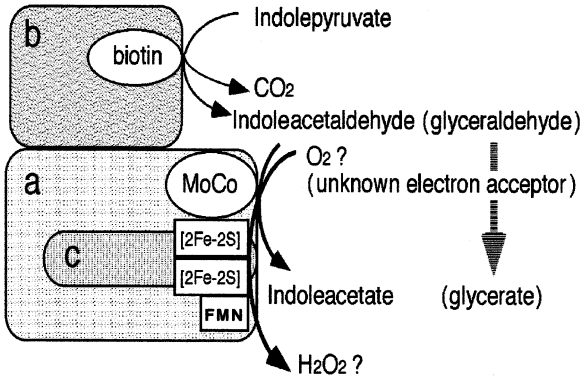

Fig. 4. Possible architecture of IMOR. MoCo is a molybdenum cofactor.

but their K_m values were much higher. Notably, indole-3-aldehyde did not react at all. Surprisingly, aldehydes without bulky side chains were also reactive. DL-Glyceraldehyde exhibited an about twice higher k_{cat} value than IPyA and IAAld. Glycolysis of *Sulfolobus* is carried out through the non-phosphorylated Entner–Doudoroff pathway [21]. The rapid oxidation of glyceraldehyde catalyzed by IMOR is a key step of the central metabolism from glucose to pyruvate in the organism, which has also been pointed out for SaAO [14].

As to the decarboxylating activity of the IMOR, the substrate specificity is quite limited. Only those compounds with a bulky side chain attached to carbon 3 of pyruvate were good substrates. The specificity to aldehydes with bulky side chains also seems to be strict: indole-3-acetaldehyde was rapidly oxidized but indole-3-aldehyde did not react at all. In conclusion, IMOR catalyzes two reaction steps: decarboxylation of IPyA to IAAld, and oxidation of IAAld to IAA. Such a pathway has so far been deduced only for plant-related bacteria producing IAA, an important plant hormone. The wide specificity of aldehyde oxidation implies the enzyme plays multifunctional roles in both the oxidative catabolism of bulky aromatic amino acids such as tryptophan, on the one hand, and the maintenance of a glycolytic energy flow via glyceraldehyde dehydrogenation, on the other hand. These reaction pathways and the molecular architecture of IMOR are schematically presented in Fig. 4. Decarboxylating activity has never been reported for any aldehyde oxidase or a related enzyme belonging to the xanthine dehydrogenase family. This novel activity is attributed to the biotin moiety bound to the middle subunit (Fig. 2C). A low rate of oxygen consumption was observed during the reaction with various aldehydes at room temperature (data not shown). Taking the optimal growth temperature of the organism into account, oxygen is one of the candidates for the physiological acceptor of electrons in these reactions.

Acknowledgements: This work was partly supported by Grants 09660074 and 12660067 from the Japan Society for the Promotion of Science to T.W.

References

[1] Zhang, Q., Iwasaki, T., Wakagi, T. and Oshima, T. (1996) J. Biochem. (Tokyo) 120, 587–599.
[2] Wakagi, T., Fujii, T. and Oshima, T. (1996) Biochem. Biophys. Res. Commun. 225, 489–493.
[3] Kerscher, L. and Oesterhelt, D. (1982) Trends Biochem. Sci. 7, 371–374.
[4] Kletzin, A. and Adams, M.W. (1996) J. Bacteriol. 178, 248–257.
[5] Heider, J., Mai, X. and Adams, M.W. (1996) J. Bacteriol. 178, 780–787.
[6] Mai, X. and Adams, M.W. (1994) J. Biol. Chem. 269, 16726–16732.
[7] Tersteegen, A., Linder, D., Thauer, R.K. and Hedderich, R. (1997) Eur. J. Biochem. 244, 862–868.
[8] Thorneley, R.N. (1974) Biochim. Biophys. Acta 333, 487–496.
[9] Basford, R.E. and Huennekens, F.M. (1955) J. Am. Chem. Soc. 77, 3873–3876.
[10] Townson, S.M., Hanson, G.R., Upcroft, J.A. and Upcroft, P. (1994) Eur. J. Biochem. 220, 439–446.
[11] Penttinen, H.K. (1979) Methods Enzymol. 62, 58–59.
[12] Gubler, C.J. and Hemming, B.C. (1979) Methods Enzymol. 62, 63–68.
[13] She, Q., Singh, R.K., Confalonieri, F., Zivanovic, Y., Allard, G., Awayez, M.J., Chan-Weiher, C.C., Clausen, I.G., Curtis, B.A., De Moors, A., Erauso, G., Fletcher, C., Gordon, P.M., Heikamp-de Jong, I., Jeffries, A.C., Kozera, C.J., Medina, N., Peng, X., Thi-Ngoc, H.P., Redder, P., Schenk, M.E., Theriault, C., Tolstrup, N., Charlebois, R.L., Doolittle, W.F., Duguet, M., Gaasterland, T., Garrett, R.A., Ragan, M.A., Sensen, C.W. and Van der Oost, J. (2001) Proc. Natl. Acad. Sci. USA 98, 7835–7840.
[14] Kardinahl, S., Schmidt, C.L., Hansen, T., Anemüller, S., Petersen, A. and Schäfer, G. (1999) Eur. J. Biochem. 260, 540–548.
[15] Iwasaki, T., Wakagi, T. and Oshima, T. (1995) J. Biol. Chem. 270, 17878–17883.
[16] Romao, M.J., Archer, M., Moura, I., Moura, J.J., LeGall, J., Engh, R., Schneider, M., Hof, P. and Huber, R. (1995) Science 270, 1170–1176.
[17] Chan, M.K., Mukund, S., Kletzin, A., Adams, M.W. and Rees, D.C. (1995) Science 267, 1463–1469.
[18] Mukund, S. and Adams, M.W. (1993) J. Biol. Chem. 268, 13592–13600.
[19] van der Oost, J., Schut, G., Kengen, S.W., Hagen, W.R., Thomm, M. and de Vos, W.M. (1998) J. Biol. Chem. 273, 28149–28154.
[20] Hans, M., Buckel, W. and Bill, E. (2000) Eur. J. Biochem. 267, 7082–7093.
[21] De Rosa, M., Gambacorta, A., Nicolaus, B., Giardina, P., Poerio, E. and Buonocore, V. (1984) Biochem. J. 224, 407–414.