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Mapping the Primary Structure of Copper/Topaquinone-Containing Methylamine Oxidase from *Aspergillus niger*

R. LENOBEL[a], M. ŠEBELA[b], I. FRÉBORT[b]

[a]*Laboratory of Growth Regulators and* [b]*Department of Biochemistry, Faculty of Science, Palacký University, 783 71 Olomouc, Czechia*

e-mail sebela@prfholnt.upol.cz

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ABSTRACT. The amino acid sequence of methylamine oxidase (MeAO) from the fungus *Aspergillus niger* was analyzed using mass spectrometry (MS). First, MeAO was characterized by an accurate molar mass of 72.4 kDa of the monomer measured using MALDI-TOF-MS and by a p*I* value of 5.8 determined by isoelectric focusing. MALDI-TOF-MS revealed a clear peptide mass fingerprint after tryptic digestion, which did not provide any relevant hit when searched against a nonredundant protein database and was different from that of *A. niger* amine oxidase AO-I. Tandem mass spectrometry with electrospray ionization coupled to liquid chromatography allowed unambiguous reading of six peptide sequences (11–19 amino acids) and seven sequence tags (4–15 amino acids), which were used for MS BLAST homology searching. MeAO was found to be largely homologous to a hypothetical protein AN7641.2 (*EMBL*/*GenBank* protein-accession code EAA61827) from *Aspergillus nidulans* FGSC A4 with a theoretical molar mass of 76.46 kDa and p*I* 6.14, which belongs to the superfamily of copper amine oxidases. The protein AN7641.2 is only little homologous to the amine oxidase AO-I (32 % identity, 49 % similarity).

The filamentous fungus *Aspergillus niger* represents a microorganism of significant biotechnological importance. In Asia, it is widely used in the manufacture of fermented foods and beverages. However, the primary use of *A. niger* resides in the production of enzymes and organic acids by fermentation (Abarca *et al*. 2004). It is able of growing on solid or in liquid media under a variety of nutritional conditions. Just 40 years ago, amine oxidase activity was detected in crude extracts of various *A. niger* strains after induction with 1-butanamine. The enzyme was purified and crystallized, at that time as the first example of amine oxidase produced by microorganisms (Yamada *et al*. 1965). Later on, two different copper/topaquinone-containing amine oxidases (CAOs; EC 1.4.3.6), named AO-I and AO-II, were isolated from the strain AKU 3302 using the same induction (Frébort *et al*. 1996). The complete amino acid sequence of AO-I (*EMBL*/*GenBank* protein-accession code AAK51081) was deduced from both cDNA and gene cloning and the protein 3-D structure was modeled (Frébort *et al*. 2003). *A. niger* strains can produce CAOs of different molecular properties and substrate specificity upon induction by adding different amines into culture media (Frébort *et al*. 1999). Surprisingly, the strain AKU 3302 does not grow on methylamine as the sole carbon and nitrogen source. However, when the mycelium is incubated with methylamine, a CAO is induced, which differs from the above AO-I and AO-II (Frébort *et al*. 1999). This methylamine oxidase (MeAO) was also purified and characterized. Northern blot analysis and sequencing of several internal peptides indicated large structural differences between MeAO and AO-I (Frébort *et al*. 1999).

Analysis of MeAO performed in this study involved intact mass determination and peptide mass fingerprinting using MALDI-TOF-MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) and *de novo* peptide sequencing using LC-ESI-MS/MS (tandem mass spectrometry with electrospray ionization coupled to liquid chromatography). MALDI-TOF-MS revealed a clear peptide mass fingerprint after tryptic digestion. Nevertheless, this fingerprint did not provide any relevant hit when searched against a nonredundant protein database. It also showed no partial agreement with that of *A. niger* amine oxidase AO-I. Fortunately, LC-ESI-MS/MS experiments allowed unambiguous reading of thirteen peptide sequences and sequence tags, which were used for MS BLAST homology searching in connection with the results of the project of genome sequencing of *Aspergillus nidulans* FGSC A4 in the Broad Institute, Cambridge, MA, USA (Birren *et al*., Genome sequence of *A. nidulans*; published on **http://www.broad.mit.edu/annotation/fungi/aspergillus/**). We found homologous or identical sequences between MeAO and

a hypothetical protein AN7641.2 from *A. nidulans* (*EMBL*/*GenBank* protein-accession code EAA61827), which comprises both the topaquinone cofactor consensus sequence TXXNY(D/E)(N/Y) and copper-binding motif HQH characteristic for all known CAOs (Frébort and Adachi 1995; Dooley 1999).

MATERIALS AND METHODS

Chemicals. The following matrices for MALDI-TOF-MS were used for peptide mass fingerprinting and intact mass determination: α-cyano-4-hydroxycinnamic acid (CHCA) from *Bruker Daltonik* (Germany) and sinapic acid (SA) from *Waters-Micromass* (UK). Raffinose-modified trypsin was prepared from bovine trypsin (*ICN Biomedicals*, USA) according to a protocol which will be published elsewhere. Common chemicals for chromatography and electrophoresis were of analytical purity and were purchased from *Sigma-Aldrich Chemie* (Germany).

Enzyme purification. MeAO was isolated according to Frébort *et al*. (1999); amine oxidase AO-I was purified according to Frébort *et al.* (1996). Aliquots of both enzymes were lyophilized and stored at –50 °C.

Electromigration methods. SDS-PAGE was done according to Laemmli (1970) using 10 % running and 4 % stacking vertical slab gels (thickness 1 mm). After fixation, proteins in gels were stained by Coomassie Brilliant Blue R-250 (CBB). Isoelectric focusing (IEF) was done using a 5 % vertical slab gel (Robertson *et al.* 1987) with Servalyte 2-11 (*Serva*, Germany). After shaking with 1 % trichloroacetic acid to remove ampholytes (1 h) and repeated washings in water (5×, 1-min intervals), IEF gels were stained with CBB.

Intact mass measurements using MALDI-TOF-MS. First, MALDI target surface was modified by a thin layer of matrix according to Vorm *et al*. 1994 (10 mg/mL SA in acetone; 0.5 μL applied per spot to spread and dry). For intact mass determinations of MeAO, 1 μL of the enzyme solution in MS-quality water (≈10 mg/mL) was mixed with 3 μL of matrix (10 mg/mL SA in 0.1 % trifluoroacetic acid–acetonitrile 1:1, *V*/*V*). Aliquots (0.6 μL) were then spotted on the modified target and left to dry. Mass spectra were recorded on a Micro MX MALDI-TOF instrument (*Waters-Micromass*) in a linear mode for positive ions. External mass calibration was done using molecular ions of bovine serum albumin. Not less than 100 single-pulse spectra were summed and smoothed by Savitzky–Golay filter.

Peptide mass fingerprinting using MALDI–TOF-MS. MeAO and AO samples (5–10 pmol) were first resolved by SDS-PAGE with CBB staining. Protein bands were excised from the gel slab, cut into small pieces and put into 0.65-mL PCR microtubes (*Roth*, Germany). Accelerated in-gel digestion (Havliš *et al*. 2003) by the modified trypsin was done at 55 °C for 6 h. MALDI probes were prepared using CHCA matrix (Havliš *et al.* 2003). Measurements were performed on a Reflex IV MALDI-TOF mass spectrometer (*Bruker Daltonik*), equipped with a Scout 384 ion source. The acquired spectra were processed by XMass 5.1.1 and BioTools 2.1 software (*Bruker Daltonik*). Proteins were identified using the Mascot 1.8 program (*Matrix Science*, UK) installed on a local server. Database searches were performed against a nonredundant protein sequence database (MSDB) downloaded from the *European Bioinformatics Institute* (EBI).

Peptide sequencing using LC-ESI-MS/MS. Experiments were done on a Q-Tof micro™ mass spectrometer (*Waters-Micromass*) coupled to a chromatographic system CapLC (*Waters*, USA). The collision-induced dissociation was used to get MS–MS data. Tryptic in-gel digestion of MeAO samples was performed as given above. Digestion products were extracted using formic acid and acetonitrile (Shevchenko *et al*. 1996). The extracts were dried in a rotary vacuum concentrator Univapo 150H (*UniEquip*, Germany). Then the respective solids were each dissolved in 30 μL of 5 % (*V*/*V*) formic acid. After preconcentration on a reversed-phase C18 cartridge Opti-Pak™ Symmetry 300™ (0.35 × 5 mm; particles 5 μm; *Waters*), the peptide solutions were separated by capillary liquid chromatography on an Atlantis™ dC18 reversed-phase column (0.32 × 150 mm; 3 μm particles; *Waters*), where a linear gradient was made by mixing the following solvents: *A*: 0.1 % (*V*/*V*) formic acid in 2 % (*V*/*V*) acetonitrile, and *B*: 0.1 % formic acid in 90 % acetonitrile. The total time of the gradient from 0 to 100 % of *B* was 90 min. The eluate was directly introduced to mass spectrometer at a flow rate of 3 μL/min and analyzed in the survey scan mode. Double- and triple-charged ions in MS mode were selected for fragmentation. MS and MS–MS data were processed using the programs MassLynx 4.0, ProteinLynx Global Server 2.2 (both *Waters-Micromass*) and Mascot Distiller 1.1.1 (*Matrix Science*). Database searches were done against nonredundant protein database MSDB using a freely accessible Internet version of the Mascot program (**`http://www.matrixscience.com`**). Homology-based database searching was performed using the program MS BLAST (**`http://dove.embl-heidelberg.de/Blast2/msblast.html`**; Shevchenko *et al*. 2001).

RESULTS AND DISCUSSION

MeAO has been described as an enzyme produced in mycelia of *A. niger* on incubation with methylamine; it was first isolated by Frébort *et al.* (1999) and shown to differ from *A. niger* amine oxidase AO-I in both kinetic and molecular properties. A hypothesis based on the results of Northern blotting was postulated at that time, that the enzyme might show large differences compared to AO-I at the level of primary structure. Indeed, five *de novo* sequenced peptides were either little homologous to amino acid sequences of CAOs known at that time or were not homologous at all (Frébort *et al.* 1999). However, obtaining more detailed information with respect to the amino acid sequence has remained necessary in order to shed light on MeAO relationships to other representatives of microbial CAOs. Here we subjected the enzyme to peptide analysis using MALDI-TOF-MS and LC-ESI-MS/MS performed to acquire complete peptide sequences or amino acid sequence tags allowing homology searching in protein databases.

MeAO was isolated as a homogeneous protein and its purity was checked by SDS-PAGE. The enzyme migrated as a single protein band referring to a molar mass of 70.6 kDa. Since this value was slightly higher than that reported previously for the MeAO subunit (66.6 kDa; Frébort *et al.* 1999), MALDI-TOF-MS in the linear mode using SA matrix was applied for final confirmation. Fig. 1 shows a typical measured mass spectrum of native MeAO with distinct peaks at *m/z* 36229, 48411, 72431 and 144983. These peaks could be regarded as displays of differently charged molecular ions. Native MeAO is a homodimeric enzyme (Frébort *et al.* 1999). As the peak at *m/z* 72431 is the highest in intensity, it probably reflects a dissociation of the molecule to monomers during ionization. The low-intensity peak at *m/z* 144983 could represent a single-charged molecular ion of the dimeric enzyme but it may also refer to a dimeric adduct ion formed from the separate monomers (Senko and McLafferty 1994). Isoelectric focusing provided a p*I* value of 5.8 matching exactly the original result (Frébort *et al.* 1999).

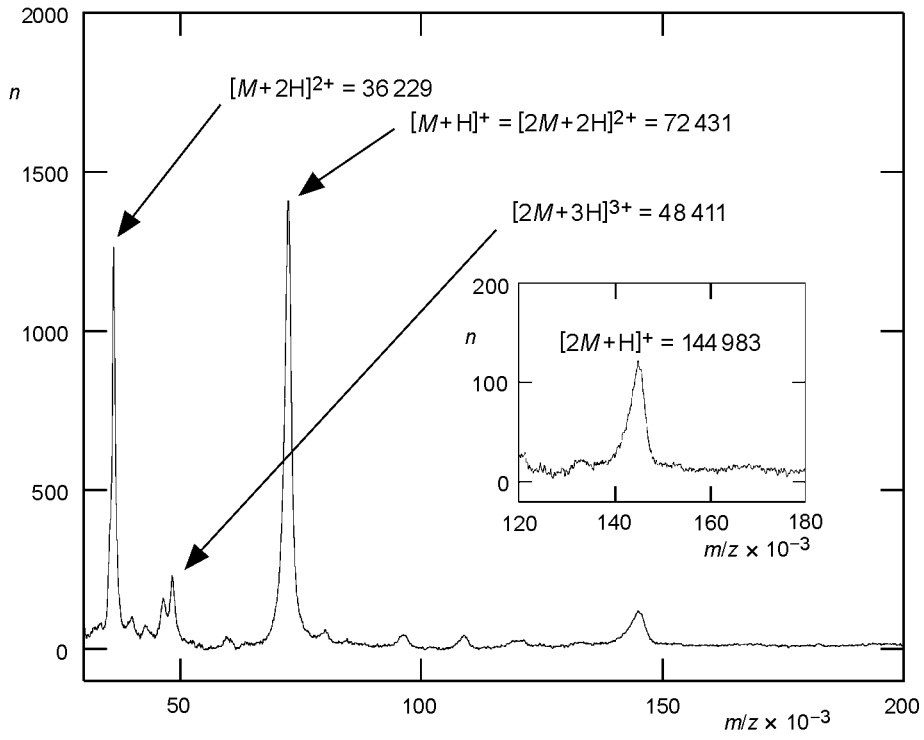


Fig. 1. MALDI-TOF mass spectrum (*m/z*) of intact MeAO; *n* – counts. Molar mass of homogeneous MeAO was obtained using a highly concentrated sample of the enzyme (10 mg/mL); SA served as a matrix. Several peaks were recorded referring to differently charged ionic forms of the enzyme molecule. The spectrum also indicates that native homodimeric MeAO somehow dissociates to its subunits during ionization (*M* and 2*M* represent molecular mass values of MeAO monomer and dimer, respectively). The *inset* shows a zoomed in region of high *m/z* values.

Peptide mass fingerprinting using MALDI-TOF-MS (CHCA matrix) revealed large differences between the primary structures of MeAO and AO-I (Fig. 2). On the first view, one can easily compare a dis-

similar distribution of intense peptide peaks in the *m/z* range 800–3000 in the respective fingerprints. Whereas for MeAO there are two main groups of peptides centered around the peaks at *m/z* 1283.7/1422.7 and 2280.3 (Fig. 2 *above*), AO-I peptides are distributed more or less regularly throughout the whole mass spectrum (Fig. 2 *below*). Database searching using the Mascot program was successful only in the case of AO-I. The fingerprint was assigned with a high probability score to the protein accession code Q96X06 (*A. niger* amine oxidase, identical with the accession code AAK51081). Conversely, there was no relevant hit registered in the database for the MeAO fingerprint.

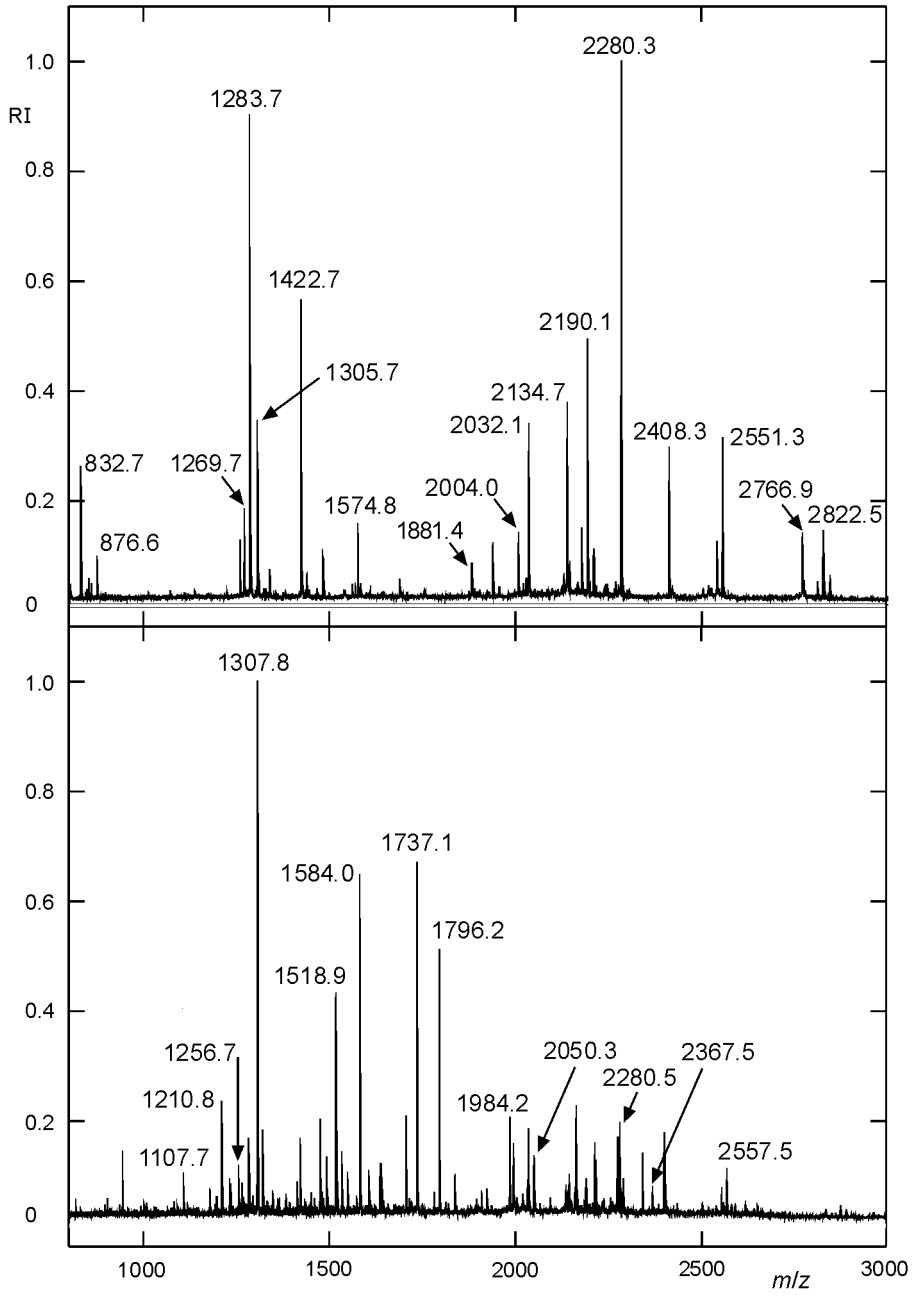


Fig. 2. MALDI-TOF peptide mass fingerprinting of MeAO and AO-I. Mass spectra of tryptic digests of MeAO and AO-I were recorded using the CHCA matrix; RI – relative intensity. Typical peptide maps of MeAO (*above*) and AO-I (*below*) are shown. For MeAO, there was no relevant hit in the MSDB protein database. For AO-I, the presented map matched the sequence of *Aspergillus niger* amine oxidase (protein accession code Q96X06) with Mowse score 122, sequence coverage 43.8 % and error 90 ppm.

Prior to peptide sequencing using tandem mass spectrometry, MeAO was in-gel digested by modified trypsin. The obtained digests were subjected to capillary chromatography after extraction and the separated

peptides were continually introduced to the electrospray ion source of Q-Tof micro instrument. Appropriate precursor ions were selected in mass spectra using the program MassLynx, isolated and fragmented in the collision cell. From thirteen fragmented peptides, the complete amino acid sequences were determined in six cases by means of the programs ProteinLynx Global Server or Mascot (Table I). For the others, sequence tags of various length (4–15 amino acids) were manually read based on *y*-ion peptide fragment series (Fig. 3). All the obtained peptide sequences and tags were subjected to homology searching in the nonredundant database nrdb-95 using the program MS BLAST, which has been developed for charting the proteomes of organisms with unsequenced genomes (Shevchenko *et al*. 2001).

Table I. Peptide sequences and sequence tags determined by *de novo* sequencing of MeAO[a]

Peptide no.	Relative mass[b]	Determined amino acid sequence (S) or sequence tag (T)	
1	1257.6	YSDDQLHPAGR	S
2	1268.7	LDLALHK	T
3	1282.7	NPALDVPPSFAR	S
4	1421.7	VNFNAVTLYEPR	S
5	1479.7 (Met_{ox})	GAIHYMDAAFVNR	S
6	1518.8	LTQPEGVSPS	T
7	1573.8	HTDFRDESVLSLR	S
8	1605.5	WQNW	T
9	1839.1	LLNTYAMNP	T
10	2003.0	EVGSAENPYGNA	T
11	2189.1 (Met_{ox})	LSLAEMVVPYGNPEHPHQR	S
12	2407.3	PLSTVELDAAVALLR	T
13	2550.3	EGLVLNNLTF	T

[a]ESI-MS/MS of precursor peptide ions was performed on *Waters-Micromass* Q-Tof micro™ instrument; a unified symbol L stands for the isobaric amino acids L and I.
[b]Methionine sulfoxide.

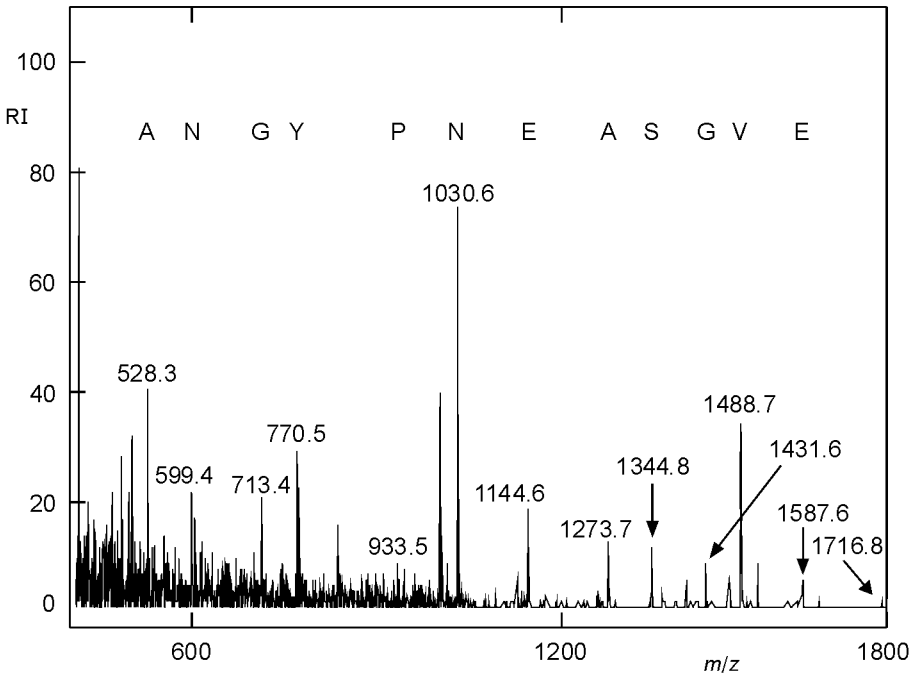
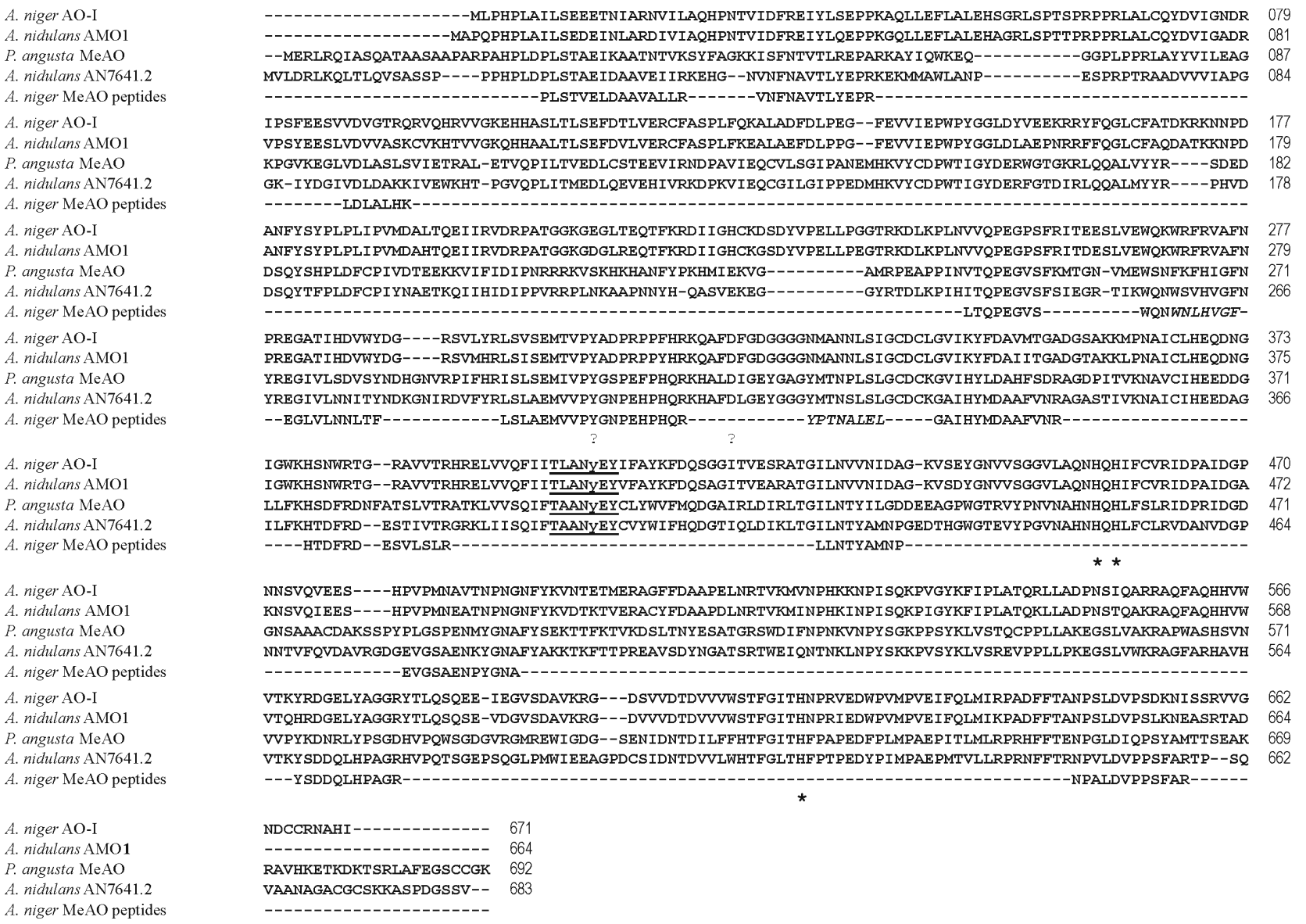


Fig. 3. LC-ESI-MS–MS *de novo* sequencing of tryptic peptide released from MeAO; RI – relative intensity; *m/z* – mass-to-charge ratio difference. After chromatographic separation of the MeAO tryptic digest, a precursor double-charged peptide with *m/z* 1002.5 was isolated and fragmented in the collision cell of Q-Tof micro™ mass spectrometer. The MS–MS spectrum was evaluated using MassLynx software. Sequencing was finally accomplished by a manual subtraction of meaningful mass differences referring to amino acid fragments generated according to the *b–y* scheme of peptide fragmentation (Shevcenko *et al.* 2000). There was an incomplete series of *y*-ions registered, which allowed to read a sequence tag EVGSAENPYGNA.


A. niger AO-I
A. nidulans AMO1
P. angusta MeAO
A. nidulans AN7641.2
A. niger MeAO peptides
MLPHPLAILSEEETNIARNVILAQHPNTVIDFREIYLSEPPKAQLLEFLALEHSGRLSPTSPRPPRLALCQYDVIGNDR 079
MAPQPHPLAILSEDEINLARDIVIAQHPNTVIDFREIYLQEPPKGQLLEFLALEHAGRLSPTTPRPPRLALCQYDVIGADR 081
MERLRQIASQATAASAAPARPAHPLDPLSTAEIKAATNTVKSYFAGKKISFNTVTLREPARKAYIQWKEQ GGPLPPRLAYYVILEAG 087
MVLDRLKQLTLQVSASSP PPHPLDPLSTAEIDAAVEIIRKEHG NVNFNAVTLYEPRKEKMMAWLANP ESPRPTRAADVVVIAPG 084
PLSTVELDAAVALLR VNFNAVTLYEPR
IPSFEESVVDVGTRQRVQHRVVGKEHHASLTLSEFDTLVERCFASPLFQKALADFDLPEG FEVVIEPWPYGGLDYVEEKRRYFQGLCFATDKRKNNPD 177
VPSYEESLVDVVASKCVKHTVVGKQHHAALTLSEFDVLVERCFASPLFKEALAEFDLPPG FEVVIEPWPYGGLDLAEPNRRFFQGLCFAQDATKKNPD 179
KPGVKEGLVDLASLSVIETRAL ETVQPILTVEDLCSTEEVIRNDPAVIEQCVLSGIPANEMHKVYCDPWTIGYDERWGTGKRLQQALVYYR SDED 182
GK IYDGIVDLDAKKIVEWKHT PGVQPLITMEDLQEVEHIVRKDPKVIEQCGILGIPPEDMHKVYCDPWTIGYDERFGTDIRLQQALMYYR PHVD 178
LDLALHK
ANFYSYPLPLIPVMDALTQEIIRVDRPATGGKGEGLTEQTFKRDIIGHCKDSDYVPELLPGGTRKDLKPLNVVQPEGPSFRITEESLVEWQKWRFRVAFN 277
ANFYSYPLPLIPVMDAHTQEIIRVDRPATGGKGDGLREQTFKRDIIGHCKGSDYVPELLPEGTRKDLKPLNVVQPEGPSFRITDESLVEWQKWRFRVAFN 279
DSQYSHPLDFCPIVDTEEKKVIFIDIPNRRRKVSKHKHANFYPKHMIEKVG AMRPEAPPINVTQPEGVSFKMTGN VMEWSNFKFHIGFN 271
DSQYTFPLDFCPIYNAETKQIIHIDIPPVRRPLNKAAPNNYH QASVEKEG GYRTDLKPIHITQPEGVSFSIEGR TIKWQNWSVHVGFN 266
LTQPEGVS WQN*WNLHVGF*
PREGATIHDVWYDG RSVLYRLSVSEMTVPYADPRPPFHRKQAFDFGDGGGGNMANNLSIGCDCLGVIKYFDAVMTGADGSAKKMPNAICLHEQDNG 373
PREGATIHDVWYDG RSVMHRLSISEMTVPYADPRPPYHRKQAFDFGDGGGGNMANNLSIGCDCLGVIKYFDAIITGADGTAKKLPNAICLHEQDNG 375
YREGIVLSDVSYNDHGNVRPIFHRISLSEMIVPYGSPEFPHQRKHALDIGEYGAGYMTNPLSLGCDCKGVIHYLDAHFSDRAGDPITVKNAVCIHEEDDG 371
YREGIVLNNITYNDKGNIRDVFYRLSLAEMVVPYGNPEHPHQRKHAFDLGEYGGGYMTNSLSLGCDCKGAIHYMDAAFVNRAGASTIVKNAICIHEEDAG 366
EGLVLNNLTF LSLAEMVVPYGNPEHPHQR *YPTNALEL* GAIHYMDAAFVNR
? ?
IGWKHSNWRTG RAVVTRHRELVVQFII TLANyEY IFAYKFDQSGGITVESRATGILNVVNIDAG KVSEYGNVVSGGVLAQNHQHIFCVRIDPAIDGP 470
IGWKHSNWRTG RAVVTRHRELVVQFII TLANyEY VFAYKFDQSAGITVEARATGILNVVNIDAG KVSDYGNVVSGGVLAQNHQHIFCVRIDPAIDGA 472
LLFKHSDFRDNFATSLVTRATKLVVSQIF TAANyEY CLYWVFMQDGAIRLDIRLTGILNTYILGDDEEAGPWGTRVYPNVNAHNHQHLFSLRIDPRIDGD 471
ILFKHTDFRD ESTIVTRGRKLIISQIF TAANyEY CVYWIFHQDGTIQLDIKLTGILNTYAMNPGEDTHGWGTEVYPGVNAHNHQHLFCLRVDANVDGP 464
HTDFRD ESVLSLR LLNTYAMNP
* *
NNSVQVEES HPVPMNAVTNPNGNFYKVNTETMERAGFFDAAPELNRTVKMVNPHKKNPISQKPVGYKFIPLATQRLLADPNSIQARRAQFAQHHVW 566
KNSVQIEES HPVPMNEATNPNGNFYKVDTKTVERACYFDAAPDLNRTVKMINPHKINPISQKPIGYKFIPLATQKLLADPNSTQAKRAQFAQHHVW 568
GNSAAACDAKSSPYPLGSPENMYGNAFYSEKTTFKTVKDSLTNYESATGRSWDIFNPNKVNPYSGKPPSYKLVSTQCPPLLAKEGSLVAKRAPWASHSVN 571
NNTVFQVDAVRGDGEVGSAENKYGNAFYAKKTKFTTPREAVSDYNGATSRTWEIQNTNKLNPYSKKPVSYKLVSREVPPLLPKEGSLVWKRAGFARHAVH 564
EVGSAENPYGNA
VTKYRDGELYAGGRYTLQSQEE IEGVSDAVKRG DSVVDTDVVVWSTFGITHNPRVEDWPVMPVEIFQLMIRPADFFTANPSLDVPSDKNISSRVVG 662
VTQHRDGELYAGGRYTLQSQSE VDGVSDAVKRG DVVVDTDVVVWSTFGITHNPRIEDWPVMPVEIFQLMIKPADFFTANPSLDVPSLKNEASRTAD 664
VVPYKDNRLYPSGDHVPQWSGDGVRGMREWIGDG SENIDNTDILFFHTFGITHFPAPEDFPLMPAEPITLMLRPRHFFTENPGLDIQPSYAMTTSEAK 669
VTKYSDDQLHPAGRHVPQTSGEPSQGLPMWIEEAGPDCSIDNTDVVLWHTFGLTHFPTPEDYPIMPAEPMTVLLRPRNFFTRNPVLDVPPSFARTP SQ 662
YSDDQLHPAGR NPALDVPPSFAR
*
NDCCRNAHI 671
664
RAVHKETKDKTSRLAFEGSCCGK 692
VAANAGACGCSKKASPDGSSV 683

MS BLAST aligned the determined sequences and tags to several homologous proteins (*not shown*). The highest total score of 943 was provided by a hypothetical protein AN7641.2 from *Aspergillus nidulans* with a theoretical molar mass of 76.46 kDa and p*I* 6.14 (*EMBL*/*GenBank* protein-accession code EAA61827). It was followed among others by a hypothetical protein NCU05518.1 from *Neurospora crassa* (accession code EAA31244, total score 688), a CAO from *Schizosaccharomyces pombe* (accession code CAB83008, total score 535) and a peroxisomal CAO from *Pichia angusta* (accession code P12807, total score 380). *A. nidulans* belongs to organisms with genome already sequenced (Birren *et al*., Genome sequence of *Aspergillus nidulans*, **http://www.broad.mit.edu/annotation/fungi/aspergillus/**). The above-mentioned hit belongs to the superfamily of CAOs (Madera *et al*. 2004) together with four other hypothetical proteins – AN1586.2 (accession code EAA64293), AN2532.2 (amine oxidase AMO1; accession code EAA64637), AN5690.2 (accession code EAA62783) and AN8454.2 (accession code EAA67076). Another hypothetical protein AN6092.2 (accession code EAA58067) contains the consensus topaquinone sequence TXXNY(D/E)(N/Y) in its primary structure; however, the conserved copper-binding sequence motif HQH is replaced by HDH in this case. Fig. 4 shows an alignment of three microbial CAOs from *A. niger* (AO-I), *A. nidulans* (AMO1) and *P. angusta* (MeAO) with the protein AN7641.2 and the determined partial sequences of MeAO from *A. niger*. Whereas AN7641.2 is only little homologous to AO-I (32 % identity, 49 % similarity), AMO1 shows a large homology (85 % identity, 92 % similarity).

Our results indicate that MeAO from *A. niger* is homologous to several other microbial CAOs. Of those, the *A. nidulans* AN7641.2 protein could be a structurally similar MeAO. As can be seen on a published model (Frébort *et al*. 2003), *A. niger* AO-I active site comprises the following amino acids: TPQ (Y405), D321, Y307, H455, H457 and H617, which have their counterparts in Y398, D314, Y300, H449, H451 and H619 of AN7641.2. If the MeAO sequence is supposed to be almost identical with that of AN7641.2, it is likely that there must be an additional conformational difference between MeAO and AO-I proteins, which would account for their different substrate specificity. Structural comparison of two similar CAOs produced by a single microorganism may have significance for understanding its metabolic adaptability.

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Fig. 4. Alignment of amino acid sequences (*numbers right*) of microbial CAOs. Four sequences were aligned with the peptide sequences and sequence tags determined for MeAO from *Aspergillus niger*: amine oxidase AO-I from *A. niger* (*EMBL/GenBank* protein accession code AAK51081), amine oxidase AMO1 from *Aspergillus nidulans* (protein accession code EAA64637), MeAO from *Pichia angusta* (formerly *Hansenula polymorpha*; protein accession code P12807) and hypothetical protein AN7641.2 from *A. nidulans* (protein accession code EAA61827). A unified symbol L stands for the isobaric amino acids L and I in the determined peptide sequences of *A. niger* MeAO. The peptides given in *italics* were determined by Frébort *et al*. (1999). The consensus cofactor sequence TXXNy(D/E-)(N/Y) of CAOs ("y" stands for the tyrosine precursor of topaquinone) is underlined, copper histidine ligands are marked with *asterisks*, and constitutive active site residues tyrosine (hydrogen bonding to O-4 of topaquinone) and aspartate (a catalytic base) are labeled with *triangle* and *circle*, respectively.

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