Biochemical and Biophysical Research Communications 465 (2015) 443–449

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Human FAD synthase is a bi-functional enzyme with a FAD hydrolase activity in the molybdopterin binding domain

Teresa Anna Giancaspero [a], Michele Galluccio [b], Angelica Miccolis [a], Piero Leone [a], Ivano Eberini [c], Stefania Iametti [d], Cesare Indiveri [b], Maria Barile [a, *]

[a] Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica, Università degli Studi di Bari "A. Moro", via Orabona 4, I-70126, Bari, Italy
[b] Dipartimento DiBEST, Biologia, Ecologia, Scienze della Terra, Unità di Biochimica e Biotecnologie Molecolari, Università della Calabria, via Bucci 4c, I-87036, Arcavacata di Rende, Italy
[c] Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, via Balzaretti 9, I-20133, Milano, Italy
[d] Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Università degli Studi di Milano, via Celoria 2, I-20133, Milano, Italy

ARTICLE INFO

Article history:
Received 6 August 2015
Accepted 9 August 2015
Available online 12 August 2015

Keywords:
Molybdopterin-binding domain
Human FAD synthase
FAD pyrophosphatase
FAD hydrolase
Cysteine
Redox switch

ABSTRACT

FAD synthase (FMN:ATP adenylyl transferase, FMNAT or FADS, EC 2.7.7.2) is involved in the biochemical pathway for converting riboflavin into FAD. Human FADS exists in different isoforms. Two of these have been characterized and are localized in different subcellular compartments. hFADS2 containing 490 amino acids shows a two domain organization: the 3′-phosphoadenosine-5′-phosphosulfate (PAPS) reductase domain, that is the FAD-forming catalytic domain, and a resembling molybdopterin-binding (MPTb) domain. By a multialignment of hFADS2 with other MPTb containing proteins of various organisms from bacteria to plants, the critical residues for hydrolytic function were identified. A homology model of the MPTb domain of hFADS2 was built, using as template the solved structure of a *T. acidophilum* enzyme. The capacity of hFADS2 to catalyse FAD hydrolysis was revealed. The recombinant hFADS2 was able to hydrolyse added FAD in a Co^{2+} and mersalyl dependent reaction. The recombinant PAPS reductase domain is not able to perform the same function. The mutant C440A catalyses the same hydrolytic function of WT with no essential requirement for mersalyl, thus indicating the involvement of C440 in the control of hydrolysis switch. The enzyme C440A is also able to catalyse hydrolysis of FAD bound to the PAPS reductase domain, which is quantitatively converted into FMN.

1. Introduction

A number of fundamental metabolic pathways, such as the oxidative metabolism of carbohydrates, amino acids and fatty acids and the functionality of the mitochondrial electron transport chain in humans and experimental organisms depend on flavoenzymes and, therefore, require the constant supply of the two redox cofactors FMN and FAD, which derive from riboflavin (Rf, vitamin B2) [1–3]. While bacteria, protists, fungi, plants and some animals can synthesize Rf, mammals must obtain this vitamin from intestinal absorption mediated by recently characterized transporters [4]. A number of other regulatory processes crucial for cell life and death, among which ROS production, antioxidant defence, protein folding and chromatin remodelling, also depend on more than one hundred different flavoproteins, 75% of which use FAD as cofactor. Thus, flavoenzyme deficiency and deregulation of flavin homeostasis have been linked to several human diseases, such as cancer, cardiovascular diseases neuromuscular and neurological disorders, and so on [*for rev. see Ref.* [5] *and Refs therein*]. The intracellular homeostasis of flavins is expected to depend on the equilibrium between synthesis and degradation of cofactors, intracellular trafficking, and assembly to nascent apo-flavoproteins, as well as recycling from holoenzyme directed to degradation pathway. A piece of work has been made to clarify the enzymatic steps required to convert Rf into the flavin cofactor FAD. This occurs via the

Abbreviations: Rf, riboflavin; RFK, riboflavin kinase; FMNAT, FMN adenylyl transferase; FADS, FAD synthase; hFADS, human FAD synthase; hFADS1, human FAD synthase isoform 1; hFADS2, human FAD synthase isoform 2; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; MPTb, molybdopterin-binding.

* Corresponding author.
E-mail addresses: teresaanna.giancaspero@uniba.it (T.A. Giancaspero), mgalluccio@unical.it (M. Galluccio), miccolis.angelica@libero.it (A. Miccolis), pieroleone87@gmail.com (P. Leone), ivano.eberini@unimi.it (I. Eberini), stefania.iametti@unimi.it (S. Iametti), cesare.indiveri@unical.it (C. Indiveri), maria.barile@uniba.it (M. Barile).

http://dx.doi.org/10.1016/j.bbrc.2015.08.035
0006-291X/

A

```
Ta         --------------------------------------------------------------------------------
Atu        --------------------------------------------------------------------------------
HsGef      --------------------------------------------------------------------------------
Dm         --TGTGTDSDRNSPYPMLPVQEVLSIIFNTVQKTANLNKILLEMNAPVNIPPFRASIKDGYAMKSTGFSGTKRVLGCIAA
Ath        ---------TEEALRIVFG-VSKRLPPVIVSLYEALGKVLAEDIRAPDPLPPYPASVKDGYAVVASDGPGEYPVITESRA
Ec         MEFTTGLMSLDTALNEMLSRVTPLTAQETLPLVQCFGRILASDVVSPLDVPGFDNSAMDGYAVRLADIASGQPLPVAGKS
HsFADS2    --------------------------------------------------------------------------------

Ta         --------------------------------------------------------------------------------
Atu        --------------------------------------------------------------MSNPSS------------
HsGef      --------------------------------------------------------------------------------
Dm         GD-SPNSLPLAEDECYKINTGAPLPLEADCVVQVEDTKLLQLDKNGQESLVDILVEPQAGLDVRPVGYDLSTNDRIFPAL
Ath        GN-DGLGVTVTPGTVAYVTTGGPIPDGADAVVQVEDTKVIGDVST--ESKRKILIQTKKGTDIRRVGCDIEKDATVLTTG
Ec         FAGQPYHGEWPAGTCIRIMTGAPVPEGCEAVVMQEQTEQMDNG-------VRFTAEVRSGQNIRRRGEDISAGAVVFPAG
HsFADS2    --------------------------------------------------------------MTSRASELSPG-------

Ta         -----------------------MKNASVITVGNEILKGRT--------VNTNAAFIGNFLTYHGYQVRRGFVVMDDLDE
Atu        --------------------TSSVVTAAMLAIGDELLSGRT--------KDKNIGHLADVLTMSGIDLKEVRIVADEEES
HsGef      ----------------QIRVGVLTVSDSCFRNLAEDRSGINLKD------------LVQDPSLLGGTISAYKIVPDEIEE
Dm         DPSPVVVKSLLASVGN--RLILSKPKVAIVSTGSELCSPRN-QLTPGKIFDSNTTMLTELLVYFGFNCMHTCVLSDSFQR
Ath        ERIGASEIGLLATAGVTMVKVYPMPIVAILSTGDELVEPTAGTLGRGQIRDSNRAMLVAAVMQQQCKVVDLGIVRDDRKE
Ec         TRLTTAELPVIASLGIAEVPVIRKVRVALFSTGDELQLPGQ-PLGDGQIYDTNRLAVHLMLEQLGCEVINLGIIRDDPHA
HsFADS2    ----------------------RSVTAGIIIVGDEILKGHT--------QDTNTFFLCRTLRSLGVQVCRVSVVPDEVAT
                                     ..:.  ..*               :     :               :: *.
                                          24                                       57

Ta         IGWAFRVALE--VSDLVVSSGGLGPTFDDMTVEGFAKCIG------------QDLRIDEDALAMIKKKYG----QADLTP
Atu        IVEALNALRS--RYDYVFTSGGIGPTHDDITADAISAAFGLPCEHDAEALRLLGDMYRVREMEFTEARKRMARMPKGAAH
HsGef      IKETLIDWCDEKELNLILTTGGTGFAPRDVTPEATKEVIE--------------REAPGMALAMLMGSLN---------V
Dm         TKESLLELFE--VVDFVICSGGVSMGDKDFVKSVLEDLQFR-------IHCGRVNIKPGKPMTFASRKDK----------
Ath        LEKVLDEAVSS-GVDIILTSGGVSMGDRDFVKPLLEEKGK--------VYFSKVLMKPGKPLTFAEIRAKPTESMLGKTV
Ec         LRAAFIEADS--QADVVISSGGVSVGEADYTKTILEELGE--------IAFWKLAIKPGKPFAFGKLSNS----------
HsFADS2    IAAEVTSFSN--RFTHVLTAGGIGPTHDDVTFEAVAQAFG-------------DELKPHPKLEAATKALG--------GE
               .    .      :. :** .    * .                              :
                          80 81     88

Ta         QRLKMAKIPPS----------------------------------------------------CRPIENPVGTAPGLICA
Atu        IANPVSVAPGFVIGNVYVMAGVPQVFQAMLDNVMPTLRTGAKVMSQAVRSPYGEGDIGTPLTAIQKAHPETSIGSYPKYD
HsGef      TPLGMLSRPVCGIRGKTLIIN-----------------------------------------------------LPGSKK
Dm         YFFGLPGNPVSAFVTFHLFALPAIRFAAGWDRCKCSLSVLNVKLLNDFSLDSRPEFVRASVISKSGE-------LYASVN
Ath        LAFGLPGNPVSCLVCFNIFVVPTIRQLAGWTSP-HPLRVRLRLQEPIKSDPIRPEFHRAIIKWKDNDGS-GTPGFVAEST
Ec         WFCGLPGNPVSATLTFYQLVQPLLAKLSGNTASGLPARQRVRTASRLKKTPGRLDFQRGVLQRNADG------ELEVTTT
HsFADS2    GWEKLSLVPSSARLHYGTDPCTG-----------QPFRFPLVSVRNVYLFPGIPELLRRVLEGMKGLFQNPAVQFHSKEL
                   *

Ta         VGGKKVIILPG--VPKEMEALLKAMEKDIIIPD------------------
Atu        GQRFSTEIVVRARDAGLLKAATEAVAAMIEAIGQEKQLAASKGDATA---------------
HsGef      GSQECFQFILPALPHAI---------------------------------------------
Dm         GNQISSRLQSIVGADVLINLPARTSDRPLAKAGEIFPASVLRFD------------------
Ath        GHQMSSRLLSMRSANALLELPATGNVLSAGSSVSAIIVSDISAFSIDKKASLSEPGSIRKEK
Ec         GHQGSHIFSSFSLGNCFIVLERDRGNVEVGEWVEVEPFNALFGGL-----------------
HsFADS2    YVAADEASIAPILAEAQAHFGRRLGLGSYPDWGSNYYQVKLTLD------------------
```

B

C