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In Silico Parameters of Methioninase of Pseudomonas Putida

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Abstract: ***Pseudomonas putida was transformed with the binary plasmid pRSET, in which the Metase gene was placed downstream of the constitutive promoters and transformants were selected on the basis of ampicillin resistance. Forty ampicillin resistant colonies were obtained and analyzed. Seven transformants showed significantly higher Methioninase activity. The Methinonisae produced by these transformants was analysed further by isolating the protein in pure form and assaying its enzyme activity. In silico studies proved that the isolated gene product codes for methioninase enzyme. Basing on the studies of the methioninase enzyme of our isolate, as the Ramachandran plot dictates, the three dimensional structure of the protein predicted was given.***

Keywords: Pseudomonas putida, In silico, Methionine gamma-lyase (L-methioninase)

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INTRODUCTION:

Cancer might affect people at all ages, even fetuses , but the threat for most varieties increases with age (Merlo *et al*., 2006). Cancer causes about 13% of all deaths (Sasco *et al.,* 2004).A methionine gamma-lyase (EC 4.4.1.11) is an enzyme that catalyzes the chemical reaction. Thus, the two substrates of this enzyme are L-methionine and H_2O, whereas its 3 products are methanethiol, NH_3, and 2-oxobutanoate.

This enzyme belongs to the family of lyases, specifically the class of carbon-sulfur lyases. The systematic name of this enzyme class is L-methionine methanethiol-lyase (deaminating 2-oxobutanoate-forming). Other names in common use include L-methioninase, methionine lyase, methioninase, methionine dethiomethylase, L-methionine Ɣ lyase, and L-methionine methanethiol-lyase (deaminating). This enzyme participates in selenoamino acid metabolism. It employs one cofactor, pyridoxal phosphate (Kreis and Hession, 1973).

Methionine dependence, the elevated methionine requirement for tumor cell proliferation, is the property of the majority of tumor cell types tested (Hoffman and Erbe 1976 and Hoffman *et al.,* 1979). There have been several therapeutic strategies to target the methionine dependence of cancer cells. Methionine starvation therapy, such as with a methionine-free diet or methioninedepleted total parenteral nutrition, prolonged the survival time of tumor-bearing rodents (Goseki *et al.,* 1992). Methionine-free total parenteral nutrition in combination with chemotherapeutic drugs extended the survival of patients with high-stage gastric cancer (Goseki *et al.,* 1995). METase from *Pseudomonas putida*, which degrades extracellular methionine to a-ketobutyrate, ammonia, and methanethiol (Tanaka *et al.,* 1977), has been demonstrated to have antitumor efficacy *in vitro* and *in vivo* (Tan *et al.,* 1997). METase has synergistic efficacy in combination with 5-fluorouracil (Yoshioka *et al.,* 1998) and cisplatin (Tan *et al.*, 1999).

Numerous cell culture studies using normal and malignant cell lines (e.g., leukemia, prostate) demonstrated that methionine restriction suppresses cancer cell growth, with little or no deleterious effect on normal cells by Poirson-Bichat (1997). Likewise, tumors are methionine dependent in vivo. Dietary methionine restriction causes regression of a variety of animal tumors and inhibits metastasis in animal models reported by Guo *et al.,* (1993) *and* Millis *et al.,* (1998).

MATERIALS AND METHODS:

Methionine Ɣ lyase (EC 4.4.1.11) (L-methioninase) *Pseudomonas putida* protein parameters were analysed by using the tool Prosite at www.expasy.org/prosite. All the protein parameters with respect to amino acid composition, secondary structure prediction, hydrophobicity, isoelectric point etc were analyzed.

RESULTS AND DISCUSSION:

ProtParam

Methionine gamma-lyase (L-methioninase) *Pseudomonas putida.*The computation has been

carried out on the complete sequence (**398** amino acids FT CHAIN 1398) Methionine gamma-lyase.

Number of amino acids: 398, **Molecular weight:** 42626.7, **Theoretical pI:** 6.21

Amino acid Composition	
Ala (A) 51	12.8%
Arg (R) 20	5.0%
Asn (N) 10	2.5%
Asp (D) 18	4.5%
Cys (C) 4	1.0%
Gln (Q) 18	4.5%
Glu (E) 18	4.5%
Gly (G) 40	10.1%
His (H) 18	4.5%
Ile (I) 16	4.0%
Leu (L) 47	11.8%
Lys (K) 9	2.3%
Met (M) 14	3.5%
Phe (F) 14	3.5%
Pro (P) 19	4.8%
Ser (S) 22	5.5%
Thr (T) 23	5.8%
Trp (W) 1	0.3%
Tyr (Y) 13	3.3%
Val (V) 23	5.8%
Pyl (O) 0	0.0%
Sec (U) 0	0.0%

(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%

Total number of negatively charged residues (Asp + Glu): 36

Total number of positively charged residues (Arg + Lys): 29

Atomic composition

Carbon	C	1889
Hydrogen	H	2974
Nitrogen	N	532
Oxygen	O	557
Sulfur	S	18

Formula: $C_{1889}H_{2974}N_{532}O_{557}S_{18}$

Total number of atoms: 5970

Extinction coefficients

Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.Ext. Coefficient 25120, Abs 0.1% (=1 g/l) 0.589, assuming ALL Cys residues appear as half cystines. Ext. coefficient 24870, Abs 0.1% (=1 g/l) 0.583, assuming NO Cys residues appear as half cystines

Estimated half-life

The N-terminal of the sequence considered is M (Met).The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).>20 hours (yeast, in vivo).>10 hours (Escherichia coli, in vivo).

Instability index

The instability index (II) is computed to be 37.09. This classifies the protein as stable.

Aliphatic index: 91.31.**Grand average of hydropathicity (GRAVY):** 0.026

Molecular weight (average): 42626.70, Theoretical **pI:** 6.21.

ProtScale

Methionine gamma-lyase (L-methioninase) of *Pseudomonas putida.*
The parameters have been computed for the following feature

FT CHAIN 1398 Methionine gamma-lyase.

The computation has been carried out on the complete sequence (**398** amino acids).

SEQUENCE LENGTH: 398

Using the scale **Hphob. / Kyte & Doolittle**, the individual values for the 20 amino acids are (Combet et al., 2000)

Ala: 1.800 Arg: -4.500 Asn: -3.500 Asp: -3.500 Cys: 2.500 Gln: -3.500
Glu: -3.500 Gly: -0.400 His: -3.200 Ile: 4.500 Leu: 3.800 Lys: -3.900
Met: 1.900 Phe: 2.800 Pro: -1.600 Ser: -0.800 Thr: -0.700 Trp: -0.900
Tyr: -1.300 Val: 4.200 : -3.500 : -3.500 : -0.490

Weights for window positions 1,..,9, using **linear weight variation model**:

1 2 3 4 5 6 7 8 9
1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00
edge center edge

MIN: -2.600

MAX: 2.000

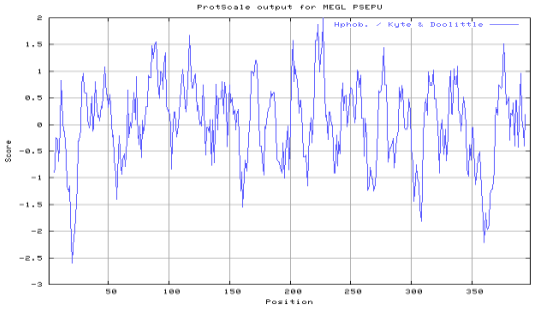

Fig.1a: ProtScale of methioninase of *Pseudomonas putida*

PepWheel

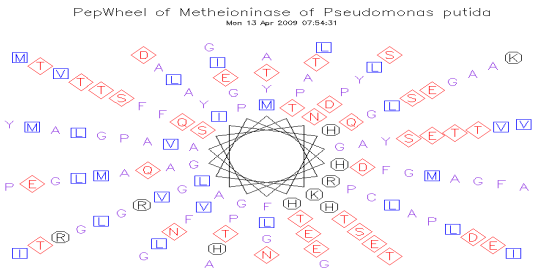

Fig.1b: Pepwheel of methioninase of *Pseudomonas putida*

SOPMA result (Geourjon and Deléage, 1995)

```
        10        20        30        40        50        60        70
        |         |         |         |         |         |         |
MHGSNKLPGFATRAIHHGYDPQDHGGALVPPVYQTATFTFPTVEYGAACFAGEQAGHF
YSRISNPTLNLL
ccccccttccheeeettccccccccccccceeeccceeecccchhhhhhhhhtccccceeeecccccchhhh
EARMASLEGGEAGLALASGMGAITSTLWTLLRPGDEVLLGNTLYGCTFAFLHHGIGEFG
VKLRHVDMADL
hhhhhhhhttchhhhhhhtthhhhhhhhhhhhcttccceeeecccccchhhhhhhhhhhttceeeeecccch

QALEAAMTPATRVIYFESPANPNMHMADIAGVAKIARKHGATVVVDNTYCTPYLQRPL
ELGADLVVHSAT
hhhhhhhcttceeeeeecccccceeehhhhhhhhhhhhttceeeeetccccccccccctttcceeeehhh
XYLSGHGDITAGIVVGSQALVDRIRLQGLKDMTGAVLSPHDAALLMRGIKTLNLRMDR
HCANAQVLAEFL
hhettccceeeeeeccchhhhhhhhhhhhhhhtcccccchhhhhhttthhhheeehhhhhhhhhhhhhhhh
ARQPQVELIHYPGLASFPQYTLARQQMSQPGGMIAFELKGGIGAGRRFMNALQLFSRAV
SLGDAESLAQH
htttteeeeecttcccccchhhhhhhhcttteeeeeeccccchhhhhhhhhhhhhhhhhcttcchheeeec
PASMTHSSYTPEERAHYGISEGLVRLSVGLEDIDDLLADVQQALKASA
Ccccccccchhhhhhhtcethheeeeeccccchhhhhhhhhhhhhhhh
```

Sequence length : 398

SOPMA :

Alpha helix	(Hh) :	168 is 42.21%
3_{10} helix	(Gg) :	0 is 0.00%
Pi helix	(Ii) :	0 is 0.00%
Beta bridge	(Bb) :	0 is 0.00%
Extended strand	(Ee) :	70 is 17.59%
Beta turn	(Tt) :	39 is 9.80%
Bend region	(Ss) :	0 is 0.00%
Random coil	(Cc) :	121 is 30.40%
Ambigous states (?)	:	0 is 0.00%
Other states	:	0 is 0.00%

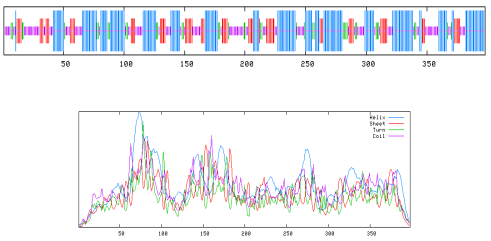

Fig.2: SOPMA of methioninase of *Pseudomonas putida*

Parameters :

Window width : 17

Similarity threshold : 8

Number of states : 4

Main Ramachandran plot

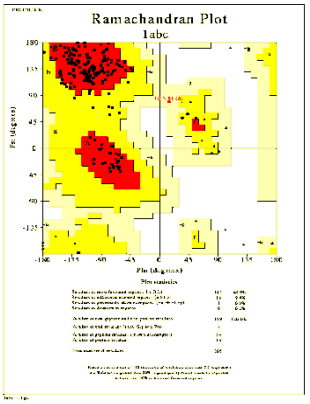

Fig.3: Main Ramachandran plot

The Ramachandran plot shows the phi-psi torsion angles for all residues in the structure (except those at the chain termini). Glycine residues are separately identified by triangles as these are not restricted to the regions of the plot appropriate to the other sidechain types. The colouring/shading on the plot represents the different regions (see below) described in Morris *et al.* (1992): the darkest areas (here shown in *red*) correspond to the "core" regions representing the most favourable combinations of phi-psi values. Ideally, one would hope to have over 90% of the residues in these "core" regions. The percentage of residues in the "core" regions is one of the better guides to stereochemical quality. The different regions on the Ramachandran plot are as described in Morris *et al.* (1992).

The regions are labelled as follows:

A	- Core alpha	**L**	- Core left-handed alpha
a	- Allowed alpha	**l**	- Allowed left-handed alpha
~a	- Generous alpha	**~l**	- Generous left-handed alpha
B	- Core beta	**p**	- Allowed epsilon
b	- Allowed beta	**~p**	- Generous epsilon
~b	- Generous beta		

The different regions were taken from the observed phi-psi distribution for 121,870 residues from 463 known X-ray protein structures. The two most favoured regions are the "core" and "allowed" regions which correspond to 10° x 10° pixels having more than 100 and 8 residues in them, respectively. The "generous" regions were defined by Morris *et al.* (1992) by extending out by 20° (two pixels) all round the "allowed" regions. In fact, the authors found very few residues in these "generous" regions, so they can probably be treated much like the "disallowed" region and any residues in them investigated more closely.

All-residue Ramachandran plots

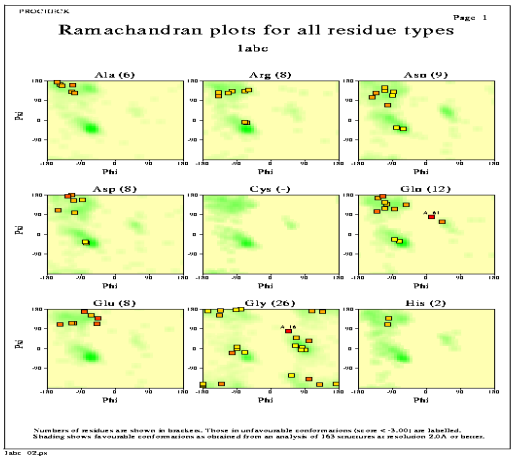

Fig.4: All-residue Ramachandran plots

The plot shows separate Ramachandran plots for each of the 20 different amino acid types. The darker the shaded area on each plot, the more favourable is the region. The data on which the shading is based has come from a data set of 163 non-homologous, high-resolution protein chains chosen from structures solved by X-ray crystallography to a resolution of 2.0Å or better and an *R*-factor no greater than 20%. The numbers in brackets, following each residue name, show the total number of data points on that graph. The red numbers above the data points are the reside-numbers of the residues in question (*ie* showing those residues lying in unfavourable regions of the plot).

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