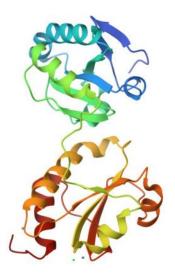




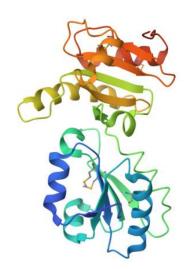
Database queries for prediction and analysis of the spatial structure of the protein disulfide isomerase enzyme of the A4 family of humans and animals

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³ Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia **Aim:** Protein disulfide isomerase of the A4 family (PDIA4) isomerase, catalyzes the formation of disulfide bonds and controls rearrangements of molecules in the EPR, which ensures their "flexibility", forms chaperone-mediated protein folding. Violation of the above functions leads to various degenerative changes in the nervous system, for example, Alzheimer's and Parkinson's diseases. The practical purpose of this work is to search for the most similar in spatial structure and functional activity of human and animal PDIA4 proteins, which can be used in medicine to create medicines based on its analogues, and for further research of the biochemical properties of biomolecules in the laboratory.



Protein disulfide isomerase of the A4 (Human)



Protein disulfide isomerase of the A4 (Rat)

Materials and methods: Protein lists were compiled based on queries to the UniProtKB and BioGraph databases. Pairwise alignment of human and animal PDIA4 amino acid sequences was performed in the Clustal Omega program. The obtained probabilities of the difference between the two sequences were entered into an Excel spreadsheet. In parallel with the above, a pairwise comparison of amino acid sequences in the BLAST system was performed. The data were processed using rank estimates, on the basis of which a summary table was obtained, including three groups of proteins: the most similar to human protein, the least similar and the median. Next, using the RCSB site, 3D models of PDIA4 proteins were built. Amino acids subject to modifications in human and animal proteins were compared.

Animals studied							
Beaver	Greater horseshoe bat						
Golden hamster	Kangaroo rat						
Horse	Bovin						
Ground squirrel	Bos indicus						
Naked mole rat	Dog						
Pig	Sheep						
Black bear	Goat						
Little brown bat	Chicken						
Leopard	Duckbill						
Cat	Mountain lion						
Mustela	Gray short-tailed opossum						
Weddell seal	American chameleon						
Seal	Zebrafish						
Rat	Gaboon caecilian						

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bit bit 475 LDEGGKKYAMEPEEFDSDALRQFVLAFKKGKLKPIVKSQPVPKNNKGPVKVVVGKTFDTI 534 bpkkDvLIEFYAPWCGHCKQLEPVYNSLAKKYKGQKGLVIAKMDATANDVPSDRYKVEGF 600 VMDPKKDVLIEFYAPWCGHCKQLEPVYNSLAKKYKGQKGLVIAKMDATANDVPSDRYKVEGF 598 bpkkDvLIEFYAPWCGHCKQLEPVYNSLAKKYKGQKGLVIAKMDATANDVPSDRYKVEGF 598 VMDPK DVLIEFYAPWCGHCKLEPVY L KKYK +K LVIAKMDATANDV +D YKVE 598 bjct 535 VMDPKNDVLIEFYAPWCGHCKLEPVYTSLGKKYKQKGKDLVIAKMDATANDV +D YKVE 594 vmbpkNDVLIEFYAPWCGHCKKLEPVYTSLGKKYKGQKDLVIAKMDATANDV +D YKVE 594 vmbpkNDVLIEFYAPWCGHCKKLEPVYTSLGKKYNPKFEGGDRDLEHLSKFIEEHATKLSRTKEEL 645 645 pTIYFAPSGDKKNPVKFEGGDRDLEHLSKFIEEHATKLSRTKEEL 643 595 GFPTIYFAPSGDKKNPVKFEGGDRDLEHLSKFIEEHATKLSRTKEEL 641	ESGKKFAMEPEEFDSDTLREFVTAFKKGKLKPVIKSOPVPKNNKGPVKVVVGKTFDSIVM 540	Query 479	LDESGKKFAMEPEEFDSDTLREFVTAFKKGKLKPVIKSOPVPKNNKGPVKVVVGKTFDSI	538
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PTIYFAPSGDKKNPVKFEGGDRDLEHLSKFIEEHATKLSRTKEEL 645 PTIYFAPSGDKKNPVKFEGGDRDLEHLSKFIEEHATKLSRTKEEL 645 Sbjct 595 GFPTIYFAPRDKKNNPIKFEGGDRDLEHLSKFIEEHATKLSRTKEEL 641		Sbjct 535	VMDPKNDVLIEFYAPWCGHCKKLEPVYTELGKKYKNEKNLVIAKMDATANDVTNDHYKVE	594
PTIYFAPSGDKKNPVKFEGGDRDLEHLSKFIEEHATKLSRTKEEL 645 GFPTIYFAP K NP+KFEGGDRDLEHLSKFIEEH TKLSRTKEEL PTIYFAPSGDKKNPIKFEGGNRDLEHLSKFIDEHATKRSRTKEEL 643 595 GFPTIYFAPRDKKNNPIKFEGGDRDLEHLSKFIEEHVTKLSRTKEEL 641		Query 599	GFPTIYFAPSGDKKNPVKFEGGDRDLEHLSKFIEEHATKLSRTKEEL 645	
	PTIYFAPSGDKKNPVKFEGGDRDLEHLSKFIEEHATKLSRTKEEL 645			
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Pairwise comparison of human PDIA4 amino acid sequences with other organisms in the BLAST system.

sp P13667 PDIA4_HUMAN sp P38659 PDIA4_RAT	MKPKKAFLLLLLLGLVQLLAVAGAEGPDEDSSNKENAIEDEEEEEEDDDEEEDDLEVKE 60 MKLRKAWLLVLLLALTQLLAAASAEDAHEDASDSENPIEDDDDEEEDEEDEDDLEVKE 58 *: ***:**:**:***.*.****.*.*****:*: ** ***:::** :*:*:**
sp P13667 PDIA4_HUMAN sp P38659 PDIA4_RAT	ENGVLVLNDANFDNFVADKDTVLLEFYAPWCGHCKQFAPEYEKIANILKDKDPPIPVAKI 120 ENGVWVLNDENFDNFVADKDTVLLEFYAPWCGHCKQFAPEYEKIASTLKDNDPPIAVAKI 118
sp P13667 PDIA4_HUMAN sp P38659 PDIA4_RAT	DATSASVLASRFDVSGYPTIKILKKGQAVDYEGSRTQEEIVAKVREVSQPDWTPPPEVTL 180 DATSASMLASKFDVSGYPTIKILKKGQAVDYDGSRTQEEIVAKVREVSQPDWTPPPEVTL 178 ******::***:*************************
sp P13667 PDIA4_HUMAN sp P38659 PDIA4_RAT	VLTKENFDEVVNDADIILVEFYAPWCGHCKKLAPEYEKAAKELSKRSPPIPLAKVDATAE 240 TLTKENFDDVVNNADIILVEFYAPWCGHCKKLAPEYEKAAKELSKRSPPIPLAKVDATEQ 238 .*******:***:*************************
sp P13667 PDIA4_HUMAN sp P38659 PDIA4_RAT	TDLAKRFDVSGYPTLKIFRKGRPYDYNGPREKYGIVDYMIEQSGPPSKEILTLKQVQEFL 300 TDLAKRFDVSGYPTLKIFRKGRPFDYNGPREKYGIVDYMVEQSGPPSKEILTLKQVQEFL 298 ************************************
sp P13667 PDIA4_HUMAN sp P38659 PDIA4_RAT	KDGDDVIIIGVFKGESDPAYQQYQDAANNLREDYKFHHTFSTEIAKFLKVSQGQLVVMQP 360 KDGDDVVILGVFQGVGDPGYLQYQDAANTLREDYKFHHTFSTEIAKFLKVSLGKLVLMQP 358 ******:*:**:* .**.* *******.***********
sp P13667 PDIA4_HUMAN sp P38659 PDIA4_RAT	EKFQSKYEPRSHMMDVQGSTQDSAIKDFVLKYALPLVGHRKVSNDAKRYTRRPLVVVYYS 420 EKFQSKYEPRMHVMDVQGSTEASAIKDYVVKHALPLVGHRKTSNDAKRYSKRPLVVVYYS 418 ********** *:*******: ******:*:*:*******
sp P13667 PDIA4_HUMAN sp P38659 PDIA4_RAT	VDFSFDYRAATQFWRSKVLEVAKDFPEYTFAIADEEDYAGEVKDLGLSESGEDVNAAILD 480 VDFSFDYRTATQFWRNKVLEVAKDFPEYTFAIADEEDYATEVKDLGLSESGEDVNAAILD 478 ********
sp P13667 PDIA4_HUMAN sp P38659 PDIA4_RAT	ESGKKFAMEPEEFDSDILREFVTAFKKGKLKPVIKSQPVPKNNKGPVKVVVGKTFDSIVM 540 ESGKKFAMEPEEFDSDALQEFVMAFKKGKLKPVIKSQPVPKNNKGPVRVVVGKTFDAIVM 538 ************************************
sp P13667 PDIA4_HUMAN sp P38659 PDIA4_RAT	DPKKDVLIEFYAPWCGHCKQLEPVYNSLAKKYKGQKGLVIAKMDATANDVPSDRYKVEGF 600 DPKKDVLIEFYAPWCGHCKQLEPVYTSLGKKYKGQKDLVIAKMDATANDITNDRYKVEGF 598 ************************************
sp P13667 PDIA4_HUMAN sp P38659 PDIA4_RAT	PTIYFAPSGDKKNPVKFEGGDRDLEHLSKFIEEHATKLSRTKEEL 645 PTIYFAPSGDKKNPIKFEGGNRDLEHLSKFIDEHATKRSRTKEEL 643

sp|P13667|PDIA4_HUMAN

Pairwise comparison of human PDIA4 amino acid sequences with other organisms in the Clustal Omega program.

Rank estimation of sequence similarity

Организм	Clustal		BLAST				Clustal		BLAST	Ранги			Средний	Общий
	р	Total	Query	E	Ident	Positives	Ранг	Total	Query	E	Ident	Positives	ранг	средний
		score	cover	value			р	score	cover	value			BLAST	ранг
Бобр Beaver	0,045	1155	100	0,00E+00	90,70	95	1	1	1	1	3	4	2,00	1,50
Хомяк Golden	0,049	1121	96	0,00E+00	90,18	96	2	2	14	1	5	1	4,60	3,30
Лошадь Horse	0,050	1118	100	0,00E+00	90,08	95	4	3	1	1	8	4	3,40	3,70
Суслик Grour	0,049	1113	90	0,00E+00	91,44	96	3	7	23	1	1	1	6,60	4,80
Голые землек	0,052	1118	100	0,00E+00	88,99	95	6	3	1	1	12	4	4,20	5,10
Свинья Pig	0,051	1117	96	0,00E+00	90,18	95	5	5	14	1	5	4	5,80	5,40
Медведь Атег	0,053	1115	96	0,00E+00	90,16	95	7	6	14	1	7	4	6,40	6,70
Малая бурая н	0,055	1108	90	0,00E+00	90,92	96	8	8	23	1	2	1	7,00	7,50
Леопард Leop	0,055	1106	100	0,00E+00	88,68	95	9	10	1	1	15	4	6,20	7,60
Kor Cat	0,056	1101	100	0,00E+00	88,70	95	10	13	1	1	14	4	6,60	8,30
Хорек Mustela	0,057	1106	96	0,00E+00	89,52	95	11	10	14	1	9	4	7,60	9,30
Тюлень Wedd	0,057	1107	96	0,00E+00	89,35	95	11	9	14	1	11	4	7,80	9,40
Морской коти	0,057	1101	96	0,00E+00	88,87	95	13	13	14	1	13	4	9,00	11,00
Крыса Rat	0,058	1106	90	0,00E+00	90,58	95	14	10	23	1	4	4	8,40	11,20
Большой под	0,062	1088	90	0,00E+00	89,37	95	15	17	23	1	10	4	11,00	13,00
Кенгуровые п	0,062	1091	95	0,00E+00	88,64	95	15	15	20	1	16	4	11,20	13,10
Бык Bovin	0,064	1081	100	0,00E+00	86,67	94	17	18	1	1	17	17	10,80	13,90
Зебу Bos indi	0,065	1090	100	0,00E+00	86,36	93	18	16	1	1	18	19	11,00	14,50
Собака Dog	0,073	1072	100	0,00E+00	85,50	93	19	19	1	1	19	19	11,80	15,40
Овца Sheep	0,074	1089	100	: 0,00E+00 :	85,12	93	20	20	1	1	20	19	12,20	16,10
Kosa Goat	0,074	1089	100	: 0,00E+00 :	85,12	93	20	20	1	1	20	19	12,20	16,10
Курица Chick	0,107	1029	100	: 0,00E+00 :	78,05	88	25	23	1	1	25	25	15,00	20,00
Утконос Duck	0,084	1084	90	0,00E+00	68,47	94	22	22	23	1	28	17	18,20	20,10
Горный лев N	0,097	957	99	0,00E+00	81,00	87	23	28	12	1	23	26	18,00	20,50
Опоссум Gray	0,105	1014	91	0,00E+00	81,15	90	24	24	21	1	22	23	18,20	21,10
Хамелеон Ате	0,115	1010	91	0,00E+00	80,65	89	28	25	21	1	24	24	19,00	22,50
Рыба-зебра Z	0,138	960	99	0,00E+00	72,34	84	27	27	12	1	27	28	19,00	23,00
Червяга Gabo	0,138	965	90	0,00E+00	75,90	87	28	26	23	1	26	26	20,40	24,20

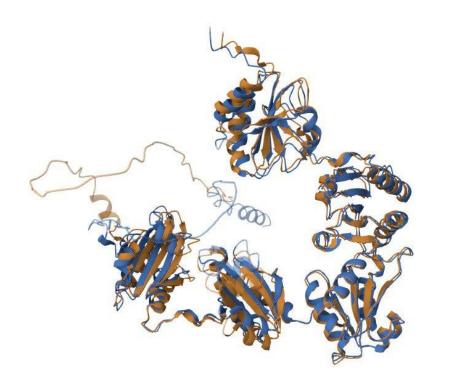
As a result of the pairwise comparisons, we obtain a large amount of data, including: Clustal P; Total score; Query cover; E-value; Indent; Positives.

We process the data obtained using rank estimates. As a result, we get the following table.

Organisms with the greatest similarity to the PDIA4 protein are colored green: Beaver, Golden hamster, Horse, Ground squirrel, Naked mole rat, Pig, Black bear, Little brown bat.

The organisms that are least similar to the human protein PDIA4 are highlighted in pink.

Yellow is the median, which is also suitable for further sequence analysis.





Rat Protein Structure

Human Protein Structure

A comparison of the 3D structures of the human and rat PDIA4 protein on the RCSB website yielded a result of 0.88 TM-store, a high percentage of correlation.

Conclusion:

- As a result of the alignment of amino acid sequences in the Clustal Omega and BLAST system, the following was revealed: the hamster protein is most similar to the human variant of PDIA4; a hamster or rat should be used for in vivo studies, since they are a standard laboratory animal and have a high level of corellation; to obtain a pure enzyme and conduct in vitro studies it is more rational to consider a pig, due to the availability of its tissues;
- A comparison of the 3D structures of the human and rat PDIA4 protein on the RCSB website yielded a result of 0.88 TM-store, a high percentage of correlation.