

Supplementary Information

Identification of lactate dehydrogenase as a mammalian pyrroloquinoline quinone (PQQ)-binding protein

Mitsugu Akagawa¹, Kenji Minematsu¹, Takahiro Shibata^{2,3}, Tatsuhiko Kondo², Takeshi Ishii⁴ & Koji Uchida²

¹ Department of Biological Chemistry, Division of Applied Life Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai 599-8531, Japan

² Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

³ PRESTO, Japan Science and Technology Agency (JST), Kawaguchi, Saitama 332-0012, Japan

⁴ Faculty of Nutrition, Kobe Gakuin University, Kobe, Hyogo 651-8586, Japan

E-mail: akagawa@biochem.osakafu-u.ac.jp (M.A.) or uchidak@agr.nagoya-u.ac.jp (K.U.)

Supplementary Figure S1

Mouse	1	ATLKDQLIYNLLKEEQAPQNKITVVGAVGMACAISILMKDLADELALVDVMEDKLLKG
Rabbit	1	AALKDQLIHNLLKEEHVPQNKITVVGAVGMACAISILMKDLADELALVDVMEDKLLKG
Human	1	ATLKDQLIYNLLKEEQTPQNKITVVGAVGMACAISILMKDLADELALVDVIEDKLLKG
Mouse	61	EMMDLQHGSFLRTPKIVSSKDYCVTANSKLVIIITAGARQQEGESRLNLVQRNVNIFKFI
Rabbit	61	EMMDLQHGSFLRTPKIVSGKDYSVTANSKLVIIITAGARQQEGESRLNLVQRNVNIFKFI
Human	61	EMMDLQHGSFLRTPKIVSGKDYVVTANSKLVIIITAGARQQEGESRLNLVQRNVNIFKFI
△		
Mouse	121	IPNIVKYSPHCKLLIVSNPVDILTYVAWKISGFPKNRVIGSGCNLDSARFRYLMGERLGV
Rabbit	121	IPNVVKYSPHCKLLVSNPVDILTYVAWKISGFPKNRVIGSGCNLDSARFRYLMGERLGV
Human	121	IPNVVKYSPNCKLLIVSNPVDILTYVAWKISGFPKNRVIGSGCNLDSARFRYLMGERLGV
△		
Mouse	181	HALSCHGWVIGEHDSSVPVWSGMNVAGVSLKSLNPELGTADKEQWKEVHKQVVD SAYE
Rabbit	181	HALSCHGWIIGEHDSSVPVWSGMNVAGVSLKTLHPDLGTADKEQWQVHKQVVD SAYE
Human	181	HPLSCHGWVIGEHDSSVPVWSGMNVAGVSLKTLHPDLGTDKDKQWKEVHKQVVD SAYE
Mouse	241	VIKLGYSWATGLSVADLAESIMKNLRRVHPVSTMIKGLYGINEDVFLSVPCILGQNGIS
Rabbit	241	VIKLGYSWATGLSVADLAESIMKNLRRVHPVSTMLKGLYGIKEDVFLSVPCILGQNGIS
Human	241	VIKLGYSWATGLSVADLAESIMKNLRRVHPVSTMIKGLYGIKDDVFLSVPCILGQNGIS
Mouse	301	DVVKVTLTPEEEARLKKSAADTLWGIQKELQF
Rabbit	301	DVVKVTLTSEEEAHLKKSAADTLWGIQKELQF
Human	301	DLVKVTLTSEEEARLKKSAADTLWGIQKELQF

Fig. S1. Alignment of the amino acid sequences of LDH-A of mouse, rabbit, and human¹. Perfect matches are enclosed in boxes with a black background. The open arrowheads indicate the position of the proposed residues involved in PQQ binding.

Supplementary Figure S2

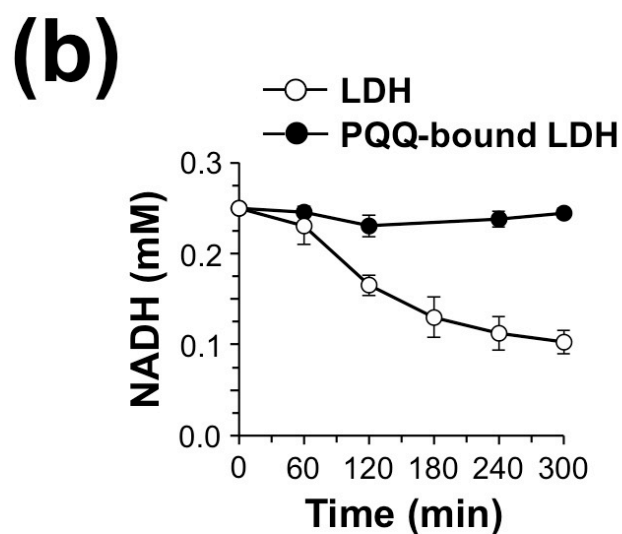
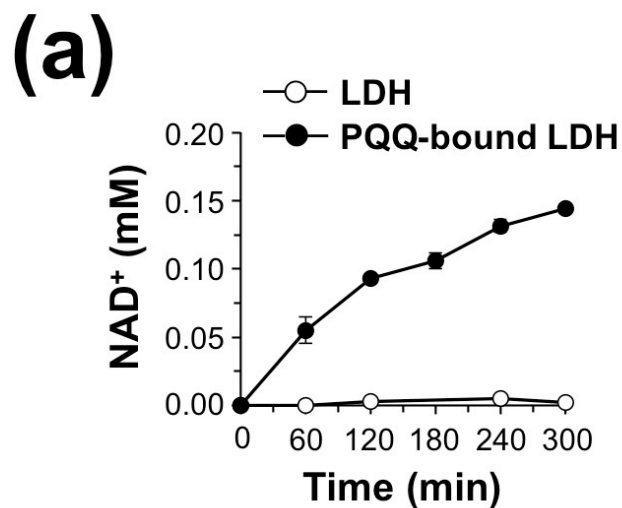


Fig. S2. Time course of NAD^+ formation by PQQ-bound LDH in the presence of NADH. Rabbit muscle LDH (600 nM) and PQQ-bound LDH (600 nM) were incubated with 0.25 mM NADH at 37 °C for the indicated time. Then, concentrations of NAD^+ (a) and NADH (b) in the reaction mixtures were determined by HPLC. The results shown are means \pm SE ($n = 3$).