

FINAL TECHNICAL REPORT

A SODIUM-CYCLE BASED ORGANISM WITH IMPROVED MEMBRANE RESISTANCE AIMED AT INCREASING THE EFFICIENCY OF ENERGY BIOTRANSFORMATIONS

THE U.S. DEPARTMENT OF ENERGY

AWARD NO. DE-FG02-97ER122214

The aim of the project was to design a functional oxidative phosphorylation system based on a Na^+ , rather than on a proton cycle. The rationale is to assemble in *E. coli* genes coding for a Na^+ -motive redox pump and genes coding for a Na^+ -driven ATPsynthase that reside in different organisms. A vector carrying genes coding for a functional Na^+ -cycle oxidative phosphorylation could then be moved to other bacteria. The presence of a sodium cycle will improve the resistance of the cell to organic solvents, detergents and other aggressive agents whose toxicity is due primarily to their causing proton leaks in the membrane. Sodium ions are more resistant to membrane leaks than are protons by several orders of magnitude. The proposed technology would be beneficial to a broad array of applications, such as improving oil desulfurization by *Rhodococci* and increasing the efficiency of ethanol and solvent production by bacteria.

A chimeric *E. coli*/*P. modestum* Na^+ ATPase has been described in the literature, and was reported to be functionally expressed in *E. coli*. Therefore the focus of our exploratory project was on cloning and functionally expressing in *E. coli* a Na^+ NADH dehydrogenase from *Haemophilus influenzae* coded by the *nqr 6*-gene operon. After many unsuccessful attempts of expressing *nqr* using a variety of host vectors and *E. coli* strains, we finally succeeded to obtain a plasmid pHC2.2 that contained the stable *nqr* insert in DH5a cells.

A number of functional test were performed to learn whether a Na^+ NADH dehydrogenase was being expressed from this vector. For this purpose, the plasmid was transformed into an *E. coli* strain lacking the two native NADH dehydrogenase loci (*nuo* and *ndh*). Recombinant cells were checked on rich medium for ampicillin resistance (carried by the backbone pSE380 vector), kanamycin and tetracycline resistance (carried by the transposon insertions disrupting the *nuo* and *ndh* genes of the host strain). All ten passed the test, indicating that the plasmid was not lost and the transposon insertions were not excised. The clones were then plated on minimal M63 medium (no sodium) with mannitol and IPTG. Growth on mannitol requires an NADH dehydrogenase, and the only possible source of this enzyme in the host deleted for NADH dehydrogenase genes would be the recombinant NQR enzyme. Experimental plates additionally contained 20mM NaCl. None of the clones plated on regular M63 medium with mannitol showed growth, but several of the plated colonies grew on this medium supplemented with NaCl. The NQR NADH dehydrogenase is stringently dependent upon Na^+ for its activity. This test indicated that the Na^+ NADH dehydrogenase from *H. influenzae* had been functionally cloned into *E. coli*.

Next, everted membrane vesicles were obtained from this recombinant strain by French press disruption/differential centrifugation, and the presence of the NADH dehydrogenase enzyme was assayed by following the decrease in added NADH (following absorbency at 340nm). Vesicles showed considerable NADH dehydrogenase activity in a medium with Na^+ , but not without Na^+ . Control vesicles from a strain lacking native or recombinant *nqr* had not NADH dehydrogenase activity. These results strongly indicate a successful heterologous expression of NADH dehydrogenase.

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The aim of the project was to express in *E. coli* components that would allow for a formation of a functional oxidative phosphorylation system based on a Na^+ , rather than on a proton cycle. The presence of a sodium cycle will improve the resistance of the cell to organic solvents, detergents and other aggressive agents whose toxicity is due primarily to their causing proton leaks in the membrane. We have cloned and expressed the *nqr* operon from *H. influenzae* in *E. coli*. Experiments with membrane vesicles indicated the presence of the functional recombinant sodium pumping NADH dehydrogenase. A gene for a hybrid *E. coli*/*P. modestum* ATPase was constructed which will enable to co-express a sodium ATPase together with a sodium NADH dehydrogenase in the same cells.

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