Purification and Properties of the NADH and NADPH Specific FMN Oxidoreductases from *Beneckea harveyi*†

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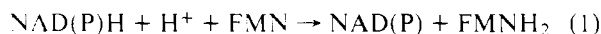
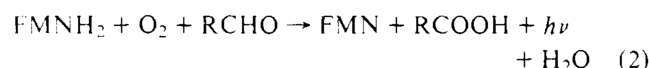
ABSTRACT: The NADH and NADPH specific FMN oxidoreductases from *Beneckea harveyi* have been purified to homogeneity as judged by single bands on sodium dodecyl sulfate gel electrophoresis. The overall purification for the NADH specific enzyme is 3000-fold and 4000-fold for the NADPH specific enzyme from a crude extract. The final step in the purification procedure is chromatography on a 5′-AMP-Sepharose 4B affinity column which results in approximately a 50-fold purification to a final specific activity of 31 μmol of NADH oxidized min^{-1} (mg of protein)$^{-1}$ for the NADH specific FMN reductase. The NADPH specific reductase has been purified to a final specific activity of 51 μmol of NADPH oxidized min^{-1} (mg of protein)$^{-1}$ using a NADP agarose affinity column, which results in a 70-fold purification. Molecular weights of 30 000 and 40 000 and K_m's of 4.75 × 10^{-5} M NADH and 4.0 × 10^{-5} M NADPH have been determined for the pure NADH and NADPH specific FMN reductases, respectively. The NADPH specific FMN reductase does not utilize NADH, while the NADH specific enzyme does dehydrogenate NADPH with a maximal velocity one-tenth of that for NADH. Separate NADH and NADPH specific FMN reductases from *Photobacterium fischeri* could not be demonstrated.

The known bioluminescent bacteria contain an enzyme, luciferase, which catalyzes the oxidation of $FMNH_2$ in the presence of a long chain aldehyde with the emission of light. It was observed (McElroy et al., 1953; Strehler and Cormier, 1953) that NADH and NADPH had a stimulatory effect on light emission in a cell-free extract of *Photobacterium fischeri*. It is now well established that these bacteria contain a NAD(PH)H:FMN oxidoreductase commonly referred to as FMN reductase or NAD(P)H dehydrogenase (Duane and Hastings, 1975). The following reactions are known to occur in these bacteria (Becvar and Hastings, 1975; Shimomura et al., 1972).



$$NAD(P)H + H^+ + FMN \rightarrow NAD(P) + FMNH_2 \quad (1)$$



$$FMNH_2 + O_2 + RCHO \rightarrow FMN + RCOOH + h\nu + H_2O \quad (2)$$

Reaction 1 is catalyzed by the reductase and the $FMNH_2$ produced is then utilized by the luciferase (eq 2) to produce light and the acid corresponding to the aldehyde (Dunn et al., 1973; McCapra and Hysert, 1973; Vigny and Michelson, 1974). There is still some question as to the stoichiometry with respect to $FMNH_2$, whether one or two reduced flavins are utilized (Lee, 1972; Lee and Murphy, 1975).

Duane and Hastings (1975) have partially purified the reductase from *P. fischeri* and *Beneckea harveyi* and Puget and Michelson (1972) report they have this reductase highly purified from *P. fischeri*. However, there are some discrepancies between these two reports regarding the final specific activity and purity of the enzyme as well as the molecular weight from *P. fischeri*. Duane and Hastings (1975) report a molecular weight of 43 000 for the enzyme and a specific activity of 50 μmol of NADH oxidized min^{-1} (mg of protein)$^{-1}$. They judged this reductase preparation to be 20% pure. Puget and Michelson (1972) found a molecular weight of 23 000 and a specific activity of 0.14 μmol of NADH oxidized min^{-1} (mg of protein)$^{-1}$. A recent paper (Gerlo and Charlier, 1975) reports that there are two distinct FMN reductases in *B. harveyi*, one which is specific for NADH and another which utilizes NADPH. These authors report the NADH dehydrogenase has a molecular weight of 19 000, and the NADPH specific enzyme is 40 000. Their maximum specific activity for the NADH and NADPH specific enzymes was 2.05 μmol of NADH and NADPH oxidized min^{-1} (mg of protein)$^{-1}$, respectively. They judged these preparations to be less than 20% pure.

Duane and Hastings (1975) report a molecular weight of 23 000 for the NAD(P)H dehydrogenase of *B. harveyi*, in agreement with the NADH specific dehydrogenase of Gerlo and Charlier (1975).

We report here the purification of the NADH and NADPH specific FMN oxidoreductases from *B. harveyi* by the use of two different affinity chromatography columns in the last step of the purification. A 5′-AMP-Sepharose 4B column binds the NADH specific enzyme and an agarose-hexane-nicotinamide adenine dinucleotide phosphate (NADP agarose) column binds the NADPH specific enzyme. Both enzymes have been obtained in pure form as judged by single bands on sodium dodecyl sulfate gel electrophoresis. The specific activity of the NADH specific anzyme is 31 μmol of NADH oxidized min^{-1} (mg of protein)$^{-1}$, while the specific activity for the NADPH specific enzyme is 51 μmol of NADPH oxidized min^{-1} (mg of protein)$^{-1}$. We could not find evidence of specific NADH: and NADPH:FMN oxidoreductases from *P. fischeri*.

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† From the Department of Chemistry, University of California, San Diego, La Jolla, California 92093. *Received January 17, 1977*. This research was supported by a grant from the National Science Foundation BMS 72-02405.

‡ Supported by a United States Public Health Service Predoctoral Training Grant.

[1] Abbreviations used: BSA, bovine serum albumin; DEAE, diethylaminoethyl; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; FMN, $FMNH_2$, flavin mononucleotide, oxidized and reduced, respectively; NAD, NADH, nicotinamide adenine dinucleotide, oxidized and reduced, respectively; NADP, NADPH, nicotinamide adenine dinucleotide phosphate, oxidized and reduced, respectively.

TABLE I: Purification of NADH:FMN Oxidoreductase.

Fraction	Vol (mL)	Total protein (mg)	Total units (μmol/min)	Spec. act. (units/mg)	% yield
1. Crude extract	2220	22400	220	0.010	100
2. DEAE cellulose peak	845	4310	165	0.038	75
3. 35–75% $(NH_4)_2SO_4$ dialysate	84	3110	156	0.050	71
4. DEAE-Sephadex A-50 peak	313	1000	154	0.154	70
5. Sephadex G-100 peak[a]	265	147	97	0.660	44
6. 5′AMP-Sepharose	90	2.7	82.5	31.0	37.5

[a] Step 5 was actually carried out with 4-mL aliquots placed onto the Sephadex column (see text).

TABLE II: Purification of NADPH:FMN Oxidoreductase.

Fraction	Vol (mL)	Total protein (mg)	Total units (μmol/min)	Spec. act. (units/mg)	% yield
1. Crude extract	2220	22400	262	0.012	100
2. DEAE-cellulose peak	845	4310	200	0.046	76
3. 35–75% $(NH_4)_2$-SO_4 dialysate	84	3100	170	0.054	65
4. DEAE-Sephadex A-50 peak	118	1266	152	0.120	58
5. Sephadex G-100 peak[a]	50	118	86	0.730	33
6. NADP agarose	18	1.5	74	51.0	28

[a] Step 5 was actually carried out with 4-mL aliquots placed onto the Sephadex column (see text).

Materials and Methods

Chemicals. BSA, DTT, NAD, NADP, NADH, and NADPH were obtained from Calbiochem. Decanal, FMN, and Coomassie brilliant blue G were from Sigma Chemicals. DEAE-cellulose (Whatman DE-32) was from H. Reeve Angel, Inc. DEAE-Sephadex A-50, Sephadex G-100, and 5′-AMP-Sepharose 4B were from Pharmacia Fine Chemicals. NADP agarose (agarose-hexane-nicotinamide adenine dinucleotide phosphate: AGNADP type 4) was from P-L Biochemicals, Inc. Luciferase was purified in our lab according to previous methods (Gunsalus-Miguel et al., 1972). Standard proteins used for molecular weight determinations were from Pharmacia Fine Chemicals. Acrylamide, methylenebisacrylamide, and *N,N,N′,N′*-tetramethylethylenediamine were from Eastman Kodak.

Bacteria. Beneckea harveyi strain No. 392 (Reichelt and Baumann, 1973) was obtained from Dr. K. Nealson at the Scripps Institute of Oceanography. Large quantities of the bacteria were grown in complete media essentially as described by Farghaly (1950) with the addition of 5 g of peptone and 3 g of yeast extract (Difco) per L. These were grown at the Oak Ridge Laboratories by Dr. John Totter and shipped to us as a frozen cell paste.

Protein Assays. Protein measurement was performed according to the method of Bradford (1972), using Coomassie brilliant blue G-250. Bovine serum albumin was used as a standard. This method was chosen because we could determine protein concentrations in the presence of DTT, NAD, and NADP in concentrations as high as 10^{-3}, 5×10^{-3}, and 5×10^{-5} M, respectively, without interference.

Enzyme Assays. Both the NADH and the NADPH specific FMN reductases were measured at 23 °C by monitoring the initial rate of oxidation of NADPH or NADH by the loss in absorbance at 340 nm using a Cary Model 14 recording spectrophotometer. Reductase activity is expressed as μmol of NADH or NADPH oxidized per min and specific activity as μmol min^{-1} (mg of protein)$^{-1}$. The assay reaction is initiated by adding 0.1 mL of 2.0×10^{-3} M NADH or NADPH in 0.1 M phosphate buffer (pH 7.0) to 1 mL of 0.015 M phosphate buffer (pH 7.0) containing 7.0×10^{-5} M EDTA, 1.3×10^{-4} M FMN, and a sample of reductase.

Reductase activity was also measured in a coupled assay with luciferase (Hastings et al., 1965) with an Aminco Chem-Glo Photometer. The maximum initial light intensity is measured upon the addition of 0.1 mL of 2×10^{-4} M NADH or NADPH, in 0.1 M phosphate buffer (pH 7.0), into 0.5 mL of 0.015 M phosphate buffer (pH 7.0) containing 1% BSA, 2.3×10^{-6} FMN, 0.0005% decanal, a fixed excess amount of purified luciferase and limiting reductase. Activity is thus expressed in arbitrary light units.

Reductase activity is dependent upon the presence of DTT (Fisher et al., 1976). During purification, activity is lost slowly, but reversibly, at a DTT concentration of 1×10^{-4} M. In order to assure maximal activity when assaying steps in the purification, fractions were removed and made 10^{-3} M in DTT by addition of the solid, incubated at 0 °C for 24 h, and then assayed.

Electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed in a Hoefer Scientific Instruments DE-100 cell driven by a Heathkit IP-17 power supply at 8 mA per gel according to Weber and Osborn (1969) with the following modifications. The proteins were prepared by incubating at 60 °C for 30 min in gel buffer plus 1% DTT and 6 M urea, adding an equal volume of tracking dye in glycerol and applying 100 μL of this resulting solution to a gel. Usually 10 μg of protein was applied per gel. The gels were 7.5% acrylamide, made by mixing 5 mL of 30% acrylamide, 0.8 mL of 2% methylenebisacrylamide, 10 mL of gel buffer, 3 mL of water, 1 mL of freshly made ammonium persulfate (12 mg per mL), and 10 μL of *N,N,N′,N′*-tetramethylethylenediamine (for 12 gels). Gel tubes were 5 mm internal diameter, 12.7 cm in length. Staining was done at 60 °C for 30 min. Destaining was done by incubating the gels in a 10% glacial acetic acid-50% methanol solution at 60 °C for 15 min; the solution was poured off and the procedure repeated one or two more times. The gels were then put in a 10% acetic acid-10% methanol solution in a Hoefer destaining chamber and allowed to destain overnight. The gels were stored in the later destaining solution.

Results

The initial procedures in the purification of NADH and NADPH specific FMN reductases from *B. harveyi* were similar to the methods reported by Gunsalus-Miguel et al. (1972) and Baldwin et al. (1975) for the purification of bacterial luciferase. Tables I and II show the data obtained from a typical purification of the two reductases starting with 371 g of frozen cells. All steps were carried out at 0–4 °C. Cells were thawed for 1 h at room temperature and placed in cold low ionic strength solution of 0.01 M potassium phosphate

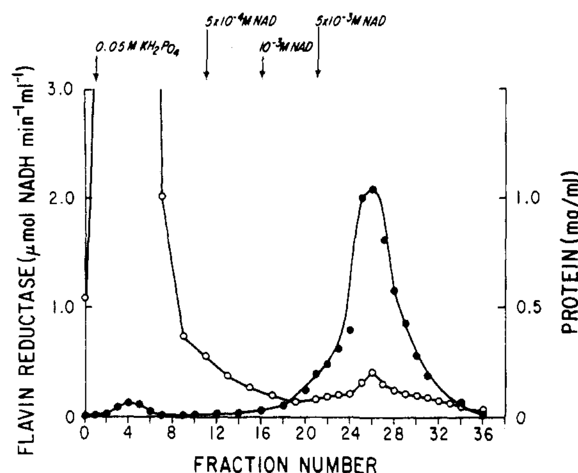

FIGURE 1: 5′AMP-Sepharose 4B affinity chromatography of partially purified NADH specific FMN reductase. (O—O) Protein concentration; (●—●) activity.

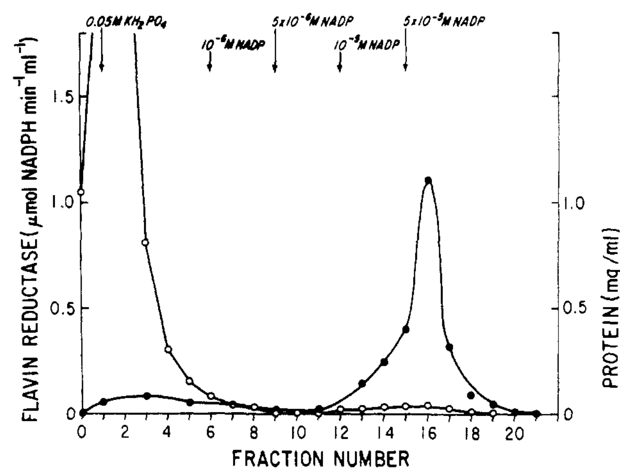

FIGURE 2: NADP agarose affinity chromatography of partially purified NADPH specific FMN reductase. (O—O) Protein concentration; (●—●) activity.

buffer (pH 7.0) containing 10^{-2} M EDTA and 10^{-4} M DTT in a ratio of 6 mL to 1 g of frozen cells. This solution was stirred slowly for 16 h with the pH maintained at 6.7–7.0 with 0.1 M KOH. Lysed cells were centrifuged for 30 min at 23 000*g* to remove cellular debris in a Sorvall centrifuge. To this crude extract 350 mL of settled DEAE-cellulose in the phosphate form (Baldwin et al., 1975) was added. After 60 min, the DEAE-cellulose was filtered out and washed with 700 mL of 0.05 M phosphate buffer (pH 7.0) containing 10^{-4} M DTT. A slurry was prepared in the same buffer and poured into a column of 5.0 cm diameter and allowed to settle. Elution was performed with a linear gradient of phosphate buffer (pH 7.0) from 0.1 to 0.7 M containing 10^{-4} M DTT. Separation of the reductases from the luciferase occurred as described previously (Puget and Michelson, 1972). The reductases were then precipitated with ammonium sulfate between 35 and 75% saturation, redissolved in 0.2 M phosphate buffer (pH 7.0) with 10^{-4} M DTT, and dialyzed against the same buffer in preparation for chromatography on DEAE-Sephadex A-50 (Gunsalus-Miguel et al., 1972). The two reductases were separated as described before (Gerlo and Charlier, 1975), and the active fractions were combined and concentrated again by ammonium sulfate precipitation. The precipitate was dissolved in 0.1 M phosphate buffer (pH 7.0) with 10^{-4} M DTT and dialyzed against the same buffer. The resulting preparation was made 10^{-3} M in DTT by addition of the solid and incubated for 24 h at 0 °C and then frozen at −20 °C.

Frozen aliquots (4 mL) were thawed as needed and chromatographed on a 2.5 cm × 80 cm Sephadex G-100 column eluted with 0.1 M potassium phosphate buffer (pH 7.0) with 5×10^{-4} M DTT (Fisher et al., 1976) and the active fractions were collected and pooled for affinity chromatography.

Affinity Chromatography of NADH Specific FMN Reductase. One gram of dry 5′-AMP-Sepharose 4B was placed in 10 mL of 0.05 M potassium buffer (pH 7.0) and poured into a column 0.7 cm × 15 cm. The column was equilibrated with 0.05 M buffer with 5×10^{-4} M DTT for several hours in the cold and resulted in a bed volume of 4.0 mL. The partially purified NADH specific FMN reductase from the G-100 column (15 mL) was applied, followed by 5.0 mL of 0.05 M buffer (pH 7.0) with 5×10^{-4} M DTT, as a wash. Elution was performed with increasing concentrations of NAD in 0.05 M phosphate buffer pH 7.0 with 5×10^{-4} M DTT (Figure 1). The NADH specific FMN reductase elutes at a concentration of 5×10^{-3} M NAD. Fractions of 1 mL were collected. The fractions containing the reductase were pooled and concentrated with an Amicon mini concentrator No. B15. The resulting enzyme preparation had a specific activity of 31 μmol of NADH oxidized min^{-1} (mg of protein)$^{-1}$ and had only a single band on sodium dodecyl sulfate–polyacrylamide electrophoresis. The single affinity column step results in a 50-fold purification based on the specific activity. A 100-fold purification results if the G-100 step is omitted.

Affinity Chromatography of the NADPH Specific FMN Reductase. NADP agarose, 2.5 mL, stored in 50% glycerol and 0.02% sodium azide at −20 °C was placed in 5 mL of 0.05 M phosphate buffer (pH 7.0) with 5×10^{-4} M DTT and poured into a column 0.5 cm × 10 cm and equilibrated for several hours in the cold with the same buffer. The pooled fractions of the NADPH specific FMN reductase from the G-100 column (15 mL) were applied followed by 5 mL of the equilibration buffer as a wash. Elution was performed with increasing concentrations of NADP in the equilibration buffer. The NADPH specific enzyme elutes at a concentration of 5×10^{-5} M NADP (Figure 2). Fractions of 1 mL were collected and concentrated with the Amicon mini concentrator No. A25. This enzyme preparation had a specific activity of 51 μmol of NADPH oxidized min^{-1} (mg of protein)$^{-1}$, and showed only a single band on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The affinity column step results in a 70-fold purification. A 200-fold purification results if the G-100 step is omitted.

Specificity. The NADH specific reductase does not bind to the NADP agarose affinity resin and is eluted immediately with the phosphate buffer wash along with most of the other protein in the partially purified preparation. The NADPH specific enzyme likewise does not bind to the 5′-AMP-Sepharose 4B affinity resin and is also eluted immediately.

The NADPH specific reductase oxidizes only NADPH. We could not detect activity of this enzyme when supplied with NADH as substrate. This is not true for the NADH specific reductase. This enzyme exhibits activity when presented with the nonspecific substrate, NADPH. The purified NADH specific reductase oxidized NADPH at 10% the rate utilizing the same concentrations of NADH. This was not due to a contamination of NADPH with NADH since the enzyme could reduce the absorbance at 340 to nearly zero and passing the purified NADH specific reductase through the NADP

agarose column did not decrease activity with NADPH, ruling out contamination by the NADPH specific reductase.

Molecular Weights. Molecular weights of the purified reductases were obtained with a calibrated Sephadex G-100 column 2.5 cm × 80 cm. Internal standards were: aldolase, 158 000; ovalbumin, 45 000; chymotrypsinogen A, 25 000; ribonuclease A, 13 700; and blue dextran 2000, 2×10^6. Each protein was eluted with 0.1 M phosphate buffer (pH 7.0) with 5×10^{-4} M DTT, the standard proteins were monitored by absorbance at 280 nm, and the reductases were assayed by the coupled assay as described in Materials and Methods. The molecular weights of the NADH specific reductase was found to be 30 000 ± 2000, somewhat higher than reported by Gerlo and Charlier (1975). The NADPH specific reductase was found to combine with blue dextran when chromatographed together on the Sephadex G-100 column. The column was subsequently run without blue dextran and a molecular weight of 40 000 ± 2000 was found, in agreement with the above authors.

Kinetic Constants. The apparent kinetic constants were determined for both reductases from initial velocity measurements using various concentrations of NADH or NADPH. The NADH specific reductase was found to have a K_m of 4.75×10^{-5} M for NADH, and a specific activity of 31.0 μmol of NADH oxidized min^{-1} mg^{-1} corresponding to a turnover number of 930 mol of NADH per mol of enzyme per min assuming a molecular weight of 30 000. The NADPH specific reductase was found to have a K_m of 4.0×10^{-5} M for NADPH and a specific activity of 51.0 μmol of NADPH oxidized min^{-1} mg^{-1} corresponding to a turnover number of 2040 mol of NADPH per mol of enzyme per min assuming a molecular weight of 40 000.

Photobacterium fischeri. We attempted to identify and purify NADH and NADPH specific reductases from *P. fischeri*. Purification was carried out as described for *B. harveyi* and the NADH/NADPH activity ratio was monitored at each step in the purification. The ratio remained at about 2 for all steps (Duane and Hastings, 1975). Furthermore, this NAD(P)H:FMN reductase did not bind to either affinity column and did not combine with the blue dextran when chromatographed together on a Sephadex G-100 column. A molecular weight of 45 000 ± 2000 was found, in agreement with Duane and Hastings (1975).

Discussion

From the information presented in this paper, it would appear that light generating systems in the luminescent bacteria are appreciably different with regard to the supplying of reduced FMN to the luciferase enzyme. One species, *B. harveyi*, has been shown to possess two highly specific reductases whereas *P. fischeri* appears to have only one, nonspecific reductase, being able to dehydrogenate both NADH and NADPH. In the case of *B. harveyi* both enzymes are able to function in the coupled assay with luciferase in vitro. The binding sites of these enzymes for NADH and NADPH appear to be very different. The two reductases of *B. harveyi* are able to bind to their respective substrates on affinity resins, while the nonspecific reductase of *P. fischeri* does not. The fact that the NADPH specific FMN reductase of *B. harveyi* can combine with blue dextran is also noteworthy. This observation has been reported elsewhere (Thompson et al., 1975) for a number of proteins. It would appear that the mechanism for directing reduced substrates for use in anabolic processes and supplying energy for luminescence and energy for oxidative phosphorylation are different in these two bacteria. *B. harveyi* may use the NADH specific FMN reductase to channel reducing power into ATP production during the cell cycle, supplying energy for growth. It has already been shown that this enzyme is constitutive with growth (Meighen et al., 1976). Luciferase, an inducible enzyme, does not appear until the culture has generated a sufficient amount of an autoinducer, at which time luciferase production exceeds the rate of growth of the cells (Nealson et al., 1970). It is conceivable that the NADPH specific reductase is also autoinducible or under cellular control such that its activity is not expressed until luciferase is produced. Such a system would act to channel away reducing power from growth into light emission only when the culture is firmly established and luciferase is available to accept hydrogens from $FMNH_2$. NADH specific reductase could also shuttle reducing equivalents into luminescence if the supply exceeds the demand by oxidative phosphorylation.

TABLE III: Total Amount of Reductases and Luciferase in One Gram of *B. harveyi*.

	μg	Mol wt	nmol	TN[a]
Luciferase	1540	79 000	20.5	8.77
NADH:FMN Reductase	19.5	30 000	0.65	930
NADPH:FMN reductase	14.5	40 000	0.36	2040

[a] Turnover number for luciferase was calculated using Nicoli's (1974) specific activity of 2.2×10^{14} quanta s^{-1} mg^{-1} luciferase with decanal and assuming a quantum yield of 0.2 and a stoichiometry of 1 with respect to flavin (Becvar et al., 1976).

The mechanism by which the cells control the flow of reducing equivalents, to the terminal cytochrome system or alternatively to luciferase, is not known. If these reductases are to supply reducing equivalents into systems other than bioluminescence, it would seem necessary for luciferase to be the limiting component in the light generating system. The data generated here and from other sources bear this out. Using the data of Tables I and II, it is possible to calculate the total amount of NADH and NADPH specific FMN reductases present in a given amount of bacteria harvested at about maximum luminescence (Table III). Luciferase was also purified from these bacteria with results similar to those reported by Gunsalus-Miguel (1972). There is then 31 and 57 times more molecules of luciferase than of NADH and NADPH specific reductase in a cell, respectively. NADH specific reductase turns over under maximal conditions in vitro at 930 mol of NADH oxidized min^{-1} (mol of reductase)$^{-1}$ and the NADPH specific reductase at 2040 mol of NADPH oxidized min^{-1} (mol of reductase)$^{-1}$. Therefore the NADH specific enzyme turns over 106 times faster than luciferase and the NADPH specific enzyme turns over 233 faster than luciferase. It may be concluded that, for *B. harveyi*, luciferase is limiting by a factor of 3.4 for NADH specific reductase and 4.1 NADPH specific reductase if these enzymes are operating under similar conditions in vivo.

A similar set of calculations may be made with *P. fischeri* luciferase and reductase from data derived from Gunsalus-Miguel (1972) and Duane and Hastings (1975). The turnover number of NAD(P)H:FMN reductase has been estimated at 1.1×10^4 mol of NADH oxidized min^{-1} (mol of reductase)$^{-1}$ and luciferase at 19 mol of $FMNH_2$ min^{-1} (mol of luciferase)$^{-1}$. This means that reductase turns over 580 times faster than luciferase in *P. fischeri*. From these same authors, luciferase comprises 13.5 nmol/g cells and reductase 0.74 nmol/g cells. Even though there is 18.2 times more luciferase

than reductase in these bacteria on a molar basis, the electron flow through luminescence is limited by luciferase due to its low turnover. For *P. fischeri* then, it appears that luciferase is limiting by a factor of 30.

NADH-dependent dehydrogenases have been isolated and studied from a variety of sources and membrane bound dehydrogenases are known to function in the first step of electron transport. *Azotobacter vinelandii,* for example, has a low-molecular-weight NADH-dependent dehydrogenase which apparently functions in phosphorylating particles (Dervartanian, 1976). This enzyme is 10% active with NADPH as substrate and has functional FMN and iron–sulfur centers. A low-molecular-weight NADH-dependent dehydrogenase of the respiratory chain is believed to be a fragmented part of a large molecular weight dehydrogenase isolated from complex I of Hatefi et al. (1962) (Singer and Gutman, 1970).

These FMN reductases may also control electron flow between the cytochromes and luminescence. In *Photobacterium phosphoreium* luciferase and NAD(P)H:FMN reductase are constitutive with growth, but in vivo luminescence increases more rapidly than growth (Watanabe et al., 1975). This has been explained as a change in the mode of competition between luciferase and the cytochrome system for NAD(P)H. Perhaps it is the reductase enzyme that controls the channeling of electrons in this system.

Studies are underway to determine if one of the two specific reductases of *B. harveyi* is primarily responsible for supplying electrons to the electron transport chain and the other responsible for shuttling electrons into luminescence. It may be that the NADH-specific FMN reductase is part of a membrane bound electron transport system which becomes solubilized upon cell lysis or the two reductases act to control electron flow as soluble components.

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