Steady-State and Time-Resolved Spectroscopy of F420 Extracted from Methanogen Cells

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Summary and Implications

The methanogen electron carrier F420 was extracted from a methanogen cell culture, *Methanobrevibacter ruminantium*, and separated from other cell components by a single anion exchange chromatographic process. The extent of separation was determined spectroscopically. The fluorescence lifetime of F420 at pH 7.5 is ~4.2 nanoseconds.

Introduction

Factor 420 (F420) is a major electron carrier in the methanogenic pathway of methanogens and is also found in other archaebacteria (1,2). It is so named due to the 420-nm absorbance maximum of its oxidized state (1). This oxidized state (Figure 1a) exhibits a bright blue-green fluorescence with a maximum around 470 nm and is a two-electron acceptor under physiological conditions (2). This fluorescence is a distinguishing feature of methanogenic bacteria and has been used for identification purposes (6). The spectral features of the oxidized F420 change with pH (2,4,7). In acidic media the absorbance maximum at 420 nm shifts to around 380 nm (3,5,6, 9). An isobestic point is reported to exist at 401 nm with an extinction coefficient of 25.9 mM^{-1} cm^{-1} (1,6). It also is reported that the extinction coefficient at 420 nm changes as a function of temperature due to a temperature effect on the pKa (6.3) of the 8-OH group (2,4). Other reported pKa values for F420 are 12.3 for the nitrogen at the 3 position (2) and 1.7 (4). The reduced form of F420 (Figure 1b) loses its absorbance at 420 nm and becomes nonfluorescent (4,5,6). The F420 was extracted from a methanogen culture and separated from other factors (F430, a nonfluorescent compound [1], and F342) and flavins by using an acetone extraction and anion exchange chromatography.

Materials and Methods

The methanogen cell paste, *M. ruminantium*, was provided by Mark Rasmussen from the National Animal Disease Center, Ames, IA. The method for extraction and isolation of the F420 was similar to that reported by Schoenheit et al. (3) and will be briefly described herein. Acetone was precooled to about -15°C. Two milliliters of the precooled acetone was added to the vial containing the cell paste and stirred using a magnetic stirrer for about 30 minutes. The suspension was centrifuged and the supernate fluid was collected. A second 2-ml volume of 50% acetone (chilled) and water was added to the pellet and stirred for another 30 minutes, followed by centrifugation. The supernate fluids from both extractions were combined and diluted 1:2 with a 0.3 M NaCl in 50 mM Tris-HCl buffer pH 7.5. The resulting solution was then applied to a Sephadex QAE A-25 ion exchange column 1 cm in diameter and 4.5 cm in length. The ion exchange resin was pre-equilibrated with the same buffer used to dilute the supernate fluids. The column was then washed with about 15 ml of the same buffer. The combined supernate fluids were then applied to the column and about 45 ml of the 0.3 M NaCl/50 mM Tris-HCl buffer was then eluted through the column. The F420 was retained on the column. To elute the F420, 15 ml of a 1.0 M NaCl/50 mM Tris-HCl buffer (pH 7.5) was then eluted through the column. During elution of the F420 several fractions of about 3 ml each were collected. The yellow F420 compound could be seen starting from the second fraction. This fraction and the subsequent fraction were then used for the steady-state and time-resolved measurements.

The absorbance spectrum of the F420 in the eluting buffer was obtained using a Perkin-Elmer Lambda 18 UV-visible spectrometer. Excitation and emission spectra were obtained using a Spex FluoroMax. Emission spectra were obtained using two excitation wavelengths, 295 nm and 420 nm. The excitation spectrum was obtained using an emission wavelength of 470 nm.

Time-correlated single photon counting measurements were performed using the apparatus described elsewhere (8). Fluorescence decays were collected over three different ranges of wavelengths, >335 nm, >400 nm, and >455 nm, and in each case yielded the same single-exponential lifetime of 4.2 nanoseconds. The time scale for the experiments was 12.5 nanoseconds and the excitation wavelength was 295 nanometers.

Anisotropy measurements also were performed on the same F420 sample. The same single-photon counting apparatus was used. A computer controlled motor was used to turn the emission polarizer to parallel and

perpendicular orientations, relative to the vertically polarized excitation beam, at alternating 2-minute intervals.

Results and Discussion

Our success in the extraction and isolation of F420 from other species in the extracted methanogen culture can be seen from the absorbance spectrum we obtained, which was identical to spectra published previously (3,4). The only exception was a small absorption band around 350 nm, which was not observed in our excitation spectrum. There was no difference in the emission spectrum when exciting at 420 nm compared with exciting at 295 nm, except in the magnitude of the intensity.

As the pH is increased through the pKa of the nitrogen at the three position a second component is observed in the fluorescence decay due to the ionization of this group. Lifetimes were not measured at low-pH values due to the change in the absorption spectrum with pH. The spectrum change was sufficient as to not allow us to excite the sample efficiently.

No effort was made to remove the buffer salts from the isolated F420 nor was the water removed. For this reason solvent-dependent studies were not performed. The extent of our solvent studies incorporated a dilution of the aqueous F420 sample with DMSO in a 1:4 F420:DMSO ratio. The decay for this solution was fit to a sum of two exponentials. The long component, which made up 72% of the decay, was ~4,660 ps, whereas the short component was ~1,730 ps. The c2 was 1.3 for this decay due to oscillations in the data.

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