BBA Report

BBA 41166

The subcellular localization of the pteridines in strain R-26 of *Rhodopseudomonas spheroides*★

DAN W. REED and BERGER C. MAYNE

C.F. Kettering Research Laboratory, Yellow Springs, Ohio 45387 (U.S.A.)

(Received January 18th, 1971)

SUMMARY

Pteridines were detected in strain R-26 of *Rhodopseudomonas spheroides* and the pteridine concentrations in subcellular fractions were measured by a sensitive fluorimetric method. The whole cell fraction contained nearly equal concentrations of pteridine and reaction center bacteriochlorophyll. The soluble subcellular fraction contained the majority of the pteridine and the photochemically active chromatophore preparations contained very little. No pteridine was detected in purified reaction center complex preparations. This subcellular localization of pteridines was not consistent with a pteridine functioning as the primary electron acceptor in this photosynthetic system.

The primary electron donor in bacterial photosynthesis, P870, has been well characterized as a bacteriochlorophyll molecule in a specialized environment[1,2]; but its reaction partner, the primary electron acceptor, has not been identified with certainty. Several types of experimental evidence have led to the suggestion that a pteridine may function in this primary role[3]. Pteridines are present in photosynthetic bacteria and their concentration increases when the bacteria are grown under photosynthetic conditions[4]. The pteridine analog, 4-phenoxy-2,6-diaminopyridine, inhibits pigmentation and development of functional membranes[5]. Pteridines interact with subchromatophore particles from *Rhodospirillum rubrum* and are photochemically reduced by them[3].

In the present investigation, we have developed a sensitive fluorimetric method to directly measure the pteridine content of crude fractions and used this assay to determine the localization of pteridines in subcellular fractions from strain R-26 of *Rhodopseudomonas spheroides*. The primary electron acceptor occurs in close association with the reaction center bacteriochlorophyll, P870. If a pteridine is this primary electron acceptor, it will be localized with P870 in the photochemically active chromatophores

★Contribution No. 421 from the C.F. Kettering Research Laboratory.

or subchromatophore reaction center complex particles.

Rhodopseudomonas spheroides, strain R-26, was grown in succinate media under photosynthetic conditions. The cells were washed, suspended in 0.01 M Tris – HCl buffer, pH 7.5, and broken at 0–10° by sonication for 8 min at full output in a 10-kcycle Raytheon sonic oscillator. The broken cell suspension was used as the whole cell fraction. The crude chromatophore membranes were recovered as a pellet separate from the soluble fraction by centrifuging the broken cell suspension of 60 min at 230000 x *g*. Chromatophores were purified from the broken cell suspension and reaction centers were isolated by the Triton X-100 fractionation procedure described previously[6]. Light-harvesting bacteriochlorophyll, B870, was determined from its absorbance at 862 nm using the extinction coefficient of 127 $mM^{-1} \cdot cm^{-1}$ (ref. 1) and the membrane preparations were diluted in the Tris – HCl buffer to 0.4 mM B870. Reaction center P870 was determined from the light-induced absorbance change at 865 nm using the differential extinction coefficient of 93 $mM^{-1} \cdot cm^{-1}$ (ref. 2). Reaction center complex preparations were 2 μM P870.

For fluorescence measurements, the preparations were treated to remove the bacteriochlorophyll which absorbed strongly at the wavelengths of maximum pteridine fluorescence excitation; and the proteins were denatured in alkaline solutions to obtain maximal fluorescence yields. Samples, 2.0 ml, were extracted 4 times with 5.0 ml of acetone–methanol (7:2, v/v). The combined organic extracts were washed 3 times with equal volumes of light petroleum (b.p. 30–60°) and the ether washes were discarded. The remaining polar organic layer was combined with the original protein precipitate and the mixture was evaporated to dryness at 40° under reduced pressure. The denatured protein residue was either solubilized in 5 ml of 0.1 M NaOH containing 1% sodium lauryl sulfate or extracted with ethanol–concentrated ammonia (2:1, v/v).

The pteridine concentrations in the subcellular fractions were determined by comparing the yield of 480 nm fluorescence from the alkaline sodium lauryl sulfate extracts with solutions of 2-amino-4-hydroxypteridine and mixtures of the pteridine and extract. Samples in a 1-cm cuvette were illuminated with 366 nm light isolated from the output of a high pressure mercury–xenon lamp (Hanovia 510B-1) with a 0.25 M Bausch and Lomb monochromator. The sample was placed at the entrance slit of a Liss prism monochromator and illuminated at 90° from the axis of the Liss monochromator. The output from the analyzing monochromator was detected with an RCA 6217 photomultiplier.

The fluorescence spectra of the extracts were very similar to that of 2-amino-4-hydroxypteridine solutions (Fig. 1). The wavelengths of maximum fluorescence emission were 470–480 nm with similar bandwidths and the fluorescence excitation maxima were near 365 nm. The slight skew and shift toward longer wavelength in the crude alkaline sodium lauryl sulfate preparations was less apparent in alkaline ethanol extracts and the spectra of preparations purified further by paper chromatography were essentially identical to that of the pteridine. Paper chromatography of the alkaline ethanol extracts in *n*-propanol–1% NH_4OH (2:1, v/v) separated three blue fluorescent compounds with mobilities of 0.3, 0.6 and 0.9 relative to 2-amino-4-hydroxypteridine. Folic acid did not fluoresce in these solvents and the oxidative degradation product of folic acid, 2-amino-4-hydroxypteridine-6-carboxylic acid, was not detected by paper electrophoresis.

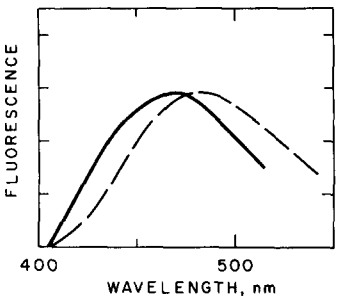

Fig.1. Fluorescence emission spectra of the whole cell extract from strain R-26 of *Rps. spheroides* (– –), and 1 μM 2-amino-4-hydroxypteridine (—) in 0.1 M NaOH containing 1% sodium lauryl sulfate. The excitation wavelength was 366 nm.

Typical results from measurements of pteridine and reaction center P870 concentrations in the subcellular fractions are listed in Table I. The concentrations of pteridine and reaction center bacteriochlorophyll were nearly equal in the whole cell fraction but the pteridine was not bound to the chromatophore membranes. By centrifuging the broken cell fraction, the majority of the pteridine in the soluble supernatant fraction was separated from the photochemically active chromatophores in the pellet. The pteridine concentration was very low in chromatophore preparations and no pteridine was detected in the purified reaction center complex preparations.

TABLE I

SUBCELLULAR LOCALIZATION OF PTERIDINE IN STRAIN R-26 OF *Rps. spheroides*

Fraction	*Composition*		
	P870 (μM)	*Pteridine (μM)*	*P870 : pteridine (mole/mole)*
Whole cell	13	10.4	1.2
Crude chromatophore	13	0.62	21
Soluble	0	4.5	–
Reaction center	0.84	< 0.1	> 8.4

Fluorescence measurements using broad-band ultraviolet excitation (Corning 7-54 filter) were also unable to detect any fluorescent compounds other than bacteriochlorophyll and bacteriopheophytin in reaction center preparations[7]. These reaction centers have previously been shown capable of photooxidizing mammalian cytochrome *c* with a quantum requirement near 1 (ref. 2) and with a halftime of 25nsec (ref. 8). The localization of the pteridines in the soluble subcellular fraction from strain R-26 of *Rps. spheroides* rather than in chromatophore or reaction center preparations is not consistent with a pteridine functioning as the primary electron acceptor in this photosynthetic system.

We are grateful to Dr. William Bulen for the generous gifts of 2-amino-4-hydroxypteridine and 2-amino-4-hydroxypteridine-6-carboxylic acid and to Dorothy A. Limbach for expert technical assistance.

REFERENCES

1 R.K. Clayton, *Photochem. Photobiol.*, 5 (1966) 669.
2 J.R. Bolton, R.K. Clayton and D.W. Reed, *Photochem. Photobiol.*, 9 (1969) 209.
3 R.C. Fuller and N.A. Nugent, *Proc. Natl. Acad. Sci. U.S.*, 63 (1969) 1311.
4 F.I. Maclean, H.S. Forest and D.S. Hoare, *Arch. Biochem. Biophys.*, 117 (1966) 54.
5 N.A. Nugent and R.C. Fuller, *Science*, 158 (1967) 922.
6 D.W. Reed, *J. Biol. Chem.*, 244 (1969) 4936.
7 D.W. Reed and B.C. Mayne, *Biophys. J.*, 10 (1970) 205a.
8 B. Ke, T.H. Chaney and D.W. Reed, *Biochim. Biophys. Acta*, 216 (1970) 373.