A C T A   U N I V E R S I T A T I S   L O D Z I E N S I S
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EFFECT OF IONIC ENVIRONMENT ON THE PROTEIN FLUORESCENCE OF BACTERIORHODOPSIN

The protein fluorescence spectra and polarization of bacteriorhodopsin excited at 293 nm and at room temperature was investigated in solution containing chlorine-, acetate- and rhodanide-ions as quenchers of fluorescence in Sörensen and Britton-Robinson buffers in the pH-range from 4 to 10. These quenchers did not affect the fluorescence and polarization spectra but considerable effect of the composition of buffers was found especially on the fluorescence polarization spectrum. The changes indicate that some of the buffer constituents (citric acid, acetic acid, phosphate ions) may exert an effect on the tertiary structure of bacteriorhodopsin in addition to the direct pH-effect.

Bacteriorhodopsin (BR) is a purple protein-pigment complex in the cell membrane of several halobacteria [11, 12], which forms the molecular basis of a new type of photophosphorylation [14]. Upon illumination BR undergoes a rather complicated photochemical cycle with a number of photometrically defined states [18]. It works simultaneously as a light driven proton-pump [17], a photoelectric generator of current [1], and a delicate regulator of the light-induced membrane potential [6]. All these functions are connected among others with conformational changes in the protein moiety. Since the conformational change of the protein is initiated by light-quanta absorbed by the retinal chromophore, an energy exchange between the retinal and the protein moiety should be assumed [4]. Attempts to reveal the routes and mechanism of this energy-exchange process have recently been made, by measuring certain fluorescence characteristics of the retinal pigment complexed with the protein [13, 7].

Seweral systematic studies have been carried out concerning

the role of the tryptophan residues in the molecular mechanisms of different functions of BR [15, 10]. In this report, in order to monitor the states and interactions of aromatic amino acid residues, especially those of tryptophans, protein fluorescence properties of BR were investigated in solutions containing Cl^-, CN^-, SCN^- ions as fluorescence quenchers at different pH-values.

Materials and methods

Purple membrane patches containing BR were isolated from Halobacterium halobium strain N_1M_1 by standard procedures of extraction and purification [16]. The membrane patches were suspended in Sörensen or Britton-Robinson buffers at pH-values between 4 and 10 and containing quenchers (KSCN, $KClO_4$, CH_3COOK) in 0.25, 0.5 and 1 M concentration. The BR concentrations of the samples were adjusted to an optical density of 0.3 at 285 nm.

The absorption spectra were measured with a UNICAM SP 1800 recording spectrophotometer. The fluorescence spectra and polarization degrees were recorded with a PERKIN-ELMER MPF-44A spectro-fluorimeter using 1 cm stoppered quartz cells. The half-widths of exciting and observation bands were 5 nm. The spectra were corrected for the spectral transmission of the emission monochromator and for the spectral sensitivity of the detector. Corrections of fluorescence spectra for reabsorption were also made; secondary fluorescence effects were negligible. The influence of light-scattering could not be eliminated, no selective scattering occured which could have falsified the spectral characteristics measured. All measurements were made on samples of the same BR extract, at room temperature.

Results and discussion

The relative absorption spectrum of native BR has two main bands centered at 280 nm (with two shoulders), and with maximum at 567 nm (Fig. 1). A small, practically constant absorption within the 300-450 nm region is probably due to light-scattering.

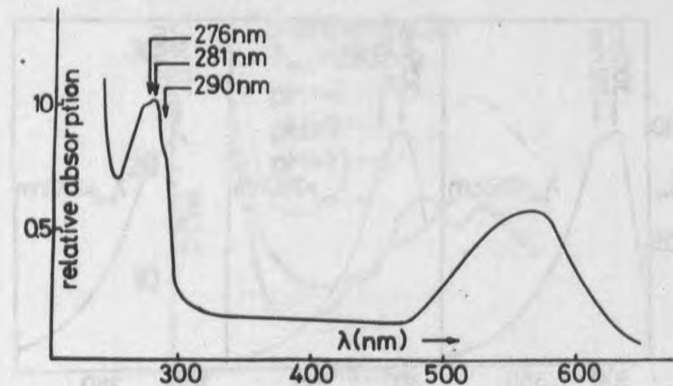

Fig. 1. Relative absorption spectrum of BR in Sörensen buffer pH = 7

Widmo różnicowe pochłaniania bakteriorodopsyny (BR) w buforze Sörensena, pH = 7

Относительный спектр поглощения бактериородопсина (БР) в буфере Сэренсена, pH = 7

The ultraviolet band consists mainly of contributions from tyrosine and tryptophan. The exact number of tryptophans and tyrosines in the complex formation is not known (see e.g. [8]). Considering the spectral properties of phenylalanine, tyrosine and tryptophan Fig. 1 indicates that oscillators absorbing at 290 nm are primarily electronic transitions of tryptophan, rather than those of the other two aromatic amino acid residues.

The fluorescence emission spectrum depends upon the wavelength of excitation (Fig. 2). At 265 nm excitation the emission spectrum displays a maximum at 300 nm and a shoulder at 312 nm. For 280 nm excitation emission with maximum 305 nm and a shoulder at 314 nm is characteristic, while with 293 nm the emission maximum is located at 311 nm and a shoulder appears at 320 nm. The fluorescence spectra are shifted towards shorter waves as compared to the usual protein fluorescence.

Very slight influence of ions usually functioning as quenchers could be observed on the emission properties even at high (about 1 molar) concentration.

pH affected the protein fluorescence, especially its polarization properties (Fig. 3). These spectra have minima in the

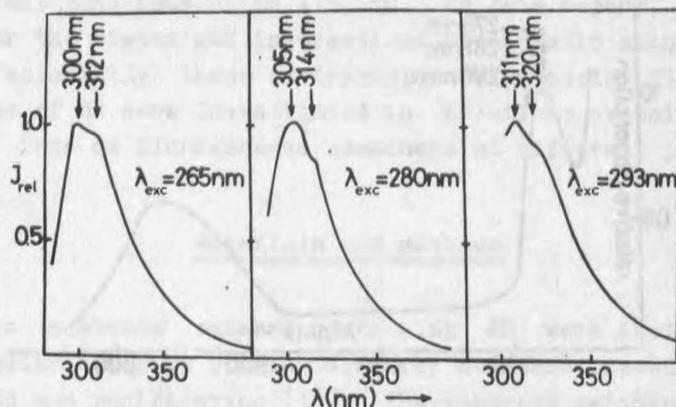

Fig. 2. Relative fluorescence spectra of BR in Sörensen buffer pH = 7, at different excitation wavelengths

Widma różnicowe fluorescencji BR w buforze Sörensena, pH = 7, dla różnych długości fal światła wzbudzającego

Относительные спектры флуоресценции БР в буфере Сэренсена, pH = 7, при различных длинах волн возбуждающего света.

degree of polarization above 310 nm and maxima in the 370-390 nm region. The polarization spectra exhibit fine structure as well. Scattering exerts its influence primarily near the excitation wavelength.

Sörensen and Britton-Robinson buffers do not influence appreciably the absorption in the ultraviolet but alter the polarization pattern (Fig. 4).

On the basis of the absorption and fluorescence emission spectra in the ultraviolet region, one may attempt to indentify the absorbing and emitting centers.

The ultraviolet absorption band consists mainly of contributions from tryptophan and tyrosine. Tyrosine, which absorbs in part in the same spectral region as tryptophan, emits a single band with maximum at 303 nm in aqueous solutions, but its fluorescence is generally quenched by tryptophan if the latter is present in the protein [9]. It is more plausible to assume an anomalously blueshifted tryptophan-fluorescence than an unusual tyrosil fluorescence which dominates the room-temperature protein fluorescence of native BR. In proteins which contain tryp-

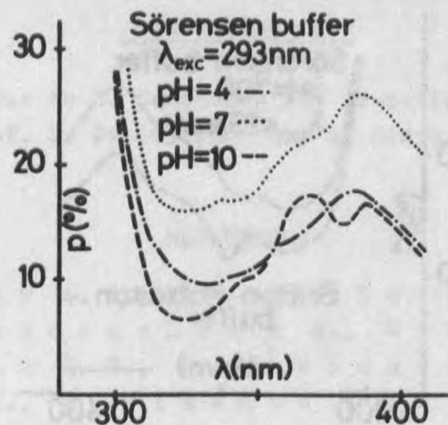

Fig. 3. Polarization spectra of protein fluorescence of BR at different pH. Buffer composition: pH = 4: 0.055 M $C_6H_8O_7$, 0.275 M NaOH, 0.045 M HCl; pH = 7: 0.027 M KH_2PO_4 0.040 M Na_2HPO_4; pH = 10: 0.065 M C_2H_5ON, 0.065 M NaCl, 0.035 M NaOH

Widma fluorescencji białkowej BR w różnych wartościach pH. Skład buforów: pH = 4: 0,055 M $C_6H_8O_7$, 0,275 M NaOH, 0,045 M HCl; pH = 7: 0,027 M KH_2PO_4, 0,040 M Na_2HPO_4; pH = 10: 0,065 M C_2H_5ON, 0,065 M Na Cl, 0,035 M NaOH

Спектры белковой флуоресценции БР в различных pH. Состав буферов: pH = 4: 0,055 M $C_6H_8O_7$, 0,275 M NaOH, 0,045 M HCl; pH = 7: 0,027 M KH_2PO_4, 0,040 M Na_2HPO_4; pH = 10: 0,065 M C_2H_5ON, 0,065 M NaCl, 0,035 M NaOH

tophan, the emission spectrum is thought to be a strong function of the environment of the tryptophans [2]. Eisinger and Navon [3] have shown that in non-polar environment the emission maximum moves by about 10-20 nm towards shorter waves. This shift can be explained by the presence of tryptophan residues situated in a low-polarity environment, and interacting with their surroundings through their iminogroups via hydrogen bonds. Such conditions may be easily attained in the sack formed by the protein in which the polyene chain of the retinal chromophore is nested, and the proteins are not accessible to simple quencher ions even if they are present in high concentration. pH seems to effect H-bonds which are not directly involved in the formation of the purple complex, at least in the pH-range 4-10. The structure may be considerably affected by the composition of the buffer even if the pH is kept constant.

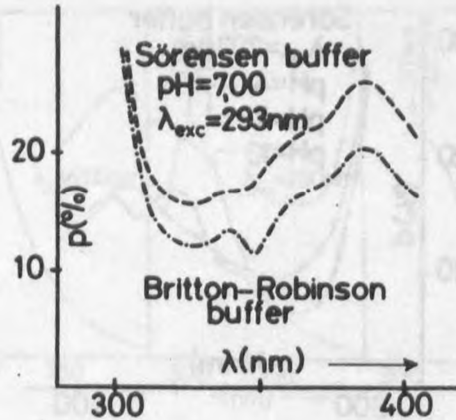

Fig. 4. Polarization spectra of protein fluorescence of BR in different buffers. Buffer composition. Sörensen: 0.027 M KH_2PO_4, 0.040 M Na_2HPO_4; Britton-Robinson: 0.026 M C_2H_4O, 0.026 M H_3BrO_4, 0.026 M H_3PO_4, 0.069 M NaOH

Widma polaryzacji fluorescencji białkowej BR w różnych buforach. Skład buforów: bufor Sörensena: 0,027 M KH_2PO_4, 0,040 M Na_2HPO_4; bufor Brittona-Robinsona: 0,026 M C_2H_4O, 0,026 M H_3BrO_4, 0,026 M H_3PO_4, 0,069 M NaOH

Спектры поляризации белковой флуоресценции БР в различных буферах. Состав буферов. Буфер Сэренсена: 0,027 M KH_2PO_4, 0,040 M Na_2HPO_4; Буфер Бриттона-Робинсона: 0,026 M C_2H_4O, 0,026 M H_3BrO_4, 0,026 M H_3PO_4, 0,069 M NaOH

With the use of P l a t t's notations [19] the probable classifications of the relevent absorption and emission transitions of tryptophan, we propose that the tryptophans of BR exist in at east two different states, one of them reflects the presence of an extremely low-permittivity microenvironment, and the structure of the emission spectra is in part due to the two-level fluorescence of tryptophan [5]. Their polarization properties are different and of opposite sign. Our final conclusion is that the retinal chromophore of bacteriorhodopsin exists in an extremly hidrophobic environment which is lined by functionally important tryptophan residues. It is possible, that excitation redistribution takes place within excited tryptophans.

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WPŁYW ŚRODOWISKA JONOWEGO
NA FLUORESCENCJĘ BIAŁKOWĄ BAKTERIORODOPSYNY

Badano widma fluorescencji białkowej i polaryzacji fluorescencji bakteriorodopsyny przy długości światła wzbudzającego 293 nm, w temperaturze pokojowej, w roztworach zawierających jony chlorkowe, octanowe i rodankowe jako wygaszacze fluorescencji, w buforach Sörensena i Brittona-Robisona, w zakresie pH 4-10. Wygaszacze nie miały wpływu na widma fluorescencji i polaryzację fluorescencji, lecz stwierdzono znaczny wpływ składu buforowego, zwłaszcza na widmo polaryzacji fluorescencji. Otrzymane wyniki wykazują, że niektóre składniki buforów (kwas cytrynowy, kwas octowy, jony fosforanowe) mogą wpływać na trzeciorzędową strukturę bakteriorodopsyny niezależnie od bezpośredniego efektu pH.

Эржебет Балинт, Беля Карвали, Зольтан Варконни

ВЛИЯНИЕ ИОННОЙ СРЕДЫ НА БЕЛКОВУЮ ФЛУОРЕСЦЕНЦИЮ БАКТЕРИОРОДОПСИНА

Исследовали спектра белковой флуоресценции и поляризации флуоресценции бактериородопсина при возбуждении в 293 нм, при комнатной температуре, в растворах содержащих хлористые, ацетатные и роданковые ионы в качестве тушителей, в буферах Сэренсена и Бриттона-Робинсона, в области pH 4-10. Использованные тушители не влияли на флуоресценцию и поляризайию флуоресценции но буферной состав оказывал существенное влияние на спектры поляризации флуоресценции. Эти изменения означают, что некоторые компоненты буферов (лимонная кислота, уксусная кислота, фосфатные ионы) могут влиять на третичную структуру бактериородопсина независимо от непосредственного влияния pH.