Biochimica et Biophysica Acta, 725 (1983) 361–367
Elsevier

BBA 41408

ISOLATION AND PROPERTIES OF A PIGMENT-PROTEIN COMPLEX ASSOCIATED WITH THE REACTION CENTER OF THE GREEN PHOTOSYNTHETIC SULFUR BACTERIUM *PROSTHECOCHLORIS AESTUARII*

HENK VASMEL, TON SWARTHOFF, HERMAN J.M. KRAMER and JAN AMESZ

Department of Biophysics, Huygens Laboratory of the State University, P.O. Box 9504, 2300 RA Leiden (The Netherlands)

(Received July 18th, 1983)

Key words: Pigment-protein complex; Linear dichroism; Circular dichroism; Reaction center; Bacterial photosynthesis; (Prosthecochloris aestuarii)

The membrane-bound pigment system of green sulfur bacteria consists of light-harvesting bacteriochlorophyll *a*-protein and a 'core complex' that is associated with the reaction center (Kramer, H.J.M., Kingma, H., Swarthoff, T. and Amesz, J. (1982) Biochim. Biophys. Acta 681, 359–364). The isolation and properties of the core complex from *Prosthecochloris aestuarii* are described. The complex has a molecular mass of 200 ± 50 kDa and contains bacteriochlorophyll *a*, carotenoid and pigments absorbing near 670 nm (probably bacteriopheophytin *c* and an unidentified pigment). Fluorescence emission spectra and sodium dodecyl sulfate polyacrylamide gel electrophoresis showed the absence of light-harvesting bacteriochlorophyll *a*-protein. The preparation showed no reaction center activity. Circular and linear dichroism spectra indicated that the structure of the core complex was basically not altered by the isolation procedure. Comparison with the CD spectrum of the intrinsic membrane-bound pigment-protein complex indicates that the latter contains 14 bacteriochlorophyll *a* molecules (two subunits) belonging to the light-harvesting protein and about 20 bacteriochlorophyll *a* molecules belonging to the core complex.

Introduction

The pigment system of the green photosynthetic bacteria (Chlorobiaceae) consists of various components. The largest of these is the chlorosome, which typically contains about 10000 BChl *c*, *d* or *e* molecules [1] and which is situated adjacent to the cytoplasmic membrane. After removal of the chlorosome, membrane vesicles have been obtained from *Prosthecochloris aestuarii* [2] and from *Chlorobium limicola* [3] which contain 80–120 BChl *a* molecules per reaction center. By detergent treatment of such membranes a pigment-protein complex of essentially the same pigment composition (the so-called photosystem-protein complex) has been isolated with a molecular mass of about 600 kDa [4,5].

Treatment of the photosystem-protein complex with the chaotropic agent guanidine hydrochloride releases two molecules of a water-soluble light-harvesting BChl *a*-protein, that has been extensively characterized [1,6]. The resulting preparation, called the reaction center pigment-protein complex, contains about 35 BChl *a* molecules per reaction center and has a molecular mass of about 350 kDa [4]. In the reaction center pigment-protein complex, electron transport at the acceptor side is strongly impaired, resulting in a low yield of stable charge separation upon illumination and a high yield of formation of the triplet of the

Abbreviations: BChl, bacteriochlorophyll; LDS, lithium dodecyl sulfate.

primary electron donor P-840 [7].

The reaction center pigment-protein complex shows two major fluorescence emission bands in the near-infrared region at low temperature, which are located at 828 and 838 nm [8]. The excitation spectrum of the 828 nm emission was virtually identical to the absorption spectrum of the soluble BChl *a*-protein, whereas the excitation spectrum for the 838 nm emission showed, in addition to bands of BChl *a*, contributions by carotenoid and pigments absorbing near 670 nm [9]. These measurements strongly suggest that the reaction center pigment-protein complex consists of at least two components: a pigment-protein complex that is spectroscopically very similar to the soluble BChl *a*-protein, and a complex that contains BChl *a*, carotenoid and pigments absorbing near 670 nm and which fluoresces at 838 nm. We shall call this complex the 'core complex'. Measurements of linear dichroism [10] agree with such a pigment arrangement. In the present communication we report the isolation and purification of the core complex and describe some of its properties.

Materials and Methods

The photosystem-protein and reaction center pigment-protein complexes were prepared from *P. aestuarii* strain 2 K as described earlier [4]. The soluble BChl *a*-protein was prepared as described in Ref. 11. Low-temperature spectra were measured in a noncrystallizing medium, obtained by addition of 40% (w/w) sucrose and mixing the solution with an equal volume of glycerol. Linear dichroism spectra were measured in a pressed polyacrylamide gel [12] as described elsewhere [13]. The gel was pressed in two perpendicular directions, so that axially symmetric samples were obtained. The increase in length along the orientation axis was by a factor of 1.5.

The apparatus for measuring circular and linear dichroism is briefly described elsewhere [13]. Low-temperature absorption and fluorescence emission spectra were measured as described in Ref. 14; excitation spectra as described in Ref. 15.

Results

Isolation

Treatment of the photosystem-protein complex with LDS resulted in a partial resolution of its constituents as follows. A solution of the photosystem-protein complex ($A_{810} = 0.9$/mm) containing 10 mM sodium phosphate, 10 mM sodium ascorbate and 10 mM glycine, pH 8.0, 40% sucrose (w/w) and 0.05% (v/v) Triton X-100, was mixed with a solution of 10% (w/v) LDS to a final concentration of 0.2% and incubated for 1 h at room temperature. The reaction mixture was subsequently diluted twice with ice-cold phosphate-ascorbate buffer (pH 7.4) and put on a 20–50% (w/w) continuous sucrose gradient. After centrifugation for 16 h at 200 000 × *g* bands were obtained at 42% and at 36% sucrose. The first one appeared to consist of undissociated photosystem-protein complex, whereas the second one was enriched in BChl *a*-protein, as judged from the absorption spectra. Of particular interest was a blue fraction obtained at 30% sucrose, which showed an enhanced absorption in the long-wavelength region. This fraction contained 5–10% of the total BChl *a*. Solubilized carotenoid and BChl *a* were present at still lower sucrose concentration.

Fig. 1 shows the room- and low-temperature absorption spectra of the blue fraction. At 77 K the preparation showed near-infrared BChl *a* absorption bands at 836, 813 and 801 nm and a shoulder near 785 nm. The band at 825 nm, typical for the BChl *a*-protein, was lacking, and comparison with the absorption spectra of the photosystem-protein and reaction center pigment-protein complexes [4] showed enhanced absorption

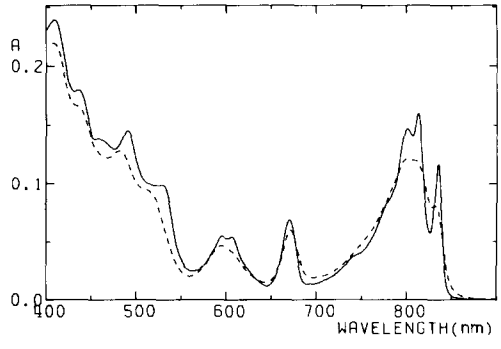

Fig. 1. Absorption spectra of the fraction obtained at about 30% sucrose (the 'core complex') in noncrystallizing medium containing 10 mM phosphate-ascorbate buffer, pH 7.4. (- - - - -) At room temperature, (———) at 77 K.

near 670 nm. In the Q_x region the spectrum showed two about equally intense BChl *a* bands at 595 and 606 nm. Below 550 nm the spectrum was similar to that of the reaction center pigment-protein complex [9]. The most prominent peptide bands upon electrophoresis on polyacrylamide gel after treatment with SDS were one at 43 kDa, earlier observed in the reaction center pigment-protein complex [4], together with a diffuse band centered at about 60 kDa. Except for a weak band near 32 kDa, bands of peptides of lower molecular mass were strongly reduced in intensity as compared to the photosystem-protein and reaction center pigment-protein complexes. The 40 kDa peptide band due to subunits of the BChl *a*-protein [11] was absent. These observations indicate that the 30% sucrose fraction consists of the core complex that, together with BChl *a*-protein, constitutes the membrane-bound photosystem of *P. aestuarii* (see introduction). From the elution volume on a Sepharose CL-6B column a molecular mass of the complex of 200 ± 50 kDa was calculated. Ovalbumin, catalase and thyroglobulin were used as marker proteins.

Optical properties

Low-temperature emission spectra of the core complex, for two different wavelengths of excitation, are shown in Fig. 2. The main emission band was at 839 nm, which band has probably the same origin as the 838 nm emission from the photosystem-protein and reaction center pigment-protein complexes [9]. The band at 828 nm that has been ascribed to the BChl *a*-protein was very weak, and only observed upon excitation in the BChl *a* Q_x band near 600 nm. The origin of the broad band around 790 nm is not clear. The excitation spectrum for the 839 nm fluorescence (Fig. 3) was similar to the absorption spectrum except for a somewhat lower amplitude of the 670 nm band, and the presence of weak carotenoid bands at 490 and 521 nm.

From the above results we conclude that treatment of the photosystem-protein complex with LDS resulted in the essentially complete removal of the light-harvesting BChl *a*-protein. This applies not only to the soluble BChl *a*-protein, but also to the bound protein that appears to be a constituent of both the pigment-protein and reaction center

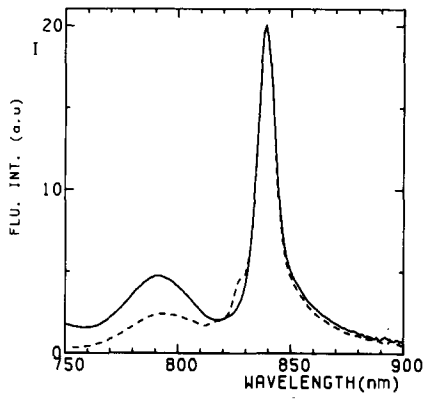

Fig. 2. Fluorescence emission spectra of the core complex, measured at 77 K. (———) Excitation at 670 nm, (- - - - -) excitation at 606 nm. The spectra were normalized at 839 nm.

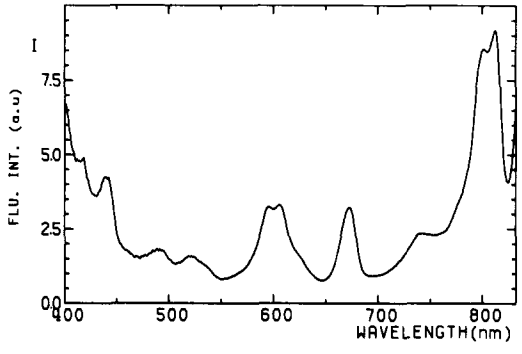

Fig. 3. Fluorescence excitation spectrum of the core complex, measured at 77 K. Detection wavelength 839 nm. The relative fluorescence intensity is plotted per incident quantum.

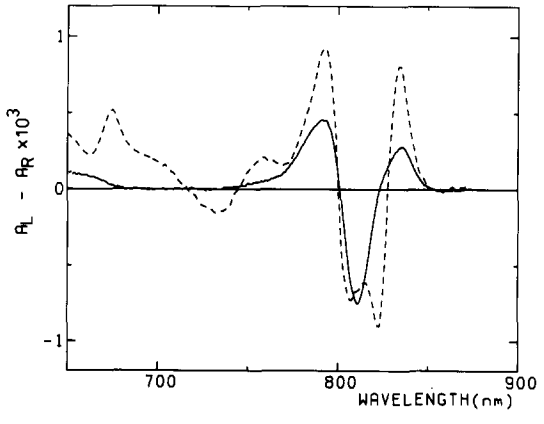

Fig. 4. CD spectra measured at room temperature of the core complex (———) and the reaction center pigment-protein complex (- - - - -).

pigment-protein complexes [9,10]. If one assumes that the fluorescence yields of the bacteriochlorophylls emitting at 828 and 838–839 nm are not changed by the isolation procedure, analysis of the fluorescence emission spectra of the photosystem-protein [8] and core complexes indicates that close to 99% of the soluble and bound BChl *a*-protein was removed from the photosystem-protein complex by LDS incubation.

The circular dichroism spectrum of the core complex, measured at room temperature, is shown in Fig. 4. The main features are a strong negative band at 810 and two positive bands at 835 and 791 nm. The first two bands correspond to the absorption bands near 836 and 813 nm; the third band may be due to the band near 800 nm in the absorption spectrum measured at 77 K. The CD spectrum of the reaction center pigment-protein complex (broken line) shows additional contributions due to the BChl *a*-protein as can be most clearly seen at 822 nm [16].

CD spectra measured at low temperature are shown in Fig. 5. Except for an enhanced resolution due to sharpening of the absorption bands, the spectrum of the core complex (Fig. 5A) is not basically different from that measured at room temperature. The main difference is the appearance of a positive band at 820 nm that is not resolved in the absorption spectrum. The CD spectrum of the BChl *a*-protein (broken line) was quite similar to that measured by Philipson and Sauer [16]. Comparison with the spectrum obtained by Olson et al. [17] (who designated the species *C. limicola* strain 2 K) shows discrepancies in the relative intensities and positions of the bands. Fig. 5B shows that a reasonable simulation of the spectrum of the reaction center pigment-protein complex in the near-infrared region can be obtained by a linear combination of the spectra of the BChl *a*-protein and the core complex. This supports the conclusion that the structure of the bound BChl *a*-protein, at least with regard to the arrangement of the bacteriochlorophylls, is identical to that of the soluble protein. The results also indicate that the structure of the core complex is left basically unchanged by the LDS treatment.

The spectrum of the photosystem-protein complex (Fig. 5C) resembles that of isolated membranes of *P. aestuarii* [17]. It is similar to that of the reaction center pigment-protein complex, except for a larger contribution by the BChl *a*-protein as can, e.g., be seen at 824 nm. The conservative band around 750 nm in the spectra of the photosystem-protein and reaction center pigment-protein complexes is probably due to some residual BChl *c*. The intensity of this band varied for different preparations.

The spectrum of the reaction center pigment-protein complex (Fig. 4) shows a conservative CD band centered at 670 nm. This band was first observed by Olson [5] in isolated membranes and the photosystem-protein complex of *C. limicola* (but not, for some reason, in the reaction center pigment-protein complex), and was attributed to a dimer of bacteriopheophytin *c* [5]. This band is absent in the core complex. Since bacteriopheophytin *c* is probably closely associated with the reaction center, and may actually function as intermediary electron acceptor in the light reaction [18], the absence of the 670 nm band may be

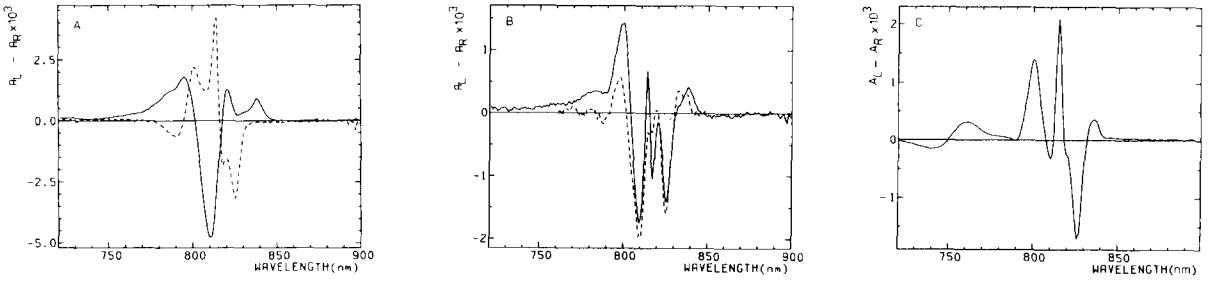

Fig. 5. CD spectra measured at 77 K. All spectra were normalized to a sample A_{810} of 1.0 at room temperature. (A) Core complex (———) and BChl *a*-protein (- - - - - -). (B) Calculated (———) (see text) and measured (- - - - - -) spectrum of the reaction center pigment-protein complex. (C) photosystem-protein complex.

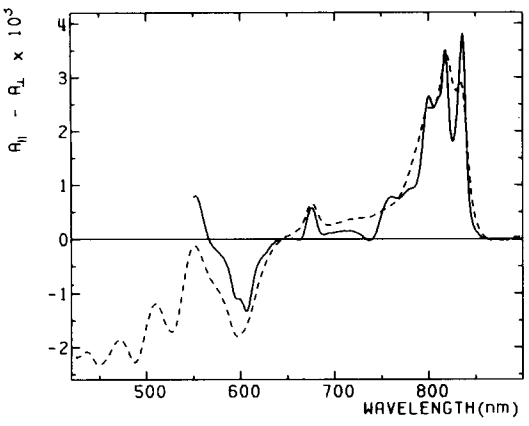

Fig. 6. LD spectra ($A_{\parallel} - A_{\perp}$) of the core complex at room temperature (- - - - - -) and at 77 K (———). $A_{810} = 0.04$ at room temperature. The sample was oriented in a polyacrylamide gel (see Materials and Methods). $A_{\parallel}$ is defined as the absorbance for light polarized parallel to the orientation axis.

related to the lack of reaction center activity to be discussed below.

Linear dichroism spectra of the core complex are shown in Fig. 6. In the near-infrared region three positive bands are visible (at 800, 818 and 836 nm at 77 K), corresponding to BChl *a* transition dipoles that make an angle smaller than the 'magic angle' (54.7°) with the orientation axis. Comparison with the LD spectrum of the membrane preparation 'Complex I' [10] indicates that this orientation axis is approximately parallel to the plane of the membrane. At 77 K the weak absorption band at 785 nm has negative polarization. The BChl *a* Q_x bands at 595 and 606 nm show negative polarization, as would be expected, because the Q_x and Q_y bands are known to be mutually perpendicular. Carotenoids show a more or less perpendicular orientation, as was also noted for the photosystem-protein and reaction center pigment-protein complexes [10]. The strong negative band at 814.5 nm of the BChl *a*-protein [10,19] is completely absent. The bands at 800 and 836 nm are obviously due to the corresponding bands in the absorption spectrum; the band at 818 nm is not resolved in the absorption spectrum, but may correspond to the transition that causes the positive band at 820 nm in the circular dichroism spectrum of Fig. 5. As was also noted for the CD spectra, around 670 nm the linear dichroism spectrum for the core complex is clearly different from that of the photosystem-protein and reaction center pigment-protein complexes: instead of two or three bands between 650 and 680 nm [10], only one, positive band at 666 nm can be seen in the spectrum of the core complex.

Reaction center activity

The presence of reaction center activity in the core complex was tested in various ways. Photooxidation of the primary electron donor P-840 was not observed, either with continuous illumination, or upon flash illumination, using an apparatus with a time resolution of 15 μs. In view of the sensitivity of the method applied, it can be concluded that, even upon prolonged illumination, the number of reaction centers that could perform a stable charge separation was less than one per 500 BChl *a* molecules, i.e., less than 5% of the number of reaction centers present in a corresponding amount of the reaction center pigment-protein complex. These results indicate that secondary electron transport was effectively absent in the core complex, but would not exclude the possibility of primary charge separation involving only the intermediary electron acceptor I. Such a reaction would give rise to a high yield of triplet formation [7], which can be observed either directly or by measuring the effect of a magnetic field on the yield of fluorescence [9]. Only very little triplet formation was observed in the core complex. Flash-induced absorbance changes at 425 nm were about 10% of those in the reaction center pigment-protein complex, and the change in fluorescence yield at 838 nm induced by a magnetic field of up to 0.12 T in the presence of 10 mM dithionite was less than 0.1%, as compared to 4% for the reaction center pigment-protein complex [9]. Thus, we conclude that the amount of photoactive reaction centers has been reduced to less than 10% of that originally present in the photosystem-protein complex.

Discussion

The results presented in this paper demonstrate that by incubation of the photosystem-protein complex from *P. aestuarii* with LDS a fraction is obtained (the core complex) from which about

99% of the light-harvesting BChl *a*-protein has been removed. The spectral data indicate that the structure of the complex is basically unchanged by this method.

The core complex showed almost no reaction center activity. This lack of activity cannot be explained only by destroyal of the iron-sulfur acceptors by LDS, because triplet formation would then be stimulated, rather than inhibited [7]. This indicates that the capacity to perform the primary charge separation is affected, which may be due to removal of a reaction center component or to a structural change induced by the detergent. The differences in the circular and linear dichroism spectra near 670 nm as compared to the photosystem-protein and reaction center pigment-protein complexes may be related to this lack of reaction center activity as discussed in Results. Another difference is in the absorption spectrum in the long-wavelength region: at 77 K the core complex shows only one band at 836 nm, whereas the larger complexes show two, albeit poorly resolved bands near 834 and 836.5 nm. However, the amplitude of these bands is variable [4,10], and in membrane preparations only one band was observed near 834 nm [10,17,20].

The spectral data presented here allow an analysis of the composition of the reaction center pigment-protein complex and provide an estimate of the number of BChl *a* molecules present in the core complex. The CD spectra are better suited to this purpose than the absorption spectra, in view of the difference in the longest-wavelength absorption bands and the spectral overlap of the other infrared bands. The best fit of the CD spectrum of the reaction center pigment-protein complex (Fig. 5B), with a correct ratio for the bands at 809 and 824 nm, was obtained by combining the spectra of the core complex and the BChl *a*-protein in a ratio that corresponded to approximately equal absorbance at 810 nm at room temperature. The integrated absorbances in the Q_x and Q_y regions indicate that this corresponds to a ratio of about 3 : 2 for the number of BChl *a* molecules in the core complex and BChl *a*-protein, respectively. Since the total number of BChl *a* molecules in the reaction center pigment-protein complex is about 35 per reaction center [4], this suggests that this complex contains two subunits of the BChl *a* complex, with a total of 14 BChl *a* molecules, and about 20 BChl *a* molecules belonging to the core complex. At least five different transitions are observed in the BChl *a* Q_y region of the core complex. This indicates that, if the core complex consists of identical subunits, each should contain at least five BChl *a* molecules. Four of these subunits, with a protein molecular mass of 43 kDa each, coupled with one reaction center, then might make up the total complex.

Acknowledgements

The authors would like to thank Mrs. L.M. Blom for her help in preparing the samples, A.H.M. De Wit for culturing the bacteria, L.J. De Vos for his assistance with the LD and CD experiments and H. Kingma for measurements of the fluorescence yield in a magnetic field. The investigation was supported by the Netherlands Foundation for Chemical Research (SON), financed by the Netherlands Organization for the Advancement of Pure Research (ZWO).

References

1 Olson, J.M. (1980) Biochim. Biophys. Acta 594, 33–51
2 Fowler, C.F., Nugent, N.A. and Fuller, R.C. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 2278–2282
3 Olson, J.M., Philipson, K.D. and Sauer, K. (1973) Biochim. Biophys. Acta 292, 206–217
4 Swarthoff, T. and Amesz, J. (1979) Biochim. Biophys. Acta 548, 427–432
5 Olson, J.M. (1981) Biochim. Biophys. Acta 637, 185–188
6 Matthews, B.W., Fenna, R.E., Bolognesi, M.C., Schmid, M.E. and Olson, J.M. (1979) J. Mol. Biol. 131, 259–285
7 Swarthoff, T., Van der Veek-Horsley, K.M. and Amesz, J. (1981) Biochim. Biophys. Acta 635, 1–12
8 Swarthoff, T., Amesz, J., Kramer, H.J.M. and Rijgersberg, C.P. (1981) Isr. J. Chem. 21, 332–337
9 Kramer, H.J.M., Kingma, H., Swarthoff, T. and Amesz, J. (1982) Biochim. Biophys. Acta 681, 359–364
10 Swarthoff, T., De Grooth, B.G., Meiburg, R.F., Rijgersberg, C.P. and Amesz, J. (1980) Biochim. Biophys. Acta 583, 51–59
11 Olson, J.M. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 161–178, Plenum Press, New York
12 Abdourakhmanov, I.A., Ganago, A.O., Erokhin, Y.E., Solov'ev, A.A. and Chugunov, V.A. (1979) Biochim. Biophys. Acta 546, 183–186
13 Vasmel, H., Meiburg, R.F., Kramer, H.J.M., De Vos, L.J. and Amesz, J. (1983) Biochim. Biophys. Acta 724, 333–339

14 Rijgersberg, C.P. (1980) Thesis, University of Leiden
15 Kramer, H.J.M., Amesz, J. and Rijgersberg, C.P. (1981) Biochim. Biophys. Acta 637, 272–277
16 Philipson, K.D. and Sauer, K. (1972) Biochemistry 11, 1880–1885
17 Olson, J.M., Ke, B. and Thompson, K.H. (1976) Biochim. Biophys. Acta 430, 524–537
18 Van Bochove, A.C., Swarthoff, T., Kingma, H., Hof, R.M., Van Grondelle, R., Duysens, L.N.M. and Amesz, J. (1983) Biochim. Biophys. Acta, submitted for publication
19 Whitten, W.D., Pearlstein, R.M., Phares, E.F. and Geacintov, N.E. (1978) Biochim. Biophys. Acta 503, 491–498
20 Stadnichuk, I.N. and Litvin, F.F. (1977) Biofizika 22, 170–174 (transl.: Biophysics U.S.S.R. 22, 175–180)