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Isolation and complete amino acid sequence of the β - and α -polypeptides from the peripheral light-harvesting pigment-protein complex II of *Rhodobacter sulfidophilus*

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Abstract The peripheral light-harvesting bacteriochlorophyll-carotenoid-protein complex B800–850 (LHII) has been isolated from membranes of semi-aerobic dark-grown cells of *Rhodobacter sulfidophilus* strain W4. A reversed-phase HPLC system resolved one β - and one α -polypeptide in the ratio 1:1. The material obtained was of high purity and suitable for direct microsequence analysis. The primary structures of the β - and α -polypeptides have been determined. The β -polypeptide consists of 51 amino acid residues, yielding a molecular mass of 5512 Da and having 64.7% hydrophobicity. The α -polypeptide consists of 52 amino acid residues, with a calculated molecular mass of 5661 Da and 75% hydrophobicity. The significance of uncommon structure motives with respect to the unusual spectroscopic characteristics of this light-harvesting complex is discussed.

Key words: α -Polypeptide; β -Polypeptide; Light-harvesting complex; *Rhodobacter sulfidophilus*; LHII

1. Introduction

Under anaerobic conditions in the light purple bacteria are capable of phototrophic growth (for review, see [1]). In the natural environment the organisms are faced with a variety of adaptation problems. Most critical for phototrophic growth is optimal adaptation to light [2], which modulates membrane differentiation and affects the rate of synthesis of the pigment-protein complexes. The photosynthetic apparatus of most purple bacteria contains two groups of antenna complexes [3–7]:

Type A: core complexes (LHI) (B890, B880, B879 or B870) associated with the reaction center, delivering excitation energy directly to it. The 2-dimensional crystal from LHI of *Rhodospirillum rubrum* has recently been reported. The authors claimed that the projection map shows 16 subunits in a 11.6 nm diameter ring with a 6.8 nm hole in the center. These dimensions are sufficient to incorporate an RC in vivo [8]. Type B: peripheral complexes (LHII) (B800–850, B800–820, etc.) thought to be located more distantly around the photoreceptor, probably forming a lake wherein the reaction center/core complexes are situated and energetically connected. The absorption

properties of the peripheral antenna complex, e.g. as B800–820, B800–830 or B800–850, depend upon the bacterial strain, species and environmental factors such as light intensity, temperature and growth medium [6,7,9,10]. Recently, it has been shown in a number of species of purple non-sulfur bacteria that not only the amount of the peripheral antenna complexes was changed under different light intensities but also the type of LHII complexes synthesized [3,4,6,7]. *Chromatium* (*C.*) *vinosum*, *Rhodopseudomonas* (*Rps.*) *palustris* and *Rps. acidophila* seemed to correlate with their capacity to grow at very low light intensities [7,9,11,12].

In recent years interest has been focused on the molecular origin of spectral multiplicity of purple bacteria antenna pigments. Chadwick et al. [13] have reported that, upon treatment of B800–850 complex (from *Rps. acidophila*) with lithium dodecyl sulfate (LDS), a decrease of the 800 nm absorption can occur [13]. However, upon removing the reagent (LDS) by dialysis, the 800 nm peak was completely restored. Clayton and Clayton [14] have also demonstrated that the 800 nm absorption band in the B800–850 complex from *Rhodobacter* (*Rb.*) *sphaeroides*, strain 2.41, can be recovered at approximately 70% of its original intensity by dialysis of the LDS-treated complex overnight against Tris-HCl buffer containing lauryldimethylamine-*N*-oxide (LDAO). They concluded that the presence of LDS weakened the binding of monomeric bacteriochlorophyll (BChl) to the protein and proposed that the equilibrium for BChl binding shifts towards unbound Bchl, while the absence of LDS favors Bchl binding to the protein [14]. In contrast, Chadwick et al. [13] proposed that LDS-induced effects on the 800 nm BChl absorption band are due to a conformational change in the protein resulting in a change in the interaction responsible for the wavelength of the monomeric BChl absorbance. Since antenna polypeptides determine the position, distance, orientation and environment of the pigment molecules, it is possible that this shift of the relative absorption strength is based on a modified interaction of the pigments with their protein matrix [3,4,15].

Recently, isolation of a B800–850 complex from the semi-halophilic purple non-sulfur bacterium *Rb. sulfidophilus*, grown either chemotrophically or phototrophically, was reported [16]. This detergent-solubilized complex exists in different spectral forms, affected by many factors like detergent concentration, salt and pH. In contrast to the above-mentioned alterations of the relative absorption of the 800 and 850 nm BChls, the spectral modifications of the *Rb. sulfidophilus* light-harvesting complex affect exclusively the 850 nm absorption and appear to be completely reversible. Solubilized in LDAO at moderate con-

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Abbreviations: ICM, intracytoplasmic membranes; LDAO, lauryldimethylamine-*N*-oxide; LDS, lithium dodecyl sulfate; LHI, light-harvesting complex I; LHII, light-harvesting complex II; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonylfluoride; RC, reaction center; RP, reversed phase; SDS, sodium dodecyl sulfate; TFA, trifluoroacetic acid; rpm, revolutions per minute.

centrations, this complex exists in a B800–826 form, which is converted to the native B800–850 form upon addition of salt [17]. It was recently reported in a symposium article that the isolated complex contains one α and two different types of β -polypeptides. Preliminary data on their primary structures were added [18]. In contrast, we present in this paper the purification and characterization of a B800–850 complex (from cells grown under semi-aerobic, dark conditions) of the same species which contain a single β - and a single α -polypeptide. The significance of possible structural elements based on the primary structure determined in the present work with respect to spectral characteristics of the B800–850 complex will be considered (see section 4).

2. Materials and methods

2.1. Growth of bacteria, membrane isolation and purification of the LHII complex

Intracytoplasmic membranes were isolated from semi-aerobically dark-grown cells of *Rb. sulfidophilus* strain W4 as described by Hagemann et al. [19]. The LHII complex was isolated and purified as described by Doi et al. [16] with small modifications as described by Hagemann et al. [19].

2.2. Isolation and purification of the LHII polypeptides

The separation of the LHII polypeptides was performed with a Beckman (System Gold) liquid chromatography system (model 110B solvent delivery module) as previously described [12]. Elution was performed with formic acid:H₂O (1:1, v/v) (solution A) and formic acid:2-propanol (1:1, v/v) (solution B). For more details see the legend of Fig. 3. Both solvents contained 0.1% (v/v) trifluoroacetic acid (TFA). Freeze-dried LHII complex (2 or 4 mg) was dissolved in 80 or 150 μ l 50% formic acid, respectively. This solution was immediately injected and separated on the RP column. The gradient was started 5 min after sample injection. The flow rate was 1.5 ml/min at room temperature. Fractions (500–1000 μ l) were collected manually on the basis of the detector signal and put in a desiccator (connected to a freezing trap) containing potassium hydroxide pellets. The samples treated were then suitable for rechromatography, enzyme digestion, chemical cleavage, electrophoresis and N-terminal sequence analysis. The material present in peaks 1 and 2 (Fig. 3A) was highly pure and suitable for direct microsequence analysis. The eluates were stored at -20°C for later use or dried directly in a desiccator.

2.3. Cyanogen bromide cleavage of the α -polypeptide and fractionation of the fragments

An aliquot of 1.5 mg (approx. 0.2 μ mol) protein was cleaved with 5.2 mg CNBr (Sigma, Deisenhofen) in 800 μ l 70% formic acid for 24 h at

room temperature. After cleavage, the mixture was concentrated to 100 μ l and the peptide mixture was separated under the same experimental conditions used to separate the LHII polypeptides (see legend to Fig. 3). Elution was carried out as for the fractionation of the LHII polypeptides (Fig. 3).

2.4. Trypsin digestion of the β -polypeptide and fractionation of the fragments

An aliquot of 220 μ g (approx. 40 nmol) of β -polypeptide was solubilized in 300 μ l 100 mM Tris-HCl, pH 8.2, containing 0.001% LDAO and 5 μ g (0.25 nmoles) trypsin. The reaction mixture was then incubated for 4 h at 37°C . The whole reaction mixture was then immediately loaded onto an HPLC column. The peptide mixture was separated under the same experimental conditions used to separate the LHII polypeptides or for fractionation of fragments resulting from cyanogen bromide cleavage. The mode of elution is given in the legend to Fig. 3. Impure peptide fractions were pooled and dried down to ca. 0.3 ml by rotary evaporation. Then formic acid was added to a final concentration of 50% for rechromatography under the same experimental conditions.

2.5. Nomenclature of peptides

The LHII proteins and the peptide obtained by cyanogen bromide cleavage or trypsin digestion are numbered in the order of their elution from the reversed-phase (C₈) columns. In the text, each peptide is named to indicate its origin (i.e. cyanogen bromide cleavage and trypsin digestion are respectively C and T). When the peptides had to be rechromatographed because of impurities, they were designated with R and renumbered.

2.6. SDS polyacrylamide gel electrophoresis (PAGE) and amino acid analysis

Samples of the purified polypeptides and membranes were electrophoresed on SDS-PAGE gradient gels (11.5–16.5%) [20]. The amino acid composition of the proteins and peptides was determined as previously described [21–23].

2.7. Amino acid sequence analysis

Proteins and peptides were sequenced by automated Edman degradation using a pulsed-liquid gas-phase sequencer (Model 477A; Applied Biosystems Inc., Foster City, CA) with an on-line identification of the phenylthiohydantoin amino acids (Model 120A; Applied Biosystems). In the case of peptides containing cysteine, the sequence determination was carried out as in [24].

2.8. Digestion with carboxypeptidase

In order to determine the C-terminal sequence the peptide sample (30 nmol) was incubated in 0.6 ml of 0.2 M sodium bicarbonate buffer (pH 8.0) with carboxypeptidase A and B (enzyme:substrate molar ratio = 1:40). Aliquots were removed after 0, 1, 3, 6, 15, 30,

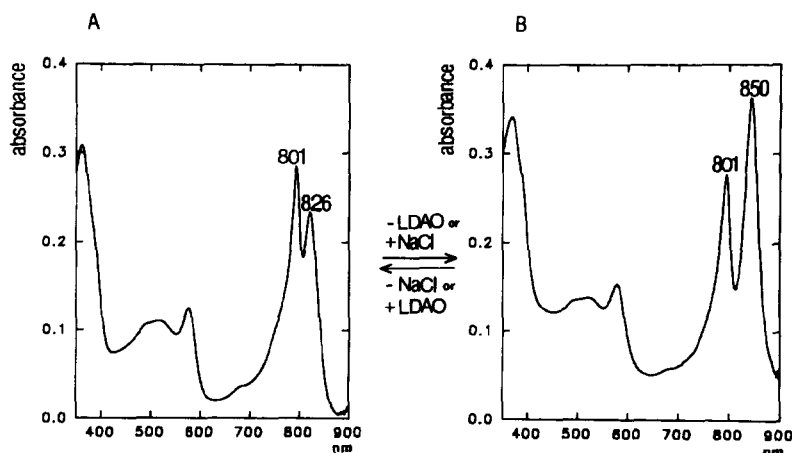


Fig. 1. Spectrophotometric analysis of pigmented fractions obtained after ion-exchange chromatography of a detergent-solubilized ICM fraction on a DEAE column. (A) Absorption spectrum of the fraction eluting at 250 mM NaCl after dialysis against Tris-HCl buffer, 20 mM, pH 7.8. (B) Absorption spectrum of the fraction eluting at 250 mM NaCl. The spectra were measured with samples of the same BChl and protein concentration.

60, 150 and 360 min. Digestion was stopped by the addition of one drop of glacial acetic acid or boiling, and the preparation was directly applied to the amino acid analyzer.

2.9. Analytical measurements

Absorption spectra were measured with a Kontron Uvikon spectrophotometer 860 and a Shimadzu UV-260 spectrophotometer. BChl and carotenoids were extracted with a mixture of acetone/methanol (7:2, v/v) and assayed spectrophotometrically. Protein was determined according to the method of Lowry et al. [25], using bovine serum albumin as a standard.

3. Results

3.1. Isolation of the polypeptides of the LHII complex

The peripheral antenna light harvesting complex (LHII) has been isolated as described in Materials and Methods. The isolated complex has two absorption bands, at 801 and 850 nm (Fig. 1B). The absorption ratio of 801/850 was about 0.77. Upon dialysing the isolated complex against 20 mM Tris-HCl, pH 7.8, to get rid of NaCl present in the preparation, the absorption band at 850 was shifted to 826 nm (Fig. 1A). The absorption ratio 801/826 was about 1.2. However, when NaCl was added to the dialysed preparation, the 850 band was completely restored. The purified native LHII complex contains two types of polypeptide, β and α , with apparent M_r of 6500 and 4000 Da, (Fig. 2, lanes 3 and 4). Both the β - and α -polypeptide components are hydrophobic and insoluble in water; formic acid (50%) was necessary to dissolve them [12]. They were then separated by RP-HPLC, and 3 peaks were resolved (Fig. 3A). The amino acid analysis of the fractions corresponding to the 3 peaks showed that only peaks 1 and 2 contained protein material as judged by amino acid composition (data not shown). Peak 3 contained mainly free pigments, i.e., carotenoids and bacteriopheophytin, which had been converted from BChl by the acid used in the eluent. The polypeptide content of peaks 1 and 2 was analyzed by SDS-PAGE and is shown in Fig. 2 (lanes 3 and 4). Judging from the molecular



Fig. 2. Protein patterns in an SDS-PAGE slab gel stained with Coomassie Brilliant Blue. Lane 1 = purified intracytoplasmic membrane; lane 2 = purified LHII complex; lane 3 = β -polypeptide; lane 4 = α -polypeptide.

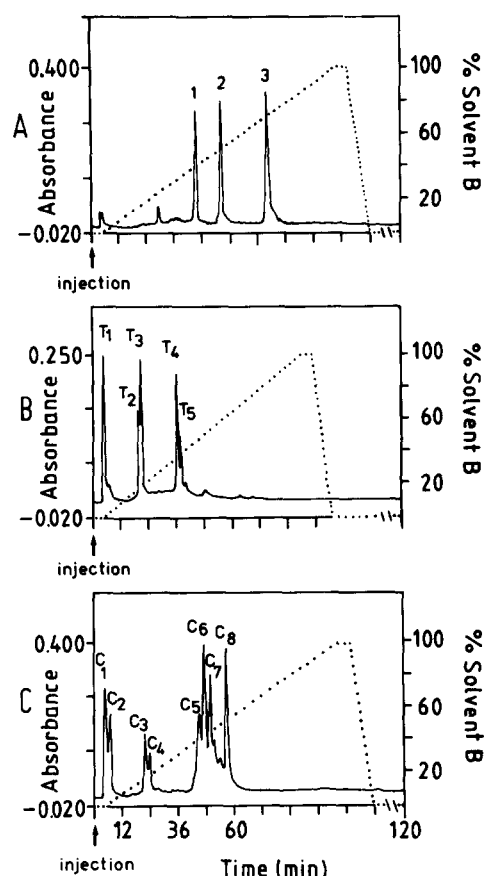


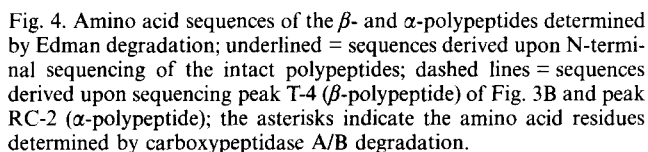
Fig. 3. Elution patterns of isolated LHII complex, separated by RP-HPLC; Octyl-LiChrosorb RP-column (C8) was used; absorption was measured at 280 nm; elution was performed with 50% formic acid with 0.1% TFA as solvent A, and 50% 2-propanol in formic acid with 0.1% TFA as solvent B; the gradient is indicated by a dotted line. (A) Elution pattern of LHII complex isolated by ion-exchange chromatography (eluted with 250 mM NaCl from the DEAE column). (B) Elution pattern of the peptide fragments generated by trypsin digestion of the β -polypeptide (peak 1 of A). (C) Elution pattern of the peptide fragments generated by cyanogen bromide cleavage of the α -polypeptide (peak 2 of A). For peptide nomenclature see section 2.

weight, we conclude that peaks 1 and 2 represent β - and α -polypeptide, respectively. The protein concentrations in peaks 1 and 2 were determined with an amino acid analyzer, revealing that the β - and α -polypeptides of the LHII complex are present in the ratio 1:1. The β - and α -polypeptides in peaks 1 and 2 were highly pure as judged by N-terminal sequence analysis.

3.2. Determination of the complete amino acid sequence of the β -polypeptide from the LHII complex

The automated Edman degradation of 5.4 μ g (approx. 0.82 nmol) yielded an N-terminal sequence of 41 residues (Fig. 4). However, approx. 90% of the β -polypeptides show the N-terminal amino acid as threonine, while the remaining 10% have methionine as their N-terminal residue (before threonine).

About 220 μ g (approx. 40 nmol) of the β -polypeptide was digested with trypsin, as described in section 2. The resulting fragments were isolated on an RP-HPLC column (Fig. 3B). Automatic Edman degradation and amino acid analysis allowed us to identify the peak materials in peaks 1–5 (Fig. 3B). Peak 4 yielded a peptide consisting of the residues Val-31 to



Gly-51 (Fig. 4). The sequence of this fragment matches the sequence analysis of the β -polypeptide exactly. This fragment most likely includes the C-terminus. Using carboxypeptidase A and B for C-terminal analysis of either the fragment in peak 4 (Fig. 4) or the whole β -polypeptide resulted in the same degradation pattern, yielding Leu/Gly in the ratio of approximately 1:1.2.

The first 43 amino acid residues of the isolated α -polypeptide were determined directly by automated Edman degradation, except that the cysteine at position 23 is not detected, and the sequence is shown in Fig. 4. Carboxypeptidase analysis (Fig. 4) of the whole α -polypeptide resulted in a degradation pattern with Glu/Ala in the ratio 1.0:2.2.

After cyanogen bromide fragmentation of α -polypeptides (Fig. 3C), 8 peaks were resolved on the HPLC column. Automated Edman degradation and amino acid analysis indicated that peak 2 (Fig. 3C) consists mainly of a fragment including residues number 2–6. Peak 3 consists mainly of a fragment with residues number 34–41, while peak 4 contains a fragment with residues number 34–51. The presence of both the latter two fragments shows that the methionine at position 41 was only partially cleaved. Sequence analysis of peaks 5–8 has revealed that they contain different peptides cross-contaminated with each other. The material present in peaks 5–8 was rechromatographed under the same conditions as used before except that the flow rate was 1.7 instead of 1.5 ml/min and the gradient from 0% solution B to 100% solution B was done in 110 min instead of ca. 75 min. Three different peaks were well resolved (named RC-1, RC-2, RC-3) and sequenced.

Peak RC-1 contained homoserine, while peak RC-2 was: Gly-Asp-Tyr-Tyr-Ser-Gly-Ser-Phe-Glu-Ala-Ala. This frag-

ment comprises the segment beginning with residue number 42 (Fig. 4). The sequence of this fragment matches its amino acid analysis exactly (data not shown). C-terminal analysis with carboxypeptidase A and B resulted in the same degradation pattern as for the whole α -polypeptide, revealing Glu/Ala in the ratio of approx. 1:2.2. The analysis of RC-3 showed that it contains mainly a fragment with residues 7–33, with cysteine at position 23, which could be identified after pyridine-ethylation [24] of the peptide.

4. Discussion

The peripheral light-harvesting pigment-protein complex (B800–850) from the photosynthetic bacterium *Rb. sulfidophilus* exhibits a blue-shift of the long wavelength absorbance maximum from 800–850 to 800–826 nm. This blue-shifted form is fully reversible upon addition of salts [16,17,19]. Currently, there are a number of complexes known to show considerable blue-shift of the absorption maximum as a result of modifications of the pigment binding proteins through site-directed mutagenesis [26,27], detergent treatments [28], or salt treatment [29]. A number of mechanisms of action can be considered as possible explanations for the ion effects on the light-harvesting complex: (A) a direct influence of the ion changes on the conjugated system of the 830 nm pigments has been suggested [17,30]; (B) a second mechanism of action might be an influence of the ion on the local protein conformation, causing the 830(850) BCHls to change their distance and/or mutual angular position, and thus their excitonic interaction and absorption maxima; (C) a third mechanism of action might involve aggregation of several pigment-protein complexes as a consequence of ion binding [31].

The elucidation of the primary structure of different types of antenna complexes, as well as their determined arrangement and orientation within the photosynthetic membrane, offered the basis for consideration of the specific microenvironment of the functional antenna pigment and its spectroscopic characteristics. Taking the information on structure mentioned above into account, we believe that the differences in the spectral properties of the isolated B800–850 complex from *Rb. sulfidophilus* are due to modification of the surroundings of the pigment, and we favor the hypothesis that ions might cause local conformational changes and/or aggregation of the protein matrix in which BChl molecules are non-covalently bound by histidine residues and other conserved amino acids [3,4,15].

Therefore, in this study we have isolated the β - and α -polypeptides belonging to the B800–850 peripheral antenna complex and determined their primary structure (Fig. 4) to find out which structural motif within the primary structure might explain the blue-shift phenomenon.

The β -polypeptide consists of 51 amino acid residues (Fig. 4), yielding a calculated molecular mass of 5512 Da, 64.7% hydrophobicity and had a net negative charge (of -4), whereas the α -polypeptide was neutral (net charge of 0) and consists of 52 amino acid residues, yielding a molecular mass of 5661 Da and 75% hydrophobic amino acid residues, typical for integral membrane proteins. Most other LHII α -polypeptides have a positive net charge [3,4].

Several features can be observed in the elucidated primary structure of the α - and β -polypeptides which are also common to those from several other bacterial peripheral antenna light-

harvesting primary structures [3,4]: (i) the primary structures presented in this study show a 3-domain structure, relatively hydrophilic N- and C-terminal regions and a highly hydrophobic central domain which spans the membrane once in a putative α -helix; (ii) the N-terminal region of the β subunit contains 6 negatively charged amino acid residues and 1 positive, while in the α subunit there are 2 positive charges (Fig. 4); and (iii) a conserved, central histidine residue (position 40 for α and 31 for β ; Fig. 4) within a cluster of hydrophobic amino acid residues. This histidine most probably provides the fifth ligand to the bacteriochlorophyll magnesium, which is known to be pentagonally coordinated [4]. In the β -polypeptide an additional histidine residue (position 22, Fig. 4) is present, which could coordinate a third bacteriochlorophyll. This was also the case in other β -polypeptides previously determined [3,4,15].

The presence of aromatic amino acids at a consensus distance from this histidine has been reported [3,5,26]. Most antenna polypeptides exhibit a Trp-9 residue C-terminal of the histidine [4]. This aromatic amino acid residue is apparently one of the structural requirements needed to form core or peripheral antenna complexes [26]. The α -polypeptide of *Rb. sulfidophilus* shows the presence of such an aromatic amino acid in the position 40, 44 or 45 (Fig. 4), 9, 13 or 14 amino acid residues from the conserved His (Fig. 4). The presence of these aromatic amino acids might be important in regulating the spectral form of the B801–850 complex of *Rb. sulfidophilus*.

There are, however, some distinct differences in the primary structure of LHII α with respect to those of other purple photosynthetic bacteria: (i) the presence of 4 Met residues in the α subunit (positions 1, 6, 33, 40, Fig. 4) has not been found in the previously determined primary structure of α -polypeptides from peripheral antenna complexes [3,4]; (ii) a carboxyl group in the C-terminal domain of the α -polypeptide (position 50, Fig. 4) as the possible binding site for cations, which might regulate the spectral form of the BChl dimer [17]; and (iii) a cysteine at position 23 (Fig. 4) in the α -polypeptide. The relevance of such amino acid residues with respect to the blue-shift phenomenon has to be ascertained by site-directed mutagenesis.

This cysteine is 8 amino acid residue N-terminal from the conserved histidine. Cysteine residues have not previously been found in the primary structure of α - or β -polypeptides determined from purple bacteria [3,4], and their structural and spectral relevance remains open. However, cysteines are often artificially inserted into polypeptides in order to provide ligands for heavy-metal substitution. Binding of heavy metals is essential for solving the crystallographic phase problem and thus for determination of the 3-dimensional structure. In this work, the presence of a cysteine in the α -polypeptide belonging to the native LHII complex has been established. Therefore, the isolated peripheral native light-harvesting complex (LHII) from *Rb. sulfidophilus* might be a promising candidate for X-ray structure determination.

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