

Amino acid composition of proteins in halophilic phototrophic bacteria of the genus *Ectothiorhodospira*

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Accepted August 25, 1983

IMHOFF, J. F., D. J. KUSHNER, and P. J. ANDERSON. 1983. Amino acid composition of proteins in halophilic phototrophic bacteria of the genus *Ectothiorhodospira*. *Can. J. Microbiol.* **29**: 1675–1679.

The amino acid composition of total proteins of eight *Ectothiorhodospira* strains with different salt optima and of separated membranes of selected strains have been determined. Amino acid compositions were compared with those reported for non-halophilic phototrophic and heterotrophic bacteria and *Halobacterium halobium*. The membrane fractions from *Ectothiorhodospira* strains requiring high salt for maximum growth contained more polar and less nonpolar amino acids than strains with low salt requirements or nonhalophilic bacteria. The content of intermediate amino acids increased with the increasing halophilic properties of the *Ectothiorhodospira* strains. Proteins which function in high-salt environments may therefore require such compositions to maintain their structures in highly ionic solutions.

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La composition en acides aminés des protéines totales a été déterminée chez huit souches de *Ectothiorhodospira* ainsi que sur des membranes détachées de souches choisies. Les compositions en acides aminés ont été comparées à celles qui furent rapportées pour les bactéries non-halophiles phototrophes et hétérotrophes et pour *Halobacterium halobium*. Les fractions de membranes des couches de *Ectothiorhodospira* requérant une teneur élevée en sels pour l'atteinte d'une croissance maximale contenaient plus d'acides aminés polaires et moins d'acides aminés non-polaires que les souches ayant de faibles exigences en sels ou que les bactéries non-halophiles. La teneur en acides aminés intermédiaires s'est accrue en même temps qu'augmentaient les propriétés halophiles des souches de *Ectothiorhodospira*. Conséquemment, les protéines que évoluent dans les milieux élevés en sels peuvent donc nécessiter de telles compositions de façon à maintenir leurs structures dans les solutions hautement ioniques.

[Traduit par le journal]

Introduction

The genus *Ectothiorhodospira* comprises six species that show a wide range of salt requirement and tolerance. *Ectothiorhodospira mobilis* has a salt (NaCl) optimum of about 5% for growth, but some strains are extremely halotolerant. *Ectothiorhodospira shaposhnikovii* and *E. vacuolata* require less than 10% salt. *Ectothiorhodospira halochloris* and *E. abdelmalekii* grow best between 15 and 20% salts, and different strains of *E. halophila* have optimal concentrations for growth ranging from 15 to 30% total salts and do not grow below 10 or 15%. Despite their differences in salt requirements, both *E. mobilis* and *E. halophila* have been isolated from the extremely concentrated brines (32%) of the Wadi Natrun (Imhoff et al. 1978; Imhoff et al. 1979).

Both *E. mobilis* and *E. halophila* are very similar in

some physiological properties, in their pigment content (Schmidt and Trüper 1971), and the structure of their photosynthetic membranes which originate and are continuous with the cytoplasmic membrane (Remsen et al. 1968; Trüper and Imhoff 1981). These membranes are present as lamellar stacks, and models which consider their continuity with the cytoplasmic membrane imply that their outside is in contact with the exterior medium of the cell.

The genus *Ectothiorhodospira* is only distantly related to the *Halobacteriaceae*, known as "extreme halophiles" or "red halophilic bacteria," which are among the "Archaeobacteria" (Balch et al. 1979). The halobacteria possess a high excess of acidic amino acids in their proteins, unlike such nonhalophilic bacteria as *Pseudomonas fluorescens* and *Sarcina lutea* (Reistad 1970; Lanyi 1974). It seemed likely that examination of the amino acid composition of the halophilic *Ectothiorhodospira* strains might provide information concerning the necessity of acidic proteins as an adaptive mechanism in extreme halophilic bacteria in general.

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Therefore, the amino acid composition of the bulk protein and of membrane proteins of representative *Ectothiorhodospira* strains were determined.

Materials and methods

Media and growth conditions for the *Ectothiorhodospira* strains were as previously described for *E. halochloris* (Imhoff and Trüper 1977), *E. abdelmalekii* (Imhoff and Trüper 1981), *E. halophila* (Imhoff et al. 1978), and *E. mobilis* and *E. vacuolata* (Imhoff et al. 1981). Cells for analysis were harvested in the late logarithmic growth phase. Amino acid compositions of total cellular proteins were determined on acetone powders of cells. Membrane proteins were prepared from cells disrupted in 2.5% NaCl by ultrasonication. Unbroken cells and cell fragments were removed by centrifugation at $10\,000 \times g$ for 15 min. Membranes were then sedimented by centrifugation at $45\,000 \times g$ for 60 min. All protein fractions were washed three times with cold acetone and dried under vacuum.

Amino acid analyses were carried out on samples to which norleucine was added as an internal standard. Protein samples were hydrolyzed for 24 h at 106°C in 6N HCl in sealed evacuated tubes. After hydrolysis, HCl was removed under vacuum. The samples were then dissolved in 1 mM HCl and aliquots were applied to a Technicon TSM amino acid analyzer equipped with an Infotronics integrator. Amino acid compositions were calculated from the color yield relative to norleucine and were obtained with a precision of $\pm 2.5\%$. Proline, cystine, and tryptophan were not determined and the calculated aspartic and glutamic acid contents of proteins includes the contribution of their amides.

Results and discussion

The amino acid compositions of the total protein of seven *Ectothiorhodospira* strains (five species) with varying salt requirements were rather similar (Table 1). There was no apparent increase in polar amino acids in the more halophilic strains. The basic amino acids lysine and arginine made up approximately 9% of the total amino acids and the ratio arginine/lysine was higher in the more halophilic strains. The content of intermediate amino acids was lower in *E. mobilis* BN 9903 and *E. vacuolata* BN 9512, two strains which required only about 5% salts for maximum growth.

We realized, however, that it might be misleading to compare total proteins of *Ectothiorhodospira* species with those of halobacteria. Though many of the former species grow in as high salt concentrations as the latter, the internal ionic environments are quite different. While halobacteria accumulate large concentrations of potassium ions (Kushner 1978), the main "compatible solute" of *Ectothiorhodospira* is betaine (Galinski and Trüper 1982). Direct measurements and the salt response of some enzymes (ribulose biphosphate carboxylase, malate dehydrogenase, and ATPase) suggest that inorganic cations are largely excluded from the cells

(Tabita and McFadden 1976; J. Imhoff, unpublished data).

However, the outer surfaces of both kinds of bacteria would, of course, be exposed to the salt concentrations of the growth media. Thus, it seemed possible that a comparison between membrane proteins would be more enlightening.

Membrane proteins of two *E. halophila* strains with different salt optima (15 and 25%) were analyzed and compared with reported values for membrane fractions from the related species *E. mobilis* (Oyewole and Holt 1976) and from *Halobacterium halobium* (Marshall et al. 1969) (Table 2). The membrane preparation of Oyewole and Holt (1976), although further purified by density gradient centrifugation, is thought to be comparable with our membrane fractions, which were obtained by differential centrifugation and may contain small amounts of "cell wall" fragments and ribosomes. Relative to *E. mobilis*, the proportion of polar amino acids is increased in the *E. halophila* strains and even more in *H. halobium*. This increase was mainly due to a higher proportion of the acidic amino acids, glutamic and aspartic acid, and (or) their amides. The membrane proteins of the *Ectothiorhodospira* strains contained significantly higher proportions of arginine than lysine, as did the whole-cell protein. This is unlike membrane proteins from the phototrophic freshwater bacteria *Rhodospseudomonas sphaeroides* (Gorchein et al. 1968) and *Thiocapsa roseopersicina* (Takacs and Holt 1971). Although the total polar amino acid content changes relatively little with increasingly higher optimal salt concentrations, because of the increase in acidic and the decrease in basic amino acid content, the more halophilic *Ectothiorhodospira* strains have more acidic membrane proteins.

In strong correlation to their dependence on higher salt concentrations, the membrane proteins of the three *Ectothiorhodospira* strains showed changes in individual amino acid contents as follows: (i) higher content of glutamic acid (not in strain BN 9628), aspartic acid, glycine, serine, and threonine (not in strain BN 9631); (ii) lower content of arginine, lysine, valine, leucine, and phenylalanine. This trend continued to *H. halobium* (content of glycine and serine were lower and of valine higher than expected from this trend), which had the highest excess of acidic amino acid residues and the highest overall polarity of its membrane proteins. Differences in the intermediate and the nonpolar amino acid contents were much more pronounced in the *Ectothiorhodospira* strains than differences in the total polar amino acid content. At increasing salt concentration, there was a gain of intermediate, and loss of nonpolar amino acids. Taken together, these differences make the membrane proteins increasingly more polar

TABLE 1. Amino acid compositions of whole cells from five different *Ectothiorhodospira* species (eight strains)

| | Species and strains (optimal salinity) | | | | | | | |
|--------------|--|--------------------------------------|--|---|---|---|---|--|
| | <i>E. vacuolata</i> BN 9512 (1-6%) | <i>E. mobilis</i> BN 9903 (5%) | <i>E. abdelmalekii</i> BN 9840 (15%) | <i>E. halochloris</i> BN 9850 (20%) | <i>E. halophila</i> SL 1 (10-20%) | <i>E. halophila</i> BN 9625 (25%) | <i>E. halophila</i> BN 9627 (25%) | <i>E. halophila</i> BN 9628 ^a (25%) |
| Asp | 9.5 | 11.0 | 9.9 | 10.0 | 10.2 | 9.5 | 10.2 | 11.0 |
| Glc | 15.2 | 13.6 | 12.7 | 13.2 | 13.3 | 12.9 | 13.5 | 11.3 |
| Arg | 6.1 | 4.9 | 6.9 | 6.2 | 8.6 | 6.4 | 6.4 | 5.8 |
| Lys | 3.7 | 3.4 | 3.0 | 3.0 | 2.8 | 3.1 | 2.8 | 2.8 |
| His | 2.3 | 2.6 | 2.7 | 3.0 | 2.6 | 2.7 | 2.6 | 3.3 |
| Thr | 5.2 | 5.4 | 5.7 | 4.8 | 4.6 | 5.0 | 5.2 | 6.0 |
| Ser | 4.9 | 4.6 | 5.5 | 6.1 | 5.4 | 4.3 | 5.3 | 6.0 |
| Gly | 9.5 | 9.6 | 10.1 | 11.0 | 11.4 | 12.0 | 11.9 | 12.0 |
| Tyr | 2.7 | 2.8 | 3.2 | 3.3 | 2.8 | 2.7 | 2.7 | 3.1 |
| Ala | 11.4 | 11.5 | 11.9 | 11.1 | 10.9 | 11.4 | 11.0 | 11.3 |
| Val | 8.5 | 9.3 | 6.4 | 6.2 | 7.4 | 9.1 | 7.6 | 7.1 |
| Met | 2.5 | 0.9 | 2.8 | 2.7 | 2.2 | 2.5 | 2.4 | 2.8 |
| Ile | 4.2 | 4.0 | 5.0 | 5.4 | 4.4 | 4.9 | 4.7 | 3.7 |
| Leu | 10.0 | 10.8 | 9.8 | 9.3 | 9.4 | 9.5 | 9.8 | 9.5 |
| Phe | 4.3 | 5.6 | 4.4 | 4.7 | 4.0 | 4.0 | 3.9 | 4.3 |
| Polar | 34.5 | 32.9 | 32.5 | 32.4 | 34.9 | 31.9 | 32.9 | 30.9 |
| Intermediate | 24.6 | 25.0 | 27.2 | 28.2 | 26.8 | 26.7 | 27.7 | 30.4 |
| Nonpolar | 40.9 | 42.1 | 40.3 | 39.4 | 38.3 | 41.4 | 39.4 | 38.7 |

NOTE: Amino acid compositions are given as percent determined amino acids. Ala, Val, Leu, Ile, Met, and Phe are classified as nonpolar amino acids; Ser, Thr, Tyr, His, and Gly are intermediate and Asp, Glu, Lys, and Arg are polar (Vanderkoi and Capaldi 1972).

^aData of *E. halophila* BN 9628 were calculated from amino acid analyses of soluble and membrane protein fractions.

TABLE 2. Amino acid composition of membrane proteins of *Ectothiorhodospira* strains with different salt optima and of *Halobacterium halobium*

| | Species and strains (optimal salinity) | | | |
|--------------|---|---|---|--|
| | <i>Ectothiorhodospira mobilis</i> ^a (3%) | <i>Ectothiorhodospira halophila</i> BN 9631 (15%) | <i>Ectothiorhodospira halophila</i> BN 9628 (25%) | <i>Halobacterium halobium</i> ^b |
| Asp | 9.0 | 10.7 | 11.3 | 13.7 |
| Glc | 10.9 | 12.2 | 10.9 | 13.3 |
| Arg | 6.5 | 6.3 | 5.6 | 4.6 |
| Lys | 3.8 | 2.4 | 2.3 | 2.2 |
| His | 2.8 | 2.6 | 4.0 | 1.0 |
| Thr | 5.8 | 4.3 | 6.4 | 8.8 |
| Ser | 5.3 | 5.8 | 6.6 | 6.1 |
| Gly | 7.3 | 10.5 | 11.5 | 9.8 |
| Tyr | 3.6 | 3.0 | 3.3 | 3.1 |
| Ala | 12.3 | 12.7 | 11.0 | 11.2 |
| Val | 8.3 | 7.1 | 6.2 | 8.5 |
| Met | 3.4 | 2.6 | 3.3 | 1.7 |
| Ile | 3.3 | 4.2 | 3.2 | 4.0 |
| Leu | 12.0 | 10.2 | 9.2 | 7.9 |
| Phe | 6.5 | 5.4 | 5.2 | 3.3 |
| Polar | 29.4 | 31.6 | 30.1 | 33.9 |
| Intermediate | 24.8 | 26.2 | 31.8 | 29.5 |
| Nonpolar | 45.8 | 42.2 | 38.1 | 36.6 |

NOTE: Percent composition and classification of amino acids are as described in Table 1.

^aOyewole and Holt (1976). Data from this reference were adapted by setting the total amino acids as 100%.

^bMarshall et al. (1969). Data from this reference were adapted by setting the total amino acids as 100%.

in the order *E. mobilis*, *E. halophila* BN 9631, *E. halophila* BN 9628, *H. halobium*.

Because different parts of the intrinsic membrane proteins are in contact with either the cell's exterior, the hydrophobic membrane continuum, or the cell's interior, the observed differences in the membrane proteins may reflect changes in different parts of the proteins. In the more halophilic strains, the part facing the cell's exterior may have higher proportions of polar residues, whereas the part in contact with the hydrophobic zone of the lipid bilayer may have higher proportions of intermediate amino acids than the less halophilic strains. Solvent effects at the cell's surface might promote hydrophobic bonding with intermediate amino acids and favor their preferred incorporation over nonpolar amino acids into the hydrophobic environment of the membrane.

Lanyi (1974) pointed out that a few tenths molarity of NaCl is sufficient to account for the charge-shielding effect of the salt, but much higher concentrations are needed to account for increased participation of intermediate amino acids in protein stabilization by hydrophobic bonding.

Two properties of the *Halobacteriaceae*, namely their highly acidic proteins and the high intracellular

potassium concentration, have been claimed to be essential features for their halophilic properties (Kushner 1968; Lanyi 1974). These arguments have been weakened by findings of elevated potassium concentrations in the cells (Sprott and Jarrell 1981) and elevated amounts of acidic amino acids in the proteins (M. Yaguchi, personal communication) of methanogenic bacteria, which are not halophilic, but are phylogenetically related to the *Halobacteriaceae* (Balch et al. 1979). These recent findings posed the question as to whether both properties common to halophilic and methanogenic "Archaeobacteria" are important to function, reflect phylogenetical relatedness, or both. The three *Ectothiorhodospira* strains, whose membrane proteins were compared (Table 2), are very closely related (two strains of one species and one strain of a very similar species), but differ in their salt requirement and tolerance. Therefore, the observed differences in their amino acid compositions might be directly related to the strains' salt response. The similarities between the amino acid compositions of membrane proteins in *Halobacterium* and *Ectothiorhodospira* species suggest that excess of acidic residues and higher proportions of intermediate residues may be essential for proteins functioning in high-salt environments.

Acknowledgements

This work was supported by grants from the Medical Research Council of Canada (P.J.A.), and the Natural Sciences and Engineering Research Council of Canada (D.J.K.). J.F.I. was the recipient of a NATO postdoctoral fellowship.

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