

Formation of Erythroascorbic Acid and 2,3-Enediol Pentonic Acid from L-Ascorbic Acid by Purple Sulfur Bacteria

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Phototrophic growth of *Chromatium vinosum* strain D on pyruvate or malate, and of *Thiocapsa roseopersicina* strain 6311 on malate or sulfide as sole electron donors was promoted by 5 mM L-ascorbate, while growth of *T. roseopersicina* on fructose was inhibited. Slow growth was obtained also with ascorbate alone. In ascorbate-containing cultures the medium developed a yellow to red-brown colour which showed an absorption maximum at 310–320 nm. As colourless products of L-ascorbic acid degradation by the two strains examined erythroascorbic acid and 2,3-enediol pentonic acid were found in cell-free culture filtrates by gas chromatographic analysis of the silylated residues and identified by their mass, IR and UV spectra.

Most species of the purple sulfur bacteria (family Chromatiaceae) are strict anaerobes and therefore usually are cultivated in completely filled screw cap bottles in media containing sulfide which serves as electron donor for photosynthesis and also removes residual oxygen. Many strains, however, are able to utilize simple organic substrates in the absence of reduced sulfur compounds, and in these cases the addition of ascorbic acid to the medium is recommended [1] to lower the redox potential sufficiently.

When we cultivated *Chromatium vinosum* and *Thiocapsa roseopersicina* on organic substrates as the only photosynthetic electron donors in the presence of ascorbic acid, we observed a browning of the liquid medium during growth, and a change of the growth rate in some cases compared to cultures grown without ascorbate. This led us to study growth of both organisms with ascorbic acid and to look for possible degradation products of ascorbate in the culture liquid.

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Materials and Methods

Chromatium vinosum strain D (DSM * No. 180) and *Thiocapsa roseopersicina* strain 6311 (DSM No. 219) were grown anaerobically in the light (incandescent lamp, 1000 lux) at 28 °C in 100 ml- or 1 l-screw cap bottles. The modified Pfenig's medium [1] was used containing either Na₂S · 9 H₂O (0.075% w/v = 3.1 mM) or an organic substrate (10 mM) as sole electron donor; in the latter case 0.04% Na₂SO₄ were added. The pH was about 7.2. Cells were grown with or without addition of 0.1% (5 mM) sodium L-ascorbate after precultivation in the same medium.

Growth was measured turbidimetrically at 700 nm with a Bausch & Lomb Spectronic 20 photometer, and by protein determination of whole cells according to Schmidt *et al.* [2].

For the examination of the culture liquid cells were removed by centrifugation and subsequent filtration through a membrane filter. Visible and UV absorption spectra of the filtrates were recorded with a Beckman Acta M VI spectrophotometer. For gas chromatographic separation filtrates were freeze-dried, and the residues (each 10 mg) were silylated with hexamethyldisilazane and trimethylchlorosilane [3]. A Hewlett Packard gas chromatograph model 5750 equipped with F. I. D. was employed, using a 1.8 m × 2.3 mm I. D. stainless steel column with 10% silicone UCCW 982 on Chromosorb W AW DMCS 80–100 mesh. Carrier gas: helium, flow rate 29 ml/min. Temperature program: 4 °C/min from 100 ° to 260 °C. Sample size: 3 µl.

The GC eluates were transferred through an open split connection [4] into the ion source of an AEI mass spectrometer model MS 30. The mass spectra were obtained at an ionization energy of 20 eV and an accelerating voltage of 4 kV. Temperature of the ion source: 200 °C; temperature of the inlet system: 220 °C.

To obtain IR and UV spectra of the eluted substances, samples were collected in microcuvettes and measured in an IR spectrometer type 257 and an UV-VIS spectrometer type 157 (Bodenseewerk Perkin-Elmer).

Results and Discussion

Addition of 5 mM L-ascorbate to a medium containing sulfide or a simple organic substrate as the

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only electron donor had a varying effect on the growth rates of *Chromatium vinosum* and *Thiocapsa roseopersicina* (Table I). Growth of *C. vino-*

Table I. Phototrophic growth of *Chromatium vinosum* and *Thiocapsa roseopersicina* in the presence of 5 mM L-ascorbate.

Substrate (10 mM)	Relative growth *	
	<i>Chromatium vinosum</i> strain D	<i>Thiocapsa roseopersicina</i> strain 6311
Acetate	90	110
Pyruvate	630	120
DL-Malate	140	160
Fructose	n. g.	20
Sulfide (3.1 mM)	100	160

* Growth was measured as optical density at 700 nm and as protein content of 1–3 days old cultures and expressed as percent of the growth of corresponding cultures without ascorbate.

n. g., no growth with or without ascorbate.

sum was markedly enhanced on pyruvate and, to a lower extent, on malate; growth of *T. roseopersicina* was promoted on malate as well as on sulfide, but was inhibited on fructose. Slow growth was obtained also with ascorbate alone. In all cultures containing ascorbate the previously colourless medium developed a yellow to red-brown colour which deepened as growth proceeded. The visible and UV spectra of the cell-free culture filtrates (with a final

pH between 7.0 and 8.1) showed an increase of absorption below 600 nm with a maximum between 310 and 320 nm. When the same medium was incubated under sterile conditions without inoculation either under vigorous aeration for 48 hours, or anaerobically for one month, no colouration was observed.

From these findings we conclude that L-ascorbate does not only act as metabolically indifferent substance by lowering the redox potential of the medium, but that it is involved in the metabolism of the two strains examined and can serve as a source of electrons for photosynthesis. The electrons may be taken up by cytochrome *c* – which is readily reduced by ascorbate in vitro [5, 6] – and from there be transferred to bacteriochlorophyll.

We analyzed the first products of ascorbate degradation to be found in the cell-free culture liquid using GC-MS coupling. The gas chromatographic separation of the silylated residues from lyophilization revealed two main peaks (Fig. 1, No. 10 and 11) from ascorbate degradation. Peak 10 belongs to a silylated compound with a molecular weight of $m/e = 362$ (Table II). From the IR absorption at 1595 cm^{-1} and 1750 cm^{-1} , and the UV absorption at 265 nm it has the nature of an α , β -unsaturated five-ring lactone. Peak 10 therefore refers to erythroascorbic acid (I). Peak 11 belongs to a silylated compound with a molecular weight of $m/e = 524$. The mass fragmentation (Table III) re-

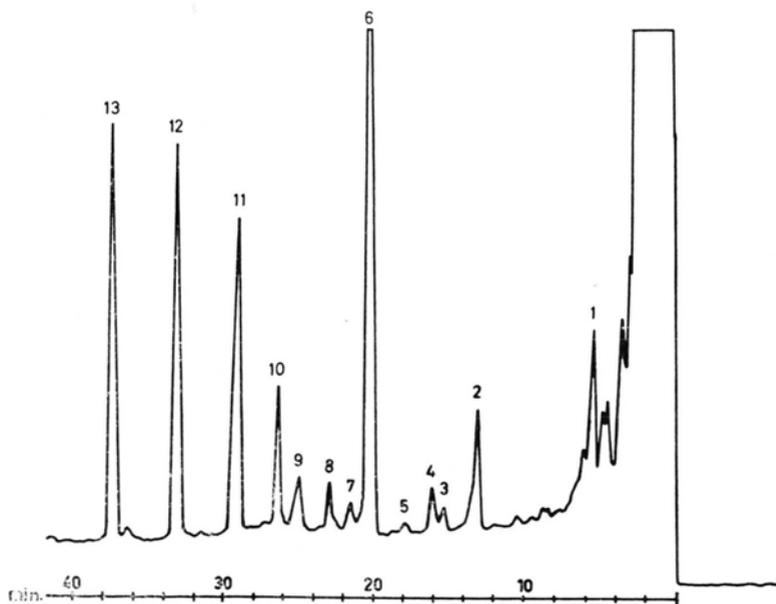


Fig. 1. Gas chromatogram of a freeze-dried and silylated culture filtrate of *Thiocapsa roseopersicina* grown on DL-malate plus L-ascorbate. 1–5, peaks from Pfennig's medium; 6, malic acid; 7–9, not identified degradation products of ascorbate; 10, erythroascorbic acid (I); 11, 2,3-enediol pentonic acid (II); 12, ascorbic acid; 13, octadecanol as internal standard.

Table II. Main peaks from the mass spectrum of erythroascorbic acid (I).

<i>m/e</i>	Relative intensity [%]	Possible coordination
362	3.0	molecule ion
347	2.6	M-CH ₃ ⁺
332	3.9	M-CH ₂ O
318	1.4	M-CO ₂
304	1.0	<i>m/e</i> 332-CO
272	0.9	M-HOT
257	4.9	<i>m/e</i> 347-HOT
75	100.0	base peak, unspecific

T, trimethylsilyl-.

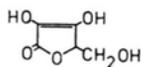
Table III. Main peaks from the mass spectrum of 2,3-enediol pentonic acid (II).

<i>m/e</i>	Relative intensity [%]	Possible coordination
524		molecule ion, not registered
509	3.5	M-CH ₃ ⁺
421	2.2	M-CH ₂ OT ⁺
393	1.3	<i>m/e</i> 421-CO
331	2.0	<i>m/e</i> 421-HOT
319	3.0	M-CH ₂ OT-CHOT ⁺
305	16.0	CHOT=COT-CHOT ⁺
292	50.5	C(OT) ₂ -CHOT ⁺
277	6.3	<i>m/e</i> 292-CH ₃
205	18.5	CH ₂ OT-CHOT ⁺
73	100.0	base peak, unspecific

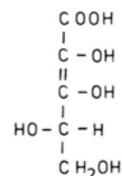
T, trimethylsilyl-.

veales an enediol compound. The absorption maxima at 1720/1630 cm⁻¹ and at 260 nm, respectively, are due to a 2,3-enediol structure. Peak 11 therefore refers to 2,3-enediol pentonic acid (II).

A semi-quantitative determination of the concentrations of the identified compounds in a culture filtrate of *T. roseopersicina* grown for five days on malate plus ascorbate resulted in approximately 38% unchanged ascorbic acid, 30% 2,3-enediol pentonic acid and 14% erythroascorbic acid. About



I



II

20% of the original ascorbate obviously are converted into yellow to brown-coloured products which are responsible for the well-known nonenzymatic browning of ascorbic acid.

Erythroascorbic acid has been reported to arise from xylonic acid in rat liver microsomes [7], from xylose in *Serratia marcescens* [8], and from various pentoses in *Candida utilis* [9]. As products of ascorbic acid catabolism dehydroascorbic acid, 2,3-diketogulonic acid [10, 11], lyxonic and xylonic acids [12], xylose [10], as well as oxalic acid and possibly threonic acid [10, 13-16] have been found in animals, while in higher plants ascorbic acid is converted to oxalic and tartaric acids [17].

The formation of these various metabolic products from L-ascorbate may be understood, when we assume two separate degradation pathways [18]: 1.) cleavage of the (bicyclic) dehydroascorbic acid [19] into a 2-carbon fragment (oxalate) and a 4-carbon fragment (threonolactone and threonate, respectively), and 2.) decarboxylation of the intermediate 2,3-diketogulonic acid to a 5-carbon compound. In *C. vinosum* and *T. roseopersicina* the second pathway seems to be realized involving oxidation of the 5-carbon compound to 2,3-enediol pentonic acid, followed by lactonization to erythroascorbic acid. So far, however, nothing is known about the enzymes which might be involved in this path of ascorbate catabolism.

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