the new metabolite. This observation, as well as chromatographical data, indicated that the new metabolite was acetylated methylhistamine or another hydrolysable conjugate of methylhistamine.

The radioactivity remaining in the fraction of the new metabolite after hydrolysis may be due to incomplete hydrolysis. However, the possibility also exists that at least part of the activity, but more than 2–3%, may stem from histaminol. By the methods used, quantitation of histaminol was impossible. To identify the unknown metabolite, it was necessary to separate it from N-acetylhistamine. The main fractions of the new metabolite were rechromatographed on an Aminex column until the metabolite seemed to move as a single compound.

The new metabolite is easily extractable from a basic solution with n-butanol and can be reextracted as histamine, from the organic solution with HCl. Butanol extraction followed by reextraction with HCl was combined with repeated column chromatography for isolation and purification. By starting with a 24-h urine, enough substance for direct mass-spectrographic analysis was obtained. The mass-spectra (figure 2a) indicate that the substance is N-acetyl-1.4-methylhistamine.

Reference N-acetylmethylhistamine was synthesized by methylation of N-acetylhistamine with dimethylsulfate and purified by means of TLC and butanol-HCl extractions. The purified compound could be visualized neither with ninhydrin nor by diazotation. The mass-spectrum for synthetic N-acetylmethylhistamine HCl (figure 2b) is nearly identical to that of the new metabolite isolated from the hens' urine. From this evidence, we conclude that N-acetyl-1.4-methyl-histamine is a metabolite of histamine in chickens.

About 90% of the radioactivity could be accounted for by identified histamine-metabolites, the remaining 10% may represent other metabolites such as fragments of the histamine-ring[6]. However, one cannot exclude the possibility that chromatographical effects may result in underestimation of some of the metabolites. The table demonstrates the metabolic pattern of exogenous histamine in chickens. The metabolic pattern of histamine in chicken differs largely from that found in other species. This difference is first and foremost a reflection of the new metabolite, N-acetylmethylhistamine, which accounted for about 20% of the excreted radioactivity, but also of the great quantitative importance of N-acetylhistamine. The identity of N-acetylhistamine was confirmed with mass-spectrography.

The present experiments support the conclusion of Shifrine et al.[3] that acetylation is of great importance in the detoxication of histamine in chicken. This might apply to fowls generally since in vitro experiments with liver slices and cell free extracts of pigeon liver are potent to conjugate histamine[7].

The fact that Bergmark and Granerus[4] found only very low radioactivity in the fractions where N-acetylmethylhistamine should occur suggests that this metabolite, quantitatively important in chickens, is negligible, if present at all, in man.

Whether acetylation or methylation of histamine is the first step in the formation of N-acetylmethylhistamine, and in which tissues these reactions take place, is under examination. Since neither N-acetylhistamine nor 1.4-methylhistamine possess biological activity, it seems likely that this is true also for N-acetyl-1.4-methylhistamine.

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Reduction of adenylylsulfate and 3′-phosphoadenylylsulfate in phototrophic bacteria

A. Schmidt and H. G. Trüper[1]

Botanisches Institut der Universität München, Menzinger Strasse 64, D–8000 München; and Institut für Mikrobiologie der Universität Bonn, Meckenheimer Allee 168, D–5300 Bonn (Federal Republic of Germany, BRD), 28 January 1977

Summary. Extracts of 14 species of phototrophic bacteria, partly grown with different sulfur compounds, were tested for their ability to form volatile sulfur compounds from adenylylsulfate (APS) and 3′-phosphoadenylylsulfate (PAPS). The Rhodospirillum species showed marked activities with both APS and PAPS while the Rhodopseudomonas species seem to prefer PAPS. The Chromatiaceae exhibited the strongest activities with APS, whereas Chlorobium limicola had equally high activity with PAPS.

During dissimilatory sulfur metabolism in Chlorobiaceae and Chromatiaceae, sulfite is oxidized by APS[2] reductase[3]. The relatively high levels of APS reductase found in Chromatium vinosum and Thiocapsa roseopersicina after photoheterotrophic growth with sulfate, as the sole sulfur source, led to the assumption that APS reductase might be involved also in assimilatory sulfur metabolism in these bacteria[4], i.e. in the reduction of APS to sulfite. On the other hand, none of the Rhodospirillaceae contain APS reductase[4], and the formation of PAPS[2] from sulfate and ATP has been reported to occur in chromatophores of Rhodospirillum rubrum[5].

The aim of this study was to test a number of representative species of the phototrophic bacteria as to whether they are able to form volatile sulfur compounds from PAPS or APS (or both).

Material and methods. The Rhodospirillaceae were grown on conventional media[6,7] with sulfate, or special media[8,9] with reduced sulfur compounds. The Chlorobium strain and the Chromatiaceae were cultivated in Pfennig's medium[10] with sulfide, C. vinosum also photoheterotrophically. Incubation occurred in 500 ml screw-capped bottles at 25–30 °C and about 2000 lux.

Frozen cells were thawed and suspended in a buffer containing 0.1 m Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 0.1 M KCl,

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2 Abbreviations. APS, Adenylylsulfate (= adenosine phosphosulfate). DTE, Dithioerythritol. PAPS, 3′-Phosphoadenylylsulfate.
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and 5 mM DTE[2]. 2 ml of buffer were used for 1.0 g of wet weight cells. These were broken at 10,000 psi (French press) and centrifuged to remove unbroken material. Protein was determined by the biuret method after trichloroacetic acid precipitation and 2fold acetone extraction to remove pigments[11]. Preparation of APS and PAPS was performed using the slightly modified Chlorella system[12,13].

Table 1. Reduction of sulfonucleotides in crude (chromatophore-containing) extracts of phototrophic bacteria

Species	Strain	Sulfur source	nmoles sulfonucleotide reduced per mg protein/h	
			APS	PAPS
Rhodospirillum				
rubrum	1761-1a	Sulfate	0.234	0.453
fulvum	BN211	Sulfate	0.168	0.173
(chromatophore fraction alone)			0.020	0.066
(supernatant alone)			0.126	0.554
tenue	BN230	Sulfate	0.206	0.493
		Cysteine	0.372	0.468
photometricum	UW	Sulfate	0	0
Rhodopseudomonas				
palustris	DSM123*	Thiosulfate	0	0.017
	DSM127	Sulfide	0	0.113
		Thiosulfate	0	0.261
	BN128*	Sulfate	0.012	0.165
viridis	BN170	Sulfate	0.006	0.461
capsulata	DSM155	Sulfate	0.099	0.145
		Sulfide	0.006	0.019
gelatinosa	DSM149	Sulfate	0.019	0.029
globiformis	DSM161	Cysteine	0	0
		Thiosulfate	0.003	0.004
		Sulfate	0.005	0.009
sulfidophila	W4	Sulfate	0	0.023
		Sulfide	0	0.001
	BN982*	Sulfide	0	0
Thiocapsa				
roseopersicina	DSM219	Sulfide	4.720	0.447
pfennigii	DSM226	Sulfide	0.700	0.012
Chromatium				
vinosum	DSM180	Sulfate	0.174	0.074
		Sulfide	0.571	0.287
Chlorobium				
limicola	DSM249	Sulfide	0.640	0.745

Conditions see table 2.
*KCl omitted from buffer mixture during preparation of extract.

Table 2. Isotope exchange reaction between labelled sulfonucleotides and unlabelled sulfite catalyzed by bacterial extracts

Organism	Formation of radioactive acid volatile sulfur (nmoles/h × mg protein) from $AP^{35}S$	$PAP^{35}S$
R. rubrum	0.005	0.027
Chr. vinosum	0.32	0.013
Tca. pfennigii	1.16	0.14
Chl. limicola	1.58	1.04

Conditions (in μmoles): Tris-HCl, pH 9.0: 100; $MgCl_2$: 10; unlabelled sulfite: 100; $AP^{35}S$: 0.035 (1 nmole ≙ 1891 cpm) when indicated; $PAP^{35}S$: 0.035 (1 nmole ≙ 1315 cpm) when indicated; bacterial extracts, total volume 1 ml. After 1 h at 37 °C under N_2, 0.1 ml of 1 M SO_3^{2-} was added and the acid volatile radioactivity trapped in 1 ml 1 M triethanolamine. The radioactivity was determined by scintillation counting[14] in a Packard Tricarb.

In order to obtain useful concentrations for a reaction mixture suitable to study volatile sulfur formation from labelled sulfonucleotides, a pilot study with R. rubrum was made. The optimal pH for PAPS reduction was 9.0. The relationship between reaction rate and protein concentrations was linear up to about 25 mg of protein per test volume. From the influence of the PAPS concentrations, an apparent K_m-value of $4 \cdot 10^{-5}$ M for PAPS was obtained by Lineweaver-Burk plot.

A suitable reaction mixture was prepared from these data, buffered at pH 9.0, containing 35 μmoles of labelled sulfonucleotide and 1–20 mg of protein per ml.

Results. Table 1 shows the result of 54 incubations carried out with extracts of 14 different species of phototrophic bacteria partly grown on different sulfur sources. The highest activities with APS were found in those bacteria that are known to contain APS reductase, the Chromatiaceae and Chlorobium. But these bacteria also showed relatively high activity with PAPS. Another group exhibiting fairly high activity with APS is found in the Rhodospirillum species (except R. photometricum), where reactivity with PAPS was only slightly higher. The lowest activities – often down to zero – for APS occur in the Rhodopseudomonas species tested, where the activity with PAPS was always higher, especially when the cells had been grown with sulfate.

The experiments were carried out with chromatophore-containing crude extracts that were only freed from unbroken cells, large fragments and sulfur globules by low speed centrifugation. It is, however, most unlikely that the extracts still contained or produced (by photophosphorylation) sufficient ATP to allow a phosphorylation of APS to PAPS by APS kinase. This is also supported by the comparison made with chromatophore-free fractions (table 1) and controls in the dark (data not shown). On the contrary, the apparent PAPS reduction also occurring in the Chromatiaceae and in Chlorobium is most likely due to the presence of a 3′-nucleotidase in the extracts, leading to an intermediary APS formation from PAPS. This assumption is supported by the isotope exchange reaction between labelled APS (or PAPS) and unlabelled sulfite (table 2). If a regular APS reductase is present, together with a small amount of AMP, one would expect reactivity in both directions[15,16]: In reaction mixtures containing ^{35}S-sulfonucleotide and unlabelled sulfite, the latter together with AMP would form unlabelled APS. This would mix with labelled APS and react back forming labelled sulfite. Thus, label in sulfite (i.e., volatile sulfur) will increase. Again, a 3′-nucleotidase would facilitate PAPS to participate via APS. This has to be postulated, because APS reductases have been shown to be specific for APS[16]. Table 2 shows the results of such isotope exchange experiments.

The organisms known to contain APS reductase show reasonable exchange rates, while in R. rubrum no substantial exchange takes place. This proves that a different, non-reversible mechanism, such as occurs in all assimilatory sulfate reducers so far studied, is present also in this species.

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The results with R. rubrum, and probably the other Rhodospirillum species (except R. photometricum), point to the possibility that in these bacteria an APS sulfotransferase like that found in algae and higher plants[13,17,18] is active. We know that our results do not present final proof for this possibility, and also that we cannot exclude the presence of APS sulfotransferase besides APS reductase in the phototrophic sulfur bacteria. Further studies with purified enzymes are under way.

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Effect of trypsin, S-adenosylmethionine and ethionine on L-serine sulfhydrase activity[1]

Darinka Koraćević and Vidosava Djordjević
Department of Biochemistry, Faculty of Medicine, Niš (Yougoslavia), 16 September 1976

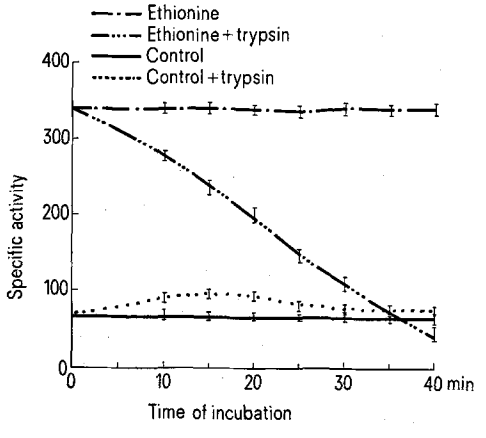
Summary. Trypsin causes an activation of serine sulfhydrase in the liver extracts from intact animals, but inhibits enzyme activity in the liver of ethionine treated rats. Trypsin also decreases an elevation of serine sulfhydrase activity caused by S-adenosylmethionine.

During the last few years, several studies have indicated that both serine sulfhydrase (EC 4.2.1.22) and cystathionine β-synthetase activities are due to a single enzyme[2–4]. Recently, it has been demonstrated that ethionine administration produces a significant activation of cystathionine β-synthetase and serine sulfhydrase in the rat tissues[4–6]. It is also found that S-adenosylmethionine affects as the activator of cystathionine β-synthetase[4,6]. Mudd and his coworkers[7] have shown that trypsin increases the activity of cystathionine-synthetase from an extract of human liver.

As the control mechanism of serine sulfhydrase action has not been known of sufficiently, we examined the interaction of trypsin, ethionine and S-adenosylmethionine in relation to the activity of this enzyme.

Effects of S-adenosylmethionine (SAM) and trypsin on L-serine sulfhydrase activity in vitro (nmoles cysteine/mg protein h)

No. of experiments	Medium for incubation (20 min at 37 °C)	Enzyme activity
9	Enzyme + SAM	99.6 ± 1.8
9	Enzyme + SAM + trypsin	81.5 ± 1.4
9	Enzyme + trypsin	97.3 ± 2.1
9	Enzyme	76.1 ± 0.9



In vitro effect of trypsin on L-serine sulfhydrase activity in the liver of rats treated with ethionine. Vertical bars indicate standard errors of the mean.

Material and methods. Albino rats weighing 160–220 g were used. DL-ethionine was dissolved in 0.9% NaCl and injected i.p. in a dose of 200 mg/kg. The control group received the corresponding volume of 0.9% NaCl. The animals were killed 3 h after the injection. Serine sulfhydrase was extracted and separated from serine dehydratase according to the Kashiwamata and Greenberg method[8]. Trypsin was dissolved in 0.1 M tris-HCl buffer pH 8.3 and added to the aliquots of enzyme extract in the amount of 0.5 y/mg protein. S-adenosylmethionine was dissolved in H_2O and added to the enzyme of control rat liver in the amount of 0.1 micromol per mg protein. Enzyme had been preincubated with S-adenosylmethionine 5 min at 37 °C before adding trypsin. After incubation at 37 °C, the activity of serine sulfhydrase was determined by the procedure of Stepien and Pieniazek[9]. Enzyme activity was expressed in nmoles cysteine per mg protein per h. Protein was estimated by the method of Lowry et al.[10]. DL-ethionine and S-adenosylmethionine were obtained from Calbiochem while trypsin was purchased from Merck.

Results and discussion. The effect of trypsin treatment on L-serine sulfhydrase activity is shown in the figure. Trypsin causes an increase in serine sulfhydrase activity in the liver extracts from intact animals. On the contrary, when trypsin was incubated with enzyme extracts prepared from the liver of the rats which had received DL-ethionine, a successive diminution in serine sulfhydrase activity was observed. The action of S-adenosylmethionine and trypsin upon serine sulfhydrase levels is presented in the table. It is evident from these data that S-adenosylmethionine as well as trypsin produce a remarkable rise in serine sulfhydrase. However, addition

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