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Note

2-Amino-2-deoxyguluronic acid: a constituent of the cell wall of *Halococcus* sp., strain 24

RAGNHILD REISTAD

Department of Microbiology, Dental Faculty, University of Oslo, Blindern, Oslo 3 (Norway)

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Cell-wall hydrolysates of *Halococcus* sp., strain 24, an extremely halophilic bacterium, contain amino acids, amino sugars, and amino sugar-like compounds[1]. We now report the identification of 2-amino-2-deoxyguluronic acid, which until now has been found in Nature only in *Vibrio parahaemolyticus* K15 antigen[2].

Materials and methods. — The 2-amino sugar was isolated from purified cell-walls (15 mg) of *Halococcus* sp., strain 24, after hydrolysis in evacuated glass tubes with 6M HCl (2 mg/ml) for 2 h at 105°. The hydrolysate was concentrated to dryness and the ninhydrin-positive compounds were separated on a column (0.62 × 125 cm) of Technicon Chromo-Beads (Type A) in a standard 21-h run as described for amino acid analyses on the Technicon AutoAnalyzer[3]. The fraction containing the 2-amino sugar was added to a column (1.1 × 3 cm) of Dowex-2(HO^-) resin. The column was washed with water, and the 2-amino sugar was then eluted with 20 ml of M acetic acid[4]. Concentration of the eluate gave 100–150 μg of the 2-amino sugar, measured as 2-amino-2-deoxyglucose[5]. The figures give little information on the true content of the 2-amino sugar in the cell walls, as extensive degradation occurs during acid hydrolysis[1].

Paper chromatography was carried out on Whatman No. 1 paper by the descending technique with: *A*, ethyl acetate–pyridine–water–acetic acid (5:5:3:1)[6] for 24 h; *B*, pentyl alcohol–pyridine–water (7:7:6)[7] for 20 h; *C*, butanone–acetic acid–saturated aqueous boric acid (9:1:1)[8] for 14 h.

Paper electrophoresis was carried out with a Shandon High Voltage Electrophoresis Apparatus SAE 2550 on Whatman No. 4 paper, at 40 volts/cm for 90 min, using the following buffer systems: *1*, pyridine–formic acid–acetic acid–water (1:1.5:10:90, pH 2.8)[9]; *2*, sodium molybdate–sulphuric acid (pH 5.0)[10]. Detection was effected with silver nitrate–sodium hydroxide[11], 0.2% ninhydrin in acetone, or aniline hydrogen phthalate[12].

Degradation of hexosamines to pentoses was carried out with ninhydrin in capillary tubes according to the method of Stoffyn and Jeanloz[13].

Reduction of the carboxyl group of the 2-amino sugar. — The 2-amino sugar (50–200 μg) was *N*-acetylated[14] and dried, and 2 ml of anhydrous methanol and

~50 mg of Dowex-50W(H^+) resin, previously washed with anhydrous methanol[15], were added. The mixture was protected from moisture and boiled overnight, then filtered and concentrated to dryness. A solution of the methyl ester methyl glycoside in 0.5 ml of 1% sodium borohydride in 0.05M H_3BO_3 (*cf.* ref. 16) was kept at room temperature overnight. Sufficient Dowex-50W(H^+) resin was added to make the pH acid. The mixture was then filtered and concentrated, and borate was removed from the residue by evaporation of methanol therefrom. The residue was treated with 4M HCl for 30 min at 100°, and the solution was concentrated to dryness.

G.l.c. and g.l.c.–m.s. — Carboxyl and hydroxyl trimethylsilylation of the 2-amino-2-deoxyhexuronic acid was carried out by the method of Romanowska and Reinhold[17]; 2-amino-2-deoxyhexoses were fully trimethylsilylated by this procedure. The derivatives were injected into an F & M gas chromatograph, Model 402, with a glass column (0.3 × 150 cm) of 3.1% SE-30 on Diatoport-S (80–100 mesh) at 190°. Mass spectra were recorded by using a combined Varian 1400 gas chromatograph with a glass column (0.3 × 250 cm) of 10% OV-17 on Gas-Chrom Q (80–100 mesh) and a Varian MAT CH7 mass spectrometer. The ionizing voltage was 70 eV, and the ion-source and separator temperatures were 250°.

DISCUSSION

The 2-amino sugar isolated from *Halococcus* sp., strain 24 (previously designated "halophilic coccus", strain 24), reacted[5,18] as a 2-amino-2-deoxy sugar unsubstituted at positions 3 and 4. With ninhydrin at 105°, it gave a yellow-brown colour turning gradually violet as reported for 2-amino-2-deoxyhexuronic acids[19].

Mass spectrometry of the trimethylsilylated 2-amino sugar gave a high-mass fragment at *m/e* 466 assigned to (M−15) since trimethylsilylated carbohydrates readily lose a methyl group[20]. A molecular weight of 481 was thus indicated, in agreement with a 2-amino-2-deoxyhexuronic acid having trimethylsilylated carboxyl and hydroxyl groups, but a free amino group[17].

Paper chromatography and electrophoresis indicated that the 2-amino-2-deoxyhexuronic acid was not identical to 2-amino-2-deoxyhexuronic acids having the *gluco*, *galacto*, or *manno* configurations.

Carboxyl reduction of the 2-amino-2-deoxyhexuronic acid followed by ninhydrin degradation of the product gave a compound with the mobility of xylose (Table I), which was clearly separated from arabinose and lyxose (R_{Rib} 0.29 and 0.46, respectively). Xylose is the expected product of ninhydrin degradation of 2-amino-2-deoxygulose and 2-amino-2-deoxyidose. The latter has been ruled out because of its much higher mobility in solvent *A* (R_{GlcN} 1.29)[21] than the carboxyl-reduced 2-amino-2-deoxyhexuronic acid from *Halococcus* sp. (Table I). The trimethylsilyl derivatives of the latter compound and 2-amino-2-deoxygulose gave peaks having identical retention times in g.l.c. (Table I). Mass spectra of the peaks were also identical. Thus, it is concluded that the 2-amino sugar isolated from hydrolysates of cell walls of *Halococcus* sp., strain 24, is 2-amino-2-deoxyguluronic acid.

TABLE I

CHROMATOGRAPHIC BEHAVIOUR OF THE 2-AMINO SUGAR AND ITS DERIVATIVES

Sugar	*Relative mobility in solvent*			*Relative mobility in buffer*		*Relative mobility in AutoAnalyzer*	*G.l.c. T (min) for main peak*
	A	B	C	1	2		
From Halococcus *sp.*							
2-Amino sugar	0.37	0.31		0.51	0.63	1.29	5.4
Reduced 2-amino sugar	1.04					1.15	4.5
Reduced 2-amino sugar, ninhydrin degraded			0.40				
Reference samples							
2-Amino-2-deoxyglucuronic acid	0.40	0.35		0.39	1.00	0.73	
2-Amino-2-deoxyglucose	1.00	1.00		1.00		1.00	
2-Amino-2-deoxygulose	1.04					1.15	4.5
Mannitol							10.2
Xylose			0.40				
Ribose			1.00				

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