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Structure of the O83-specific polysaccharide of *Escherichia coli* O83:K24:H31

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Abstract

The polysaccharide moiety of the O83 antigen (lipopolysaccharide, LPS) consists of D-glucose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and D-glucuronic acid in the molar ratios 1:2:1:1. Methylation analysis of the polysaccharide and derived oligosaccharides as well as one- and two-dimensional ^{1}H and ^{13}C NMR spectroscopy of the polysaccharide at pD 1 and 6 showed that the O83 polysaccharide has the primary structure

→ 6)-α-D-Glc*p*-(1 → 4)-β-D-Glc*p*A-(1 → 6)-β-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 →

Key words: E. Coli, O83 antigen; Polysaccharide structure; NMR spectroscopy

1. Introduction

Escherichia coli O83:K24:H31 is a frequent cause of sepsis and pyelonephritis [1]. There are two strains expressing different forms of the O83 antigen (LPS), *E. coli* H17a with an O83-specific polysaccharide of normal size distribution, as observed by SDS-PAGE, and *E. coli* H45 with a short O83-specific oligosaccharide (maximally four repeating units, as observed by SDS-PAGE). *E. coli* H17a with the normal-sized LPS, the O83 test strain, is serum resistant, and *E. coli* H45 with the short chain LPS, the K24 test strain, is serum sensitive (Ørskov, personal

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communication). Thus, serum resistance, an important virulence property [2], can be critically dependent on the nature of the LPS, irrespective of the presence of a capsule. In this communication we present the chemical analysis of the long chain O83 LPS and the structure of its repeating unit.

2. Results and discussion

Isolation and characterization of the O83-specific polysaccharide.—E. coli H17a, after growth in liquid culture, was extracted with 45% aqueous phenol and the aqueous phase was subjected to ultracentrifugation [3]. The sediment contained an LPS fraction (LPS I) that consisted of short-chain LPS and R-LPS. The supernatant solution contained an LPS (LPS II) that was isolated by fractional precipitation with cetyltrimethylammonium bromide (CTAB) [3,4]. LPS II, which had a long O-specific polysaccharide chain, was used for further structural studies.

LPS II was subjected to mild acid hydrolysis, the lipid A moiety was removed by centrifugation, and the polysaccharide was purified by gel permeation chromatography. The polysaccharide contained glucose (D-Glc), galactose, (D-Gal), *N*-acetylglucosamine (D-GlcNac), and glucuronic acid (D-GlcA) in the molar ratios 1:2:1:1. Periodate oxidation destroyed all sugar constituents, with the exception of GlcNAc.

The ^{13}C NMR spectrum of the O83 polysaccharide (Fig. 1) contained five signals in the anomeric region. Chemical shift values and the results of a gated decoupling experiment at 300 MHz showed that the signal at δ 99.1 ($^{1}J_{C,H}$ 172 Hz)

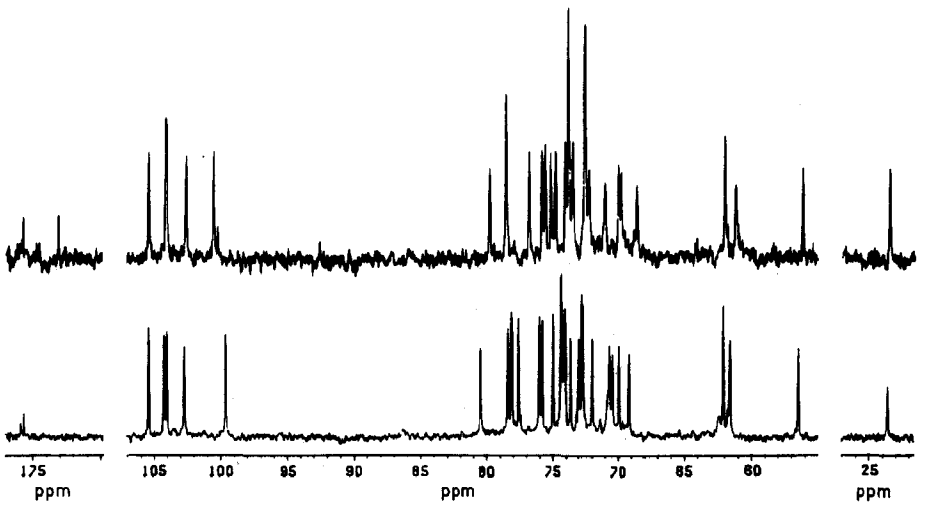

Fig. 1. 75 MHz NMR spectra (δ 22–27; 55–107; 170–177) of the O83 polysaccharide at pD 1 (top) and pD 6 (bottom), recorded in D_2O (80°C) with acetone (δ_C 31.45) as internal standard.

was from a residue having the α configuration and that the other residues were β (δ 102.6, 104.1, 104.15, and 105.4 with $^1J_{C,H}$ 161–163 Hz). Signals at δ 56.2, 175.7, and 23.5 indicated the presence of an amino sugar with a CH–$NHCOCH_3$ group. An APT spectrum [5] indicated that two hydroxymethyl groups were substituted (signals at δ 69.0 and 70.65 at pD 6). The repeating units also contained a uronic acid, as evidenced by the signal of the –COOH group (δ 176.0 at pD 6 and 173.1 at pD 1).

Methylation analysis.—The polysaccharide was methylated [6] with KH–MeI in Me_2SO. The purified (Sep-Pak C_{18}) product was hydrolysed [7], reduced with sodium borodeuteride, and per-*O*-acetylated, and the resulting partially methylated alditol acetates were characterized by GLC–MS. In another experiment, the carboxyl groups of GlcA were reduced in the intact polysaccharide [8] before the methylation analysis. The results indicated that the O83 polysaccharide contained one residue each of 6-linked Glc, 6-linked Gal, 4-linked Gal, 4-linked GlcA, and 4-linked GlcNAc. These data show that the O83 polysaccharide contains an unbranched sequence of 4- and 6-linked sugar units.

Two disaccharides, one trisaccharide and one tetrasaccharide were obtained by partial acid hydrolysis (1 M trifluoroacetic acid, 100°C, 30 min) and isolated by high voltage paper electrophoresis (42 V/cm, pH 5.4, 60 min at 10°C). With the exception of the tetrasaccharide they were reduced with sodium borodeuteride, methylated, and subjected to MS. The mass spectra showed that they can be formulated as HexA-(1 → 6)-Hex, Hex-(1 → 4)-HexNAc, and HexA-(1 → 6)-Hex-(1 → 4)-Hex. The fragmentation pattern of the deutero-reduced and methylated trisaccharide is shown in Fig. 2. The reduced tetrasaccharide was subjected to the usual methylation analysis. GLC–MS revealed the presence of 1-deutero-4-*O*-acetyl-1,2,3,5,6-penta-*O*-methylgalactitol, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl galactitol, and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl glucitol in approximately equal amounts. The data showed that the tetrasaccharide has the structure Hex-(1 → 4)-HexA-(1 → 6)-Hex(1 → 4)-Hex.

NMR analysis.—The ^{1}H-NMR spectrum of the O83 polysaccharide at pD 6 (Fig. 3) contained four signals of sugars with the *β-gluco/galacto* configuration ($J_{1,2}$ 7.5–8.0 Hz) and one signal of a sugar with the *α-gluco/galacto* configuration. Assignments of the signals (Table 1) were obtained with a sequential selective spin

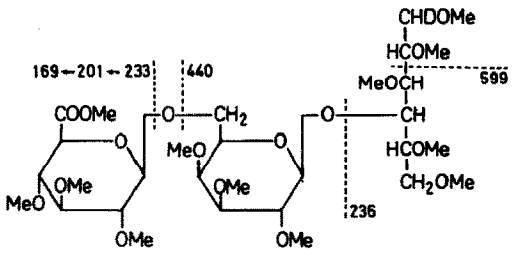

Fig. 2. Structure and mass-spectrometric fragmentation pattern of the reduced and methylated trisaccharide, obtained from the O83 polysaccharide by partial and hydrolysis.

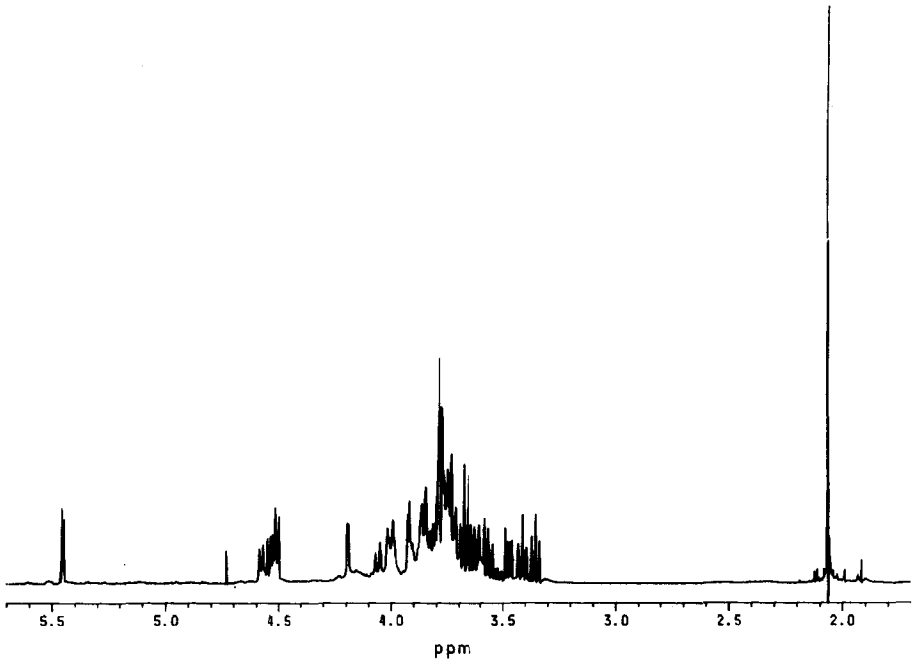

Fig. 3. 500-MHz NMR spectrum of the O83 polysaccharide at pD 6, recorded in D_2O (80°C) with acetone (δ_H 2.225) as internal standard.

decoupling procedure [9], 2D-COSY, and one- and two-step relayed coherence transfer COSY [10,11].

Attempts to analyse the sequence of the sugar residues with a 2D-ROESY spectrum [12] failed, due to the overlap of signals (at 300 and 500 MHz). The only information available from this experiment was a cross-peak between H-1 of one galactose (residue C) and H-4 of the second galactose unit in the polysaccharide (residue D), indicative of a Gal-(1 → 4)-Gal sequence. In a 1D-NOE experiment at pD 1 (Fig. 4), preirradiation of the anomeric proton of the glucose residue (residue A) resulted in a response at H-4 of glucuronic acid (residue B). This interaction is only possible with α glucose as a glycosylating sugar for the glucuronic acid residue. It also explains the pD dependence of the chemical shift for the anomeric carbon of α glucose in the ^{13}C NMR spectrum as due to close steriochemical apposition. To analyze the sequence position of GlcNAc in the polysaccharide, a 1D-NOE experiment was performed in which the methyl protons of the *N*-acetamido group were preirradiated (Fig. 5). An intraring long-range interaction between the methyl protons and H-1, H-2, and H-3 of GlcNAc (residue E) as well as an interring interaction with H-4 of the glucose residue (residue A) were detected. The H-Me (E) → H-4 (A) interaction is only possible in the configuration given by a E-(1 → 6)-A linkage.

The signals of the ^{13}C NMR spectrum were assigned (Table 1) with a 2D heteronuclear COSY spectrum (Fig. 6). The absolute configurations of all sugar

Table 1
^{1}H NMR and ^{13}C NMR data for the O83 polysaccharide [a]

Residue	Chemical shifts (δ/ppm) and coupling constants (Hz)								$^1J_{C,H}$
		pD 6	pD 1	$^3J_{H,H}$	Hz		pD 6	pD 1	
→6)-α-D-Glc*p*-(1→	H-1	5.45	5.41	$J_{1,2}$	3	C-1	99.1	100.5	172
	H-2	3.47	3.53	$J_{2,3}$	10	C-2	72.8	72.55	
A	H-3	3.67	3.64	$J_{3,4}$	10	C-3	74.0	73.9	
	H-4	3.42	3.42	$J_{4,5}$	10	C-4	70.6	70.0	
	H-5	3.75	3.71	$J_{5,6}$	⩽2	C-5	71.8	72.25	
	H-6	4.06	4.05	$J_{6,6'}$	11	C-6	69.0	68.6	
	H-6′	3.84	3.89	$J_{5,6'}$	6				
→4)-β-D-Glc*p*A-(1→	H-1	4.505	4.61	$J_{1,2}$	<8	C-1	104.2	104.15	161
	H-2	3.35	3.41	$J_{2,3}$	9	C-2	74.3	73.9	
B	H-3	3.74	3.80	$J_{3,4}$	10	C-3	77.8	76.8	(+0.8) [b]
	H-4	3.76	3.86	$J_{4,5}$	10	C-4	78.0	78.55	
	H-5	3.79	4.12			C-5	77.55	75.2	
						C-6	176.0	173.1	
→6)-β-D-Gal*p*-(1→	H-1	4.58	4.59	$J_{1,2}$	7.5	C-1	105.3	105.4	163
	H-2	3.56	3.59	$J_{2,3}$	10	C-2	72.6	72.6	
C	H-3	3.67	3.69	$J_{3,4}$	4	C-3	73.9	73.5	
	H-4	3.92	3.92	$J_{4,5}$	<2	C-4	69.8	69.8	
	H-5	3.86	3.81			C-5	74.8	74.1	
	H-6,6′	~3.78	~3.85			C-6	70.65	71.0	
→4)-β-D-Gal*p*-(1→	H-1	4.52	4.53	$J_{1,2}$	7.5	C-1	104.0	104.1	163
	H-2	3.62	3.64	$J_{2,3}$	10	C-2	72.5	72.5	
D	H-3	3.80	3.82	$J_{3,4}$	4	C-3	74.2	73.9	
	H-4	4.19	4.19	$J_{4,5}$	<2	C-4	78.3	78.5	
	H-5	3.80	3.81			C-5	75.7	75.6	
	H-6	4.00	4.01			C-6	62.0	62.0	(−0.2) [b]
	H-6′	3.85	3.87						
→4)-β-D-Glc*p*NAc-(1→	H-1	4.54	4.56	$J_{1,2}$	8	C-1	102.7	102.6	163
	H-2	3.75	3.77	$J_{2,3}$	9	C-2	56.3	56.2	
E	H-3		3.84	$J_{3,4}$	9	C-3	73.55	74.8	(−1.5) [b]
	H-4	3.72	3.71	$J_{4,5}$	9	C-4	80.2	79.8	
	H-5	3.60	3.60	$J_{5,6}$	<2	C-5	75.9	75.9	
	H-6	4.00	4.01	$J_{6,6}$	11	C-6	61.4	61.2	
	H-6′	3.86	3.83	$J_{5,6'}$	3	-NAc	23.5	23.5	
							175.7	175.7	

[a] Recorded in D_2O (30°C), with acetone (δ_H 2.225; δ_C 31.45) as internal standard.
[b] Glycosylation effects (at pD 6).

units were determined as D by calculating the experimental glycosylation effects [13,14] (Table 1), with D-glucose (residue A) as a basis, the configuration of which was proved by its reactivity with D-glucose oxidase.

The results obtained, which are corroborated by those of the methylation analysis, permit the formulation of the O83 polysaccharide as

A B C D E

→6)-α-D-Glc*p*-(1→4)-β-D-Glc*p*A-(1→6)-β-D-Galp-(1→4)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→

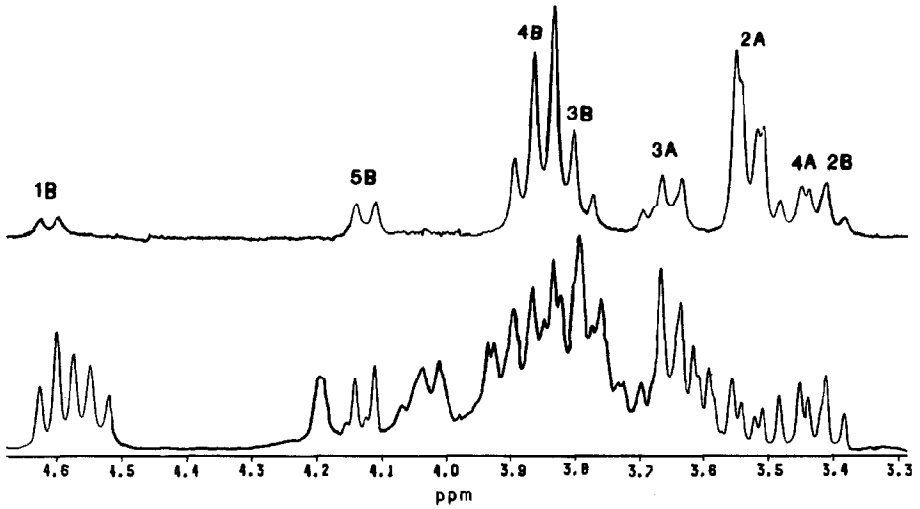

Fig. 4. 300-MHz spectrum (δ 3.3–4.7) of the O83 polysaccharide at pD 1 (bottom) and the NOE difference spectrum (top) after preirradiation of the anomeric proton (δ 5.41) of α-Glc*p* (residue A). Small signals of protons A-3 and A-4, and of protons 1, 2, 3, and 5 of β-Glc*p*A (residue B) are due to spin diffusion.

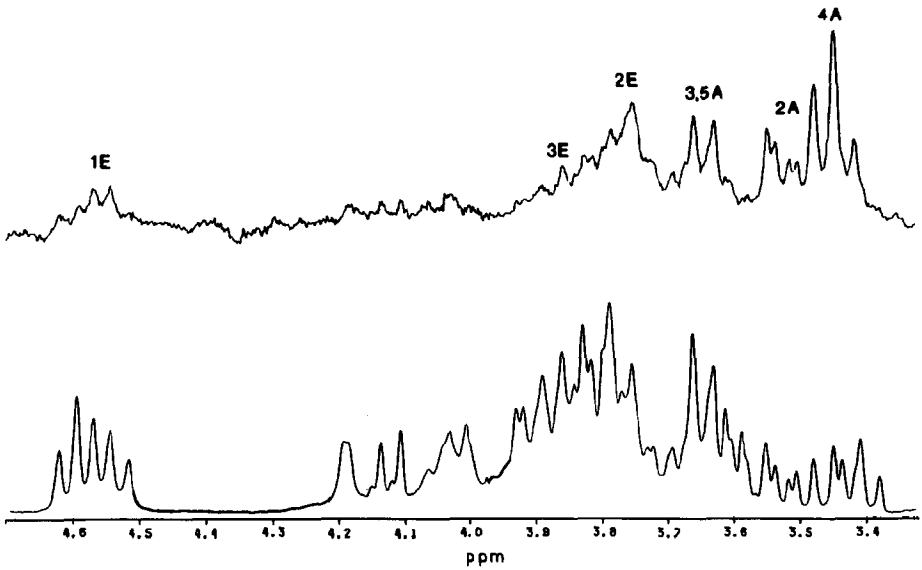

Fig. 5. 300-MHz spectrum (δ 3.3–4.7) of the O83 polysaccharide at pD 1 (bottom) and the NOE difference spectrum (top) after preirradiation of the methyl protons of the *N*-acetyl group of β-Glc*p*NAc (residue E). Note the strong interring response with H-4 of α-Glcp (residue A).

3. Experimental

Bacteria and cultivation.—*E. coli* strain H17a (O83:K24.H31) was grown at 37°C to the late logarithmic phase in a fermenter in 10-L batches of standard I broth (Merck).

Isolation of the polysaccharide.—The bacteria were extracted with 45% aq phenol and the dialyzed aqueous phase was subjected to ultracentrifugation [3]. From the supernatant solution, the O83 polysaccharide was obtained by fractional precipitation with CTAB [4] and the complex precipitated was converted into the sodium salt as described [4].

Methylation.—The O83 polysaccharide was methylated [6] with KH–MeI in Me_2SO. The purified (Sep-Pak C_{18}) product was hydrolysed [7] and the constituents were characterised by GLC–MS as their partially methylated alditol acetates. Oligosaccharides, obtained by partial hydrolysis (1 M CF_3CO_2H, 100°C, 30 min) were reduced with $NaBD_4$ before methylation and, after purification, methylated and analyzed in GLC–MS with and without hydrolysis.

Analytical methods.—Glucose, galactose, and glucosamine were determined as their alditol acetates by GLC and with the Elson–Morgan reaction [15], respectively. Glucuronic acid was determined with the carbazole reagent. The absolute

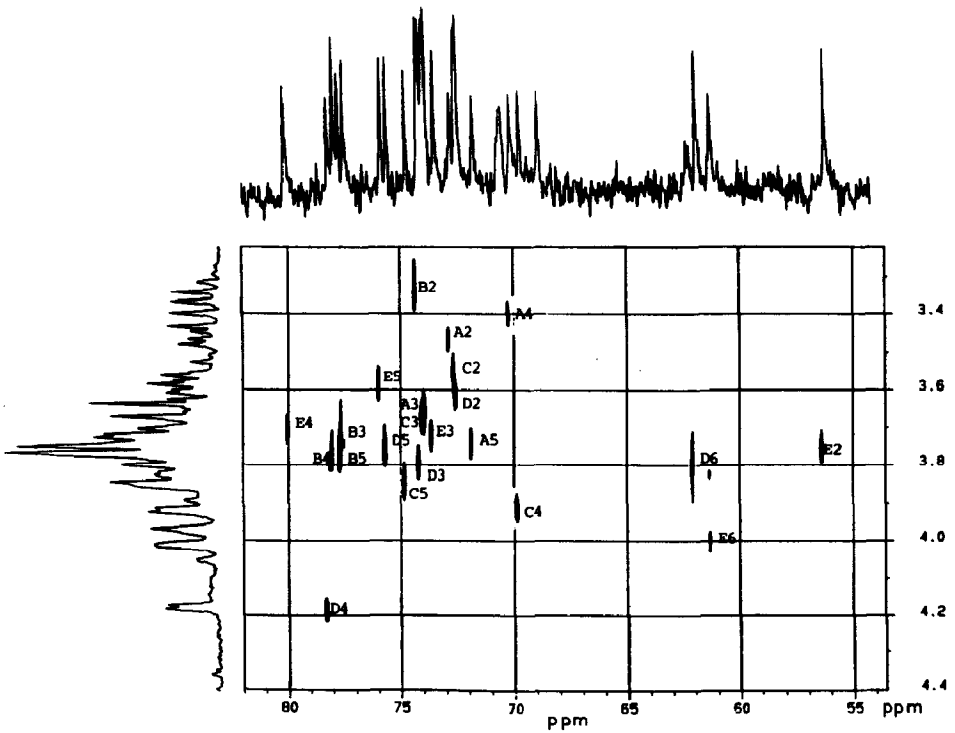

Fig. 6. 2D 300-MHz heteronuclear $^{13}C/^{1}H$ COSY spectrum of the O83 polysaccharide at pD 6.

configuration of glucose was determined with D-glucose oxidase. The periodate oxidation has been described [16]. GLC–MS was carried out with a Hewlett–Packard 5988A instrument, using a DB1 capillary column (0.25 mm × 30 m) with He as carrier gas and a temperature program of 50 → 180°C at 70°C/min and then 180 → 250°C at 5°C/min. EI mass spectra were obtained with an ionising energy of 70 eV. NMR spectra were recorded with Bruker AM 300 and AM 500 instruments with acetone as internal standard (δ_H 2.225; δ_C 31.45). Standard Bruker software was used for homonuclear ^1H, ^1H COSY (COSYHG); one- and two-step ^1H-relayed homonuclear 2D ^1H, ^1H COSY (COSYRCT and COSYRCT2); and ^1H, ^{13}C heteronuclear 2D COSY (XHCORRD). The 1D NOE experiment was performed in the truncated driven (TOE) mode [17] with the Bruker NOEMULT program and the 2D ROESY spectrum was acquired with the Rance pulse sequence [18] with a spin lock time of 0.2 s.

Acknowledgments

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