

碰撞诱导解离和高能碰撞解离方式 在褐藻胶寡糖结构分析中的比较和应用

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摘要: 利用组合式高分辨质谱仪(LTQ Orbitrap XL)对均一褐藻胶寡糖, 包括甘露糖醛酸寡糖和古洛糖醛酸寡糖进行二级质谱分析, 比较了常规的碰撞诱导解离(CID)和高能碰撞解离(HCD)在寡糖结构分析中的差异, 并应用 HCD 技术分析甘露糖醛酸寡糖和古洛糖醛酸寡糖的结构差异。研究发现, HCD 可避免 CID 中的 1/3 效应, 而且可以提供更多的糖环断裂碎片信息, 如 $^{2,5}A_2(m/z\ 291)$ 、 $^{2,4}A(m/z\ 235, 411)$ 、 $^{2,5}A$ 脱水($m/z\ 101, 277, 453$)以及 Z_2 脱羧碎片($m/z\ 307, 483, 659$)等, 进一步验证了 Z_2 脱羧碎片是区别甘露糖醛酸与古洛糖醛酸的特征碎片。同时, HCD 能够提供丰富的裂解碎片信息, 这为褐藻胶寡糖的结构解析提供了依据。

关键词: 组合式高分辨质谱仪(LTQ Orbitrap XL); 褐藻胶寡糖; 碰撞诱导解离(CID); 高能碰撞解离(HCD)

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Comparison and Application of Collision Induced Dissociation and High Energy Collision Induced Dissociation for Alginate Oligosaccharides Analysis

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Abstract: Alginate homooligosaccharides including oligomannuronate (M) and oligoguluronate (G) with degree of polymerization from 2 to 7 were analyzed by linear ion trap-Orbitrap mass spectrometer (LTQ Orbitrap XL) using collision induced dissociation (CID) and high energy collision induced dissociation (HCD). First of all, comparison of CID MS/MS and HCD MS/MS of either M or G was conducted. Taking the trimannuronate (M3) as an example, CID MS/MS showed the $m/z\ 369, 193$ and 175 ions were

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assigned to C_2/Y_2 , C_1/Y_1 and B_1/Z_1 , respectively, which were formed due to single glycosidic bond cleavage, while HCD MS/MS caused cleavage of all glycosidic bonds and showed weak A-type ions, such as $^{0,2}A$ (m/z 309, 485), $^{2,5}A$ (m/z 291, 467) and $^{0,4}A_3$ ions (m/z 471). Interestingly, an internal M produced a $Z_{int}-CO_2$ ions at m/z 307, which was unique and found only in HCD MS/MS. Furthermore, CID MS/MS and HCD MS/MS of M3 and triguluronate (G3) were executed. For CID, M3 and G3 did not show significant difference, and they gave identical glycosidic bond cleavage ions C_1 (m/z 193), B_1 (m/z 175) together with the dehydrated and decarboxylated ions (m/z 157 and m/z 131). These results suggest that it is impossible to differentiate the two residues at a nonreducing terminal of M3 and G3 by CID. However, a significant difference was observed in HCD spectra for M3 and G3. It was found that besides decarboxylation fragment m/z 307, debris $^{2,4}A_3$ (m/z 411), $^{2,5}A_3$ (m/z 467) and $^{0,4}A_3$ (m/z 471) were found in M3, whereas these ions were not found in HCD MS/MS of G3, thus demonstrating that HCD MS/MS can be used to differentiate M3 and G3. In the CID MS/MS of pentamannuronate (M5), fragment ions $^{0,2}A_3$ (m/z 837), $^{0,4}A_3$ (m/z 823) and $^{2,5}A_3$ (m/z 819) was found apparently, however, HCD displayed rich fragment ions in the low mass region, which should be ascribed to the characteristics of high-energy dissociation of HCD. For heptamannuronate (M7) and heptaguluronate (G7), doubly charged protonated species were collected as parent ions for CID and HCD. HCD MS/MS provided more fragment ions than CID MS/MS, especially, $Z_{int}-CO_2$ ion (m/z 307, 483, 659, 835 and 1 011) signal was apparently showed in M7 HCD spectra. Taken together, this work demonstrates that HCD MS/MS provides rich product ions and has no low mass cut off. The fragment ions in HCD spectra have high mass accuracy and resolution. These characteristics of HCD spectra complement the power of CID and allow easy spectrametric interpretation and high confidence in structural elucidation for alginic oligosaccharides.

Key words: linear ion trap-Orbitrap mass spectrometer (LTQ Orbitrap XL); alginate homooligosaccharides; collision induced dissociation (CID); high energy collision induced dissociation (HCD)

褐藻胶是一种直链的多糖类化合物,结构单元是 β -(1 $\rightarrow$ 4)连接的 *D*-甘露糖醛酸(M)和 α -(1 $\rightarrow$ 4)连接的 *L*-古洛糖醛酸(G),其在人类疾病、植物生长和抑菌等方面具有生物活性。褐藻酸及其寡糖的活性和应用与褐藻酸中甘露糖醛酸和古洛糖醛酸的比例以及位置有着密切的关系^[1-2]。近年来,电喷雾离子化-碰撞诱导解离-串联质谱(ESI-CID-MS/MS)技术促进了寡糖结构及其序列分析的发展^[3-8]。如,张真庆等^[9]利用 Q-TOF 质谱仪的 CID 技术对褐藻酸中甘露糖醛酸和古洛糖醛酸的异构体进行了 MS/MS 序列分析。

高能碰撞解离(HCD)是一项新型的质谱裂

解技术,与传统的碰撞诱导解离(CID)相比,HCD能够提供稳定的高能裂解模式,可以改善 CID 裂解中产生的低质量碎片丢失(1/3 cut-off)效应。因此,将 HCD 应用到寡糖序列分析中,利用其高能特点可以提供不同断裂位点的糖链碎片;另外,因其与 Orbitrap 相连能够提供高精度的质量数($<3 \times 10^{-6}$),可以直接给出元素组成,这更有助于寡糖碎片的结构分析^[10-14]。

本研究将利用 HCD 对均一结构的褐藻胶寡糖,包括甘露糖醛酸寡糖和古洛糖醛酸寡糖进行二级质谱分析,并与传统的 CID 方式的结果进行比较,探索两种不同的碎裂方式在褐藻寡糖结构分析中的差异,以扩展高分辨质谱仪

LTQ Orbitrap XL 的高能碰撞解离模式在褐藻寡糖结构分析中的应用。

1 实验部分

1.1 仪器和试剂

LTQ-Orbitrap XL 线性离子阱静电场轨道阱高分辨组合式质谱仪;美国 Thermo Fisher 公司产品。寡糖标准品:由中国海洋大学医药学院赵峡课题组提供,溶于 50% 乙腈水溶液中;乙腈(色谱纯):美国 Honeywell 公司产品;实验用水:Milli-Q 制备的超纯水。

1.2 质谱条件

电喷雾离子源(ESI),喷雾电压 3 kV,离子传输毛细管温度 275 °C;干燥气为氮气,压力 0.05 MPa;CID 和 HCD 碰撞气均为氦气,负离子模式检测;CID 归一化碰撞能量采用 15%~25%,HCD 碰撞能量采用 45~60 eV。

2 结果与讨论

2.1 甘露糖醛酸及古洛糖醛酸二糖、三糖的二级质谱解析

分析了较复杂的褐藻胶三糖——甘露糖醛酸三糖和古洛糖醛酸三糖,其二级质谱解析示于图 1^[10]。甘露糖醛酸三糖的分子离子峰为 m/z 545,糖苷键断裂产生 m/z 369、193 和 175 碎片离子,分别记做 C_2/Y_2 、 C_1/Y_1 和 B_1/Z_1 ,它们是源于还原端和非还原端的离子碎片,是吡喃糖醛酸三糖的特征碎片离子。

本研究还分析了甘露糖醛酸三糖 CID 和 HCD 谱图的差异,其二级质谱采集信息示于图 2。将产生跨环断裂碎片的区域放大 10 倍后,CID MS/MS 给出了所有的糖苷键断裂碎片 C_2/Y_2 (m/z 369)、 B_2/Z_2 (m/z 351)、 C_1/Y_1

(m/z 193)和 B_1/Z_1 (m/z 175)。糖环断裂仅显示了^{0,2}A (m/z 309 和 m/z 485),而 HCD MS/MS 除了给出糖苷键断裂碎片外,还给出了所有的 A 型碎片离子,如^{0,2}A (m/z 309 和 m/z 485)、^{2,5}A (m/z 291 和 m/z 467)、^{0,4}A₃ (m/z 471)和^{2,4}A (m/z 235 和 m/z 411),以及甘露糖醛酸三糖区别于古洛糖醛酸三糖特殊的 Z_{int}脱羧碎片 m/z 307^[10],其中^{2,4}A (m/z 235 和 m/z 411)是首次被检测到。

对比甘露糖醛酸三糖和古洛糖醛酸三糖的 CID 和 HCD 谱图的主要对照特征峰和峰高可以发现,两种寡糖的 CID 谱图没有明显差异,所有差异均显示在 HCD 谱图中。除 Z₂ 脱羧碎片 m/z 307 外,在古洛糖醛酸三糖的 HCD 中未见碎片^{2,4}A₃ (m/z 411)、^{2,5}A₃ (m/z 467)和^{0,4}A₃ (m/z 471),这可作为区别两种寡糖的特殊碎片。

通过比较甘露糖醛酸二糖和古洛糖醛酸二糖的 CID 和 HCD 二级质谱图发现,同一二糖的 CID 和 HCD 之间无明显差异。这是因为在二糖中没有中间糖环,在原三糖 HCD 谱中发现的脱羧碎片也就不存在了。

2.2 甘露糖醛酸和古洛糖醛酸四、五糖的二级质谱解析

从实验数据可以看出,四糖和五糖具有相同的结果,本研究以甘露糖醛酸五糖和古洛糖醛酸五糖为例进行解析,结果示于图 3。甘露糖醛酸五糖和古洛糖醛酸五糖的 CID 和 HCD 谱图示于图 4。通过比较甘露糖醛酸五糖的 CID 和 HCD 谱图可以发现:HCD 二级质谱图给出了通过糖苷键断裂产生的所有表征它们直链连接方式的碎片离子 m/z 175/193、 m/z 351/369、 m/z 527/545 和 m/z 703/721;而在

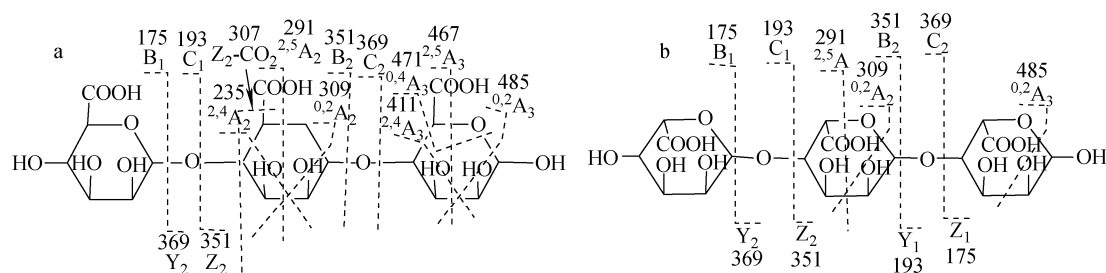


图 1 甘露糖醛酸三糖(a)和古洛糖醛酸三糖(b)的二级质谱解析

Fig.1 MS/MS analysis of trimannuronate (a) and trigluronate (b)

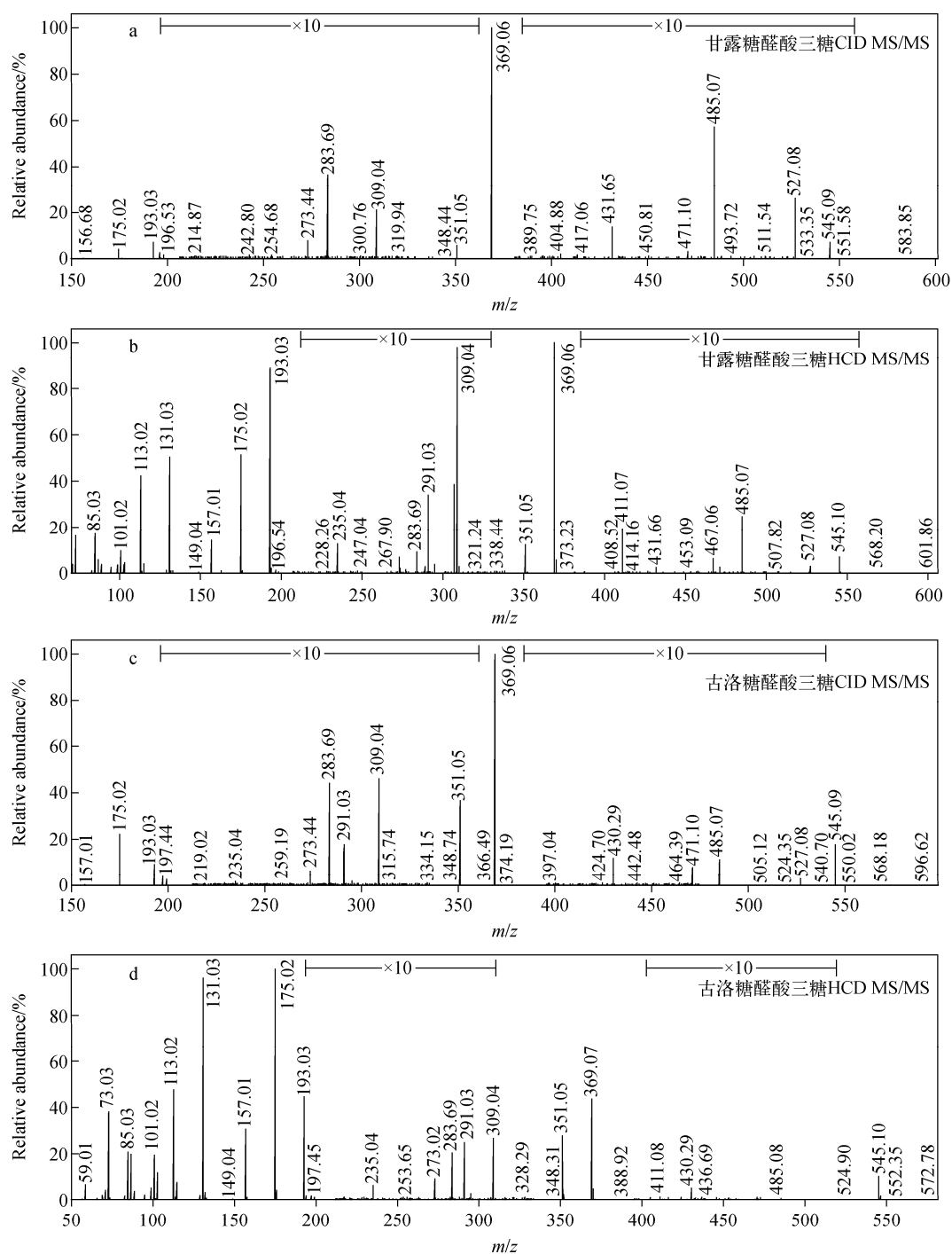


图2 甘露糖醛酸三糖和古洛糖醛酸三糖的CID(a, c)和HCD(b, d)谱图

Fig. 2 CID (a, c) and HCD (b, d) MS/MS spectrum of trimannuronate and triguluronate

CID谱图中,因1/3效应缺失了 m/z 175/193。但是,在CID谱图中可见跨环断裂产生的碎片离子 $^{0,2}A_5$ (m/z 837)、 $^{0,4}A_5$ (m/z 823)和 $^{2,5}A_5$ (m/z 819),而在HCD谱图中未见这3个特征峰。与三糖相同,甘露糖醛酸区别于古洛糖醛酸残基的Z离子脱羧碎片 $Z_{int}-CO_2$

(m/z 307和 m/z 483)仅出现在HCD谱图中。还可以看到,HCD在低质荷比端提供了大量的碎片离子,弥补了CID的1/3效应,HCD谱比CID谱在低质荷比区域的信号和峰度高,CID谱则反之,这是由于HCD高能裂解的特点。

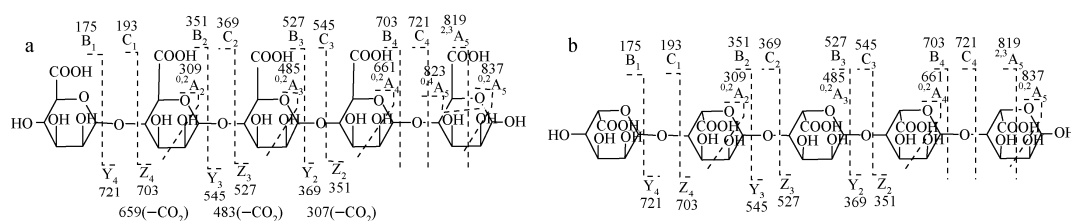


图3 甘露糖醛酸五糖(a)和古洛糖醛酸五糖(b)的质谱解析

Fig. 3 MS/MS analysis of pentamannuronate (a) and pentagulonate (b)

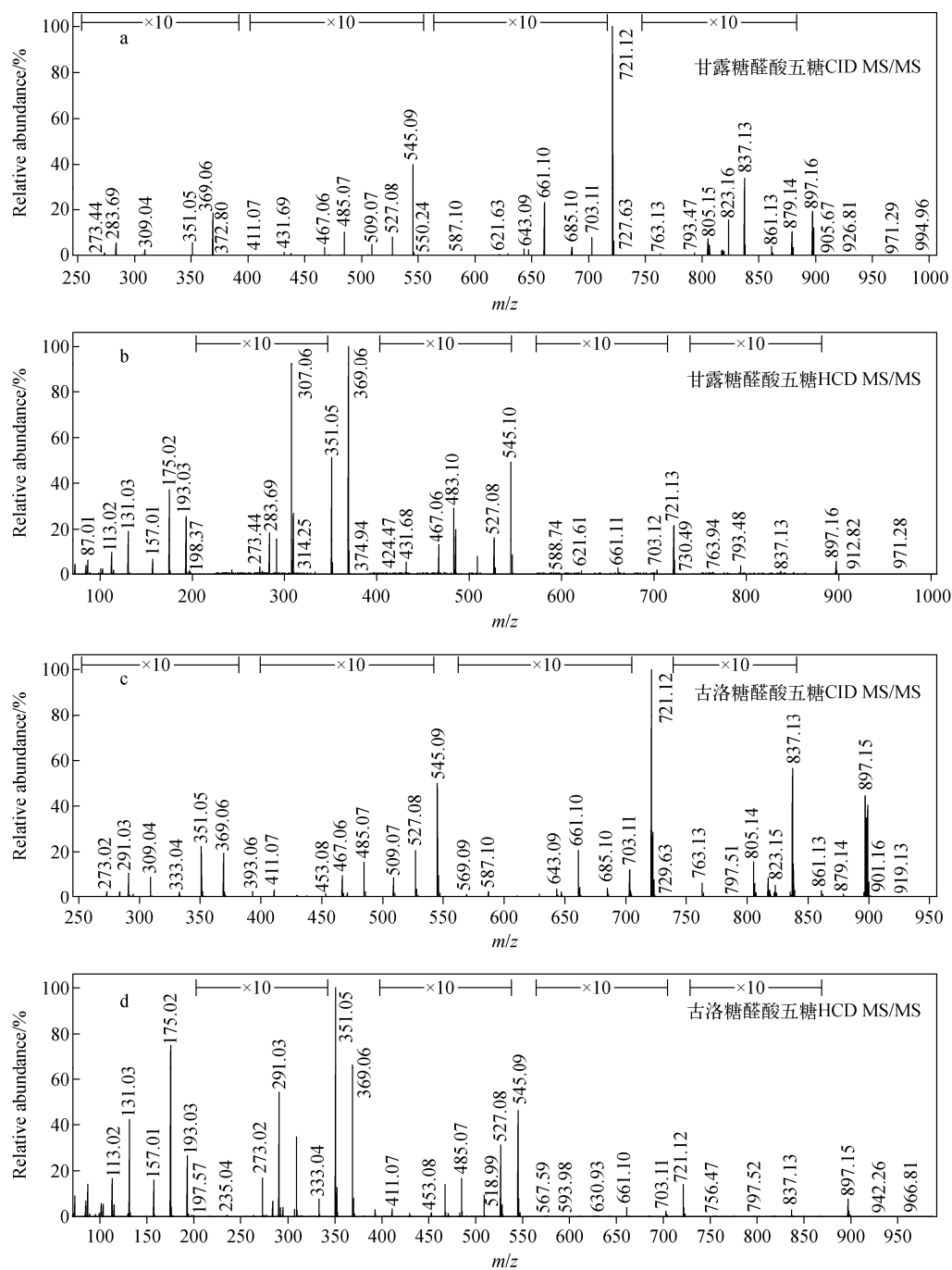


图4 甘露糖醛酸五糖和古洛糖醛酸五糖的CID(a,c)谱和HCD(b,d)谱

Fig. 4 CID (a, c) and HCD (b, d) MS/MS spectrum of pentamannuronate and pentagulonate

比较甘露糖醛酸五糖和古洛糖醛酸五糖的 CID 以及 HCD 差异,发现与三糖的解析结果有相同的规律,M5 和 G5 的 CID 谱图没有明显差异,M5 的 HCD 谱图较 G5 仅多了 $Z_{\text{int}}\text{-CO}_2$ (m/z 307 和 m/z 483),进一步验证了 $Z_{\text{int}}\text{-CO}_2$ 碎片离子是

区别甘露糖醛酸寡糖与古洛糖醛酸寡糖的重要标志。通过比较发现,需要将 CID 和 HCD 结合起来才能得到五糖完整的解析信息。聚合度为 2~5 的两种类型寡糖的 CID MS/MS 和 HCD MS/MS 详细二级碎片信息列于表 1。

表 1 8 种寡糖在 CID 和 HCD 方式下的质谱碎片峰比较
Table 1 Comparison of CID and HCD for 8 oligosaccharides

化合物 Compound	$[M-H]^-$	CID	HCD
甘露糖醛酸二糖	m/z 369	113.02(13%),131.03(23%), 157.01(5%),175.02(100%), 193.03(89%),309.04(31%), 369.06(35%)	73.03(28%),85.03(49%),89.02(10%),95.01(7%), 101.02(13%),103.00(5%),113.03(83%),131.03(37%), 157.01(8%),175.02(21%),193.03(100%),235.04(9%), 309.04(5%),369.06(10%)
古洛糖醛酸二糖	m/z 369	113.02(13%),131.03(20%), 157.01(6%),175.02(100%), 193.03(82%),309.04(20%), 351.05(50%),369.06(31%)	73.03(21%),85.03(39%),89.02(11%),95.01(5%),99.0 (8%),101.02(26%),103.0(9%),113.02(83%),131.03 (100%),157.01(25%),175.02(79%),193.03(99%), 235.04(6%),309.04(4%),369.07(23%)
甘露糖醛酸三糖	m/z 545	175.02(3%),193.03(7%), 309.04(3%),351.05(6%), 369.06(100%),485.07(5%), 545.09(6%)	73.03(17%),85.03(18%),87.01(6%),101.02(10%), 103.00(5%),113.02(43%),131.03(52%),157.01(14%), 175.02(52%),193.03(92%),307.06(4%),309.04(10%), 351.05(12%),369.06(100%),411.07(1%),467.07(1%), 485.07(2%),545.10(8%)
古洛糖醛酸三糖	m/z 545	175.02(23%),193.03(9%), 291.03(2%),309.04(5%), 351.05(39%),369.06(100%), 485.07(12%),527.08(2%), 545.09(78%)	73.03(38%),85.03(38%),87.01(20%),99.01(5%), 101.02(19%),103.04(11%),113.02(48%),115.00(7%), 131.03(99%),157.01(30%),175.02(100%),193.03 (45%),273.02(2%),291.03(2%),309.04(2%),351.05 (27%),369.07(44%),545.09(10%)
甘露糖醛酸四糖	m/z 721	309.04(1%),351.05(10%), 369.06(50%),485.07(3%), 527.09(10%),545.10(100%), 661.10(6%),703.12(4%), 721.12(4%)	73.03(12%),85.03(13%),87.01(10%),89.02(3%), 101.02(7%),103.04(4%),113.02(30%),115.00(4%), 131.03(40%),157.01(12%),175.02(50%),193.03 (65%),307.06(8%),309.04(7%),351.05(24%),369.06 (100%),411.07(1%),467.06(1%),485.07(3%),527.08 (4%),545.09(17%),721.12(3%)
古洛糖醛酸四糖	m/z 721	291.03(1%),309.04(1%), 351.05(19%),369.06(24%), 485.08(2%),527.09(10%), 545.10(62%),721.13(21%)	73.03(8%),85.03(6%),87.01(10%),101.02(5%), 103.04(5%),113.02(14%),131.03(41%),157.01(13%), 175.02(78%),193.03(26%),333.04(6%),351.05 (100%),369.06(97%),527.0(16%),545.09(87%), 721.13(35%)
甘露糖醛酸五糖	m/z 897	351.05(6%),369.06(18%), 485.07(1%),527.08(8%), 545.09(41%),703.11(8%), 721.12(100%),823.16(2%), 837.13(3%),879.14(11%), 897.15(16%)	73.02(4%),85.03(4%),87.01(6%),113.02(10%), 131.03(19%),157.01(7%),175.02(37%),193.03(26%), 307.06(9%),309.04(3%),351.05(51%),369.06(100%), 483.10(3%),527.08(17%),545.10(40%),721.13(22%), 897.16(5%)
古洛糖醛酸五糖	m/z 897	351.05(4%),369.06(3%), 485.07(3%),527.08(6%), 545.09(43%),703.11(2%), 721.12(100%),837.13(8%), 897.15(35%)	73.03(6%),87.01(9%),113.02(11%),131.03(31%), 157.01(10%),175.02(65%),193.03(16%),333.04(5%), 351.05(100%),369.06(64%),527.08(40%),545.10 (53%),721.13(17%),897.16(17%)

2.3 高聚合度甘露糖醛酸及古洛糖醛酸的二级质谱解析

对于聚合度高于 5 的寡糖,二级质谱采集选择双电荷或三电荷离子作为母离子。本研究以七糖为例进行解析,甘露糖醛酸七糖和古洛糖醛酸七糖的 CID 和 HCD 谱图示于图 5。M7 选择 m/z 624 双电荷离子作为母离子,从图中

可以看出,HCD 谱给出的信息远多于 CID 谱,在糖苷键断裂的碎片中有丰富的糖环断裂碎片,尤其是 $Z_{int}-CO_2$ 离子(m/z 307、483、659、835、1 011)的信号在 M7 的 HCD 谱中很明显,但是在 G7 的 HCD 谱中未见,因此可以很容易地区分这两种寡糖。对于高聚合度的寡糖,HCD 的优势明显高于 CID。

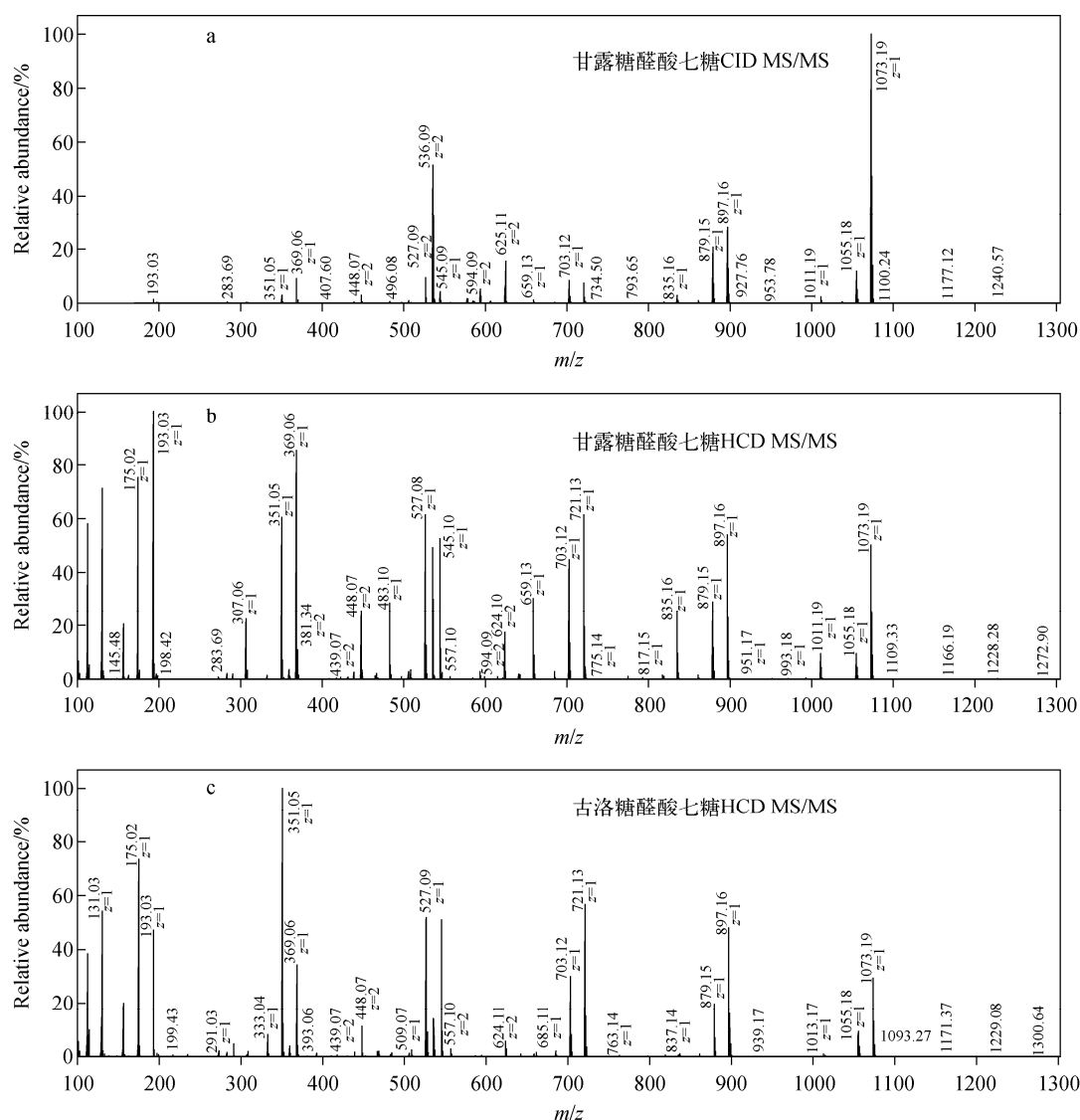


图 5 甘露糖醛酸七糖和古洛糖醛酸七糖的 CID 谱和 HCD 谱

Fig. 5 CID and HCD MS/MS of heptamannuronate and heptagulonate

3 结论

通过分析褐藻胶寡糖的 HCD 和 CID 二级质谱数据发现:对于聚合度较低的寡糖,HCD 较 CID 能够给出更多的糖环断裂碎片和低质

荷比碎片等信息,这是寡糖结构解析的关键;对于聚合度为 4 和 5 的寡糖,在高质荷比区域的 CID 信息较 HCD 信息更为丰富,因此需要综合两种裂解模式提供的信息进行解析;对于聚

合度高于 5 的寡糖, HCD 提供的信息远远多于 CID 模式。从数据分析发现, HCD 通过检测 $Z_{\text{int}}\text{-CO}_2$ 离子可以清晰地判断寡糖类型。对于褐藻胶寡糖的二级质谱分析, 将 HCD 高能裂解的特点应用到寡糖结构解析, 可以提供更多的糖环断裂碎片与高质量精度和高灵敏度的二级质谱数据, 并且避免了 CID 技术中低质荷比数据缺失, 有助于褐藻胶寡糖的结构解析。

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