

## 腐殖物质特异性及其产生机制\*

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**摘要** 腐殖物质(HS)是天然有机质的主体,其组成、结构十分复杂。之所以称其为一类物质,就是基于他们具有不同于其形成前体的化学组成与结构特征的共性,或称“特异性”,即在腐殖化过程中形成新的化学组合,甚至是“特异的”结合方式。但经历近200年的探索,人们对这种“特异性”的认识仍然不清楚,并缺少实验上的证据。加之近几年,一些研究结果被不适当的推演,HS的“特异性”更加受到质疑。HS到底有无“特异性”?“特异性”是什么?这涉及HS生物化学的根基,必须进行深入研究和回答。本文对已有的一些研究报道,包括HS的特异性、生物分子单体与HS结构特征的比较、基于生物多样性(进化)的天然生物材料与HS形成、HS形成机理及胡敏酸(HA)、富里酸(FA)形成顺序、矿物黏粒催化与HS形成、异核单量子相干谱(HSQC)和总相关谱(TOCSY)、傅里叶变换离子回旋共振质谱(FT-ICR-MS)和C1s软X射线近边吸收精细结构(NEXAFS)同步辐射技术应用等进行综合评述,并提出HS化学进一步研究的方向。

**关键词** 腐殖物质; 结构特征; 特异性; 模拟腐殖化; 形成机制

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腐殖物质(Humic substances, HS)既是天然有机质(natural organic matter, NOM)的主体,也是土壤有机质(soil organic matter, SOM)的主体。传统上分为胡敏酸(Humic acid, HA)、富里酸(Fulvic acid, FA)和胡敏素(Humin, Hu)三个组分<sup>[1]</sup>。HS既是释放CO<sub>2</sub>的重要来源,又是截获CO<sub>2</sub>的重要载体,在全球碳循环中具有重要作用,对土壤肥力和环境保护具有重要功能<sup>[2]</sup>。

自从1830年Berzelius对HS进行分类以来,已有近200年的研究历史,人们对HS的形成、本性、作用和应用进行了广泛的研究,发表了大量的研究资料和论著。HS的最大特点是其本身的“不均一性”,就像同一种树的树叶一样,一方面各个树叶有相似的特征;另一方面每个树叶在细节上又都不完全相同<sup>[3]</sup>。这种不均一性实质上反映了HS形成环境、途径和稳定条件的复杂性,但是这种不均一性同时也使HS组成、结构变得极为复杂,研究难

度极大。

但无论HS多么复杂,它毕竟是一类天然存在的、可以提取出来的、有“共性”的化学物质(如碱溶性、暗色),之所以称其为一类物质,就是基于它具有不同于其形成前体的化学组成与结构特征。即在腐殖化过程中,单纯来源于动物、植物、微生物的前体材料发生了新的生物化学反应(称为二次反应,secondary reaction),形成了特殊的化学组合比例、特殊化学结合方式,甚至是形成了某种有机化学结构未知的化合物,这就是HS的特异性。人们一直想找到(证明)这种“特异性”,但受技术手段限制和HS本身“不均一性”的影响,至今仍未能实现。

最近, Kelleher和Simpson应用二维核磁共振技术——异核单量子相干谱(HSQC)研究了常见的四大类生物分子(蛋白质、多糖、脂质、木质素)及其混合物的结构特征,并与国际腐殖物质学会

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(IHSS)泥炭HA (IHSS-PT-HA)进行对比,结果大部分信号重合,因此得出HS没有特殊的化学结合(特异性)的结论<sup>[4]</sup>。这一研究结果被不适当的推演,似乎有否定HS“特异性”的趋势<sup>[5]</sup>。这涉及到HS化学的根基,必须进行深入研究和回答。我们认为,第一,HSQC技术在检测大型化合物、混合物的基团方面,如信号有差异则可以肯定来源样品成分的差异性;但反之却不行,即如果信号没有差异,只能说明碳基团化学环境相同,并不一定说明整个大型分子或混合物的结构相同。第二,信号并未完全重合,还是说明PT-HA与一般生物分子的化学结构是不同的。第三,HS分布广泛,泥炭来源的HA与土壤来源的HA可能会有区别,并不能代表整个HS。此外,我们采用液体纯培养法,研究不同微生物利用不同基质(葡萄糖、纤维素、木质素、秸秆、锯末)形成类腐殖物质(HLS)的情况表明:这样形成的类HA(HLA)的摩尔H/C均大于1.5,与土壤HA(1.0)相差很大。这说明HS前体材料或简单培养产物并不具有真正HS的特性。因此,目前否定HS“特异性”的依据尚不充分。

## 1 腐殖物质(HS)的特异性

HS是经腐殖化作用而形成的、具有特异性的、多相分布的类高分子化合物<sup>[6]</sup>。“特异性”的涵义原本在于标准物与对比物之间的明显区别,之所以用于HS的描述,我们认为:微生物参与下的腐殖化作用是使原本结构明确的四大类生物分子单体(蛋白质、多糖、脂质和木质素)经缩合,转化为功能相似但结构各异的HS,所形成的HS并不是前体生物分子单体间的简单加和,而是形成了具有独特结构属性的系列高分子化合物或混合物,因此有关其结构特异性的研究备受关注。

尽管HS的形成过程已经争议了数十年,但都不否认可萃取的HS的结构是通过不同的生物转化过程形成的。腐殖物质的特异性形成于特定环境,具有一些特定的生物标识物(包括由各种表皮和木质素衍生的结构),以及存在除已知的化学成分性质外的额外特征,自身相互交联形成新的大分子结构。多年来,已经提出了大量的HA模型。由Schulten和Schnitzer提出的模型可能是最广为接受的<sup>[4]</sup>。在Schulten所描述的模型中,HA是由长链

脂族结构链接多个芳香环而形成,在芳香环和脂族侧链上还存在许多由羟基和羧基所构成的活性官能团组<sup>[7-8]</sup>。

作为HS的重要组成部分之一,HA在水溶液中有与有机聚合大分子相似的典型结构和流体特征,较低pH和高浓度电解质可使之呈无规则的卷曲状,而相反条件则使之呈现有高弹性的线状结构<sup>[9]</sup>。在此基础上,Piccolo等<sup>[10-14]</sup>又提出了新的假设,即作为超级大分子的HA是由具有异质功能的小分子通过较弱疏水作用和氢键链接在一起的。

Hur和Schlautman<sup>[14]</sup>指出,不同来源的HS之间存在颜色、光谱特征及化学组成上的差异。同一来源、不同分子量片段的HS组分间也存在着结构上的异质性。Christl等<sup>[15]</sup>采取中空的纤维超滤法获取了4个不同粒级的HA并对其化学不均一性进行了细致表征。研究表明,不同分子量的HA组分有着不同的化学组成,这对于其环境行为的调控有着重要的影响。Li等<sup>[16]</sup>采用元素分析、Py-GC-MS、FTIR、UV-vis以及<sup>13</sup>C-NMR技术对泥炭HA依照分子大小所分离的8个次级样品进行化学和分子异质性的表征,结果指出,即使生物地球化学条件相同、不同来源的有机物料也能促使所形成的HA有着化学上、结构上和分子上的差异。

## 2 环境与HS的特异性

不同环境条件所形成的HS之间同样存在着结构上的差异<sup>[17]</sup>。与陆地环境相比,海洋环境所形成的HS,其分子中含有更多支链脂肪族结构和相对少量的芳香族结构<sup>[18]</sup>。不同地理和气候区对HA的结构性质亦会产生影响<sup>[19]</sup>。此外,在堆肥过程<sup>[20]</sup>及植物凋落物腐解<sup>[21]</sup>的不同阶段,HS组分间也在不断转化,作为研究整体,HS组分间的异质组合致使其具有“特异性”是值得肯定的。但这方面的实际严格对比研究很少。高氧分压有利于HA稳定,而高水活度、酸度和二氧化碳分压有利于FA稳定<sup>[3]</sup>。

## 3 四类生物分子单体与HS结构特征

为充分探讨HS的化学结构特征,我们必须检验形成HS的原料即植物残体与微生物体的化合物组成。木质素、糖类、脂质和蛋白质正是构成植物



和微生物体的四种具代表性且最重要的大分子物质，同时它们也是自然界存在的具有广谱化学结构和生物功能的有机化合物，是植物和微生物体内能量和物质积累的重要代表物，也是各类生命活动的主要代谢物。木质素、多糖和脂族化合物（包括膜脂和植物角质层）是构成细胞壁及细胞膜的结构材料，而蛋白质是细胞内重要的贮藏物质<sup>[22]</sup>。这些生物聚合物很有可能以特殊形式体现在于HS中，使HS结构研究非常困难，而非传统意义上所说的“仅仅与HS伴随”存在。

**木质素：**对HS的形成影响颇大，多数学者认为陆地上HS是由木质素转化而来的，且土壤中真菌在这个过程中发挥一定的作用<sup>[23-25]</sup>。但检测手段显示HS和木质素的分子结构相似程度并不高，HS的结构更为复杂<sup>[26-27]</sup>。

**糖类：**以纤维素为原料形成HS的研究相对较少，且形成机理也不清楚<sup>[28-29]</sup>。一般而言，糖类的生物可降解性较好，在植物残体中最先被微生物同化，那些未被降解的部分可能直接进入HS结构中，成为HS的一部分<sup>[30-31]</sup>。此外有学者研究不同来源HS的水解产物，发现获得的糖类物质种类大同小异，说明大多HS中均含有糖类<sup>[32]</sup>。

**脂类：**是脂肪酸和醇所形成的酯及其衍生物，包括磷脂、角质、蜡质等。放线菌可利用甘油合成类腐殖酸物质<sup>[33]</sup>。大量研究表明，土壤和其他来源的腐殖酸中均含有脂类成分，通过化学热解可将脂类组分从腐殖酸中脱离出来进行检测<sup>[34-36]</sup>。但人们对脂类组分在HS中的比重大小观点不一。有学者认为，脂类并非HS分子的主要组分，它可能是以非共价键或者氢键等较弱的力与主体结构相连接的，对HS的化学和光谱学结构分析造成干扰<sup>[37-38]</sup>。但另有学者在研究污泥来源的腐殖酸中，二元羧酸和脂肪酸较芳香环类有机物含量丰富<sup>[39]</sup>。

**蛋白质：**氨基酸分子可以作为原料，在微生物作用下合成类HS<sup>[40]</sup>。但新的研究发现，蛋白质或多肽可能通过共价交联的方式进入HS结构中，使土壤中氮素得以保存，也使HS结构更为复杂<sup>[41-44]</sup>。

四类大分子有机物均能以原料形式参与形成HS或类HS，它们或在微生物作用下相互转化或以分子形式直接进入HS结构中，如此复杂的形成方式可能正是HS特殊结构产生的原因<sup>[45-46]</sup>。

## 4 基于生物多样性（进化）的天然生物材料与HS形成

我们希望能通过来源广泛且典型的原料找出其形成的HS的特殊共性。而在漫长的地球生物化学进化史上，在土壤形成前后，地球上经历了不同主导性微生物和主导性植被。这里结合了物种与时间的概念，各种各样的天然生物作为原料或多或少参与了HS的合成与土壤肥力的进化。由此我们联想到了生物进化树<sup>[47]</sup>，期望从中找出典型且包含历史进化意义的生物材料。

**蓝细菌：**从土壤样品中分离得到6种蓝细菌，进行扩培后将细胞破碎并经土壤微生物降解作用，发现有大量藻类组分中的碳进入腐殖酸中<sup>[48]</sup>，这说明蓝细菌残体是HS形成的一类原材料。从大分子组分来看，蓝细菌的细胞及胞外、胞内代谢物中均含有大量蛋白质、多糖，有的甚至含类腐殖酸物质<sup>[49-52]</sup>。

**真菌：**可产生多种酶类物质，如漆酶、酪氨酸酶等，通过降解木质素、纤维素，转化酚及醌类物质，参与土壤有机质的循环和HS的合成<sup>[53-55]</sup>。真菌中的担子菌在其中发挥作用最大、研究最多<sup>[56-60]</sup>。双孢蘑菇（*Agaricus bisporus*）是自然界分布广泛且可人工种植的一类大型担子菌真菌，近来其基因组序列被破解，从中发现一系列多糖和木质素降解基因<sup>[61-62]</sup>，为其可在木质原料上生长繁殖的行为提供了强有力的理论解释。此外，Wu等<sup>[63]</sup>和Weijn等<sup>[64]</sup>研究表明，有26对基因通过转录多酚氧化酶参与双孢菇表皮黑色素的形成；而真菌黑色素与HS之间有诸多相似之处<sup>[54, 65-66]</sup>。

**真核藻类：**藻类被认为是现今陆地植物的先祖，它们能够依靠光合作用自养而广泛存在于海洋中。海洋悬浮物及海底沉积物中的腐殖类物质极有可能是它们的残骸转化来的<sup>[67-69]</sup>。在历史变迁中，这些沉积物经由海洋转移到陆地，为土壤的形成与增肥提供物质基础。从结构上看，海洋与陆地起源的腐殖物质有某些共同特征<sup>[70]</sup>；从物质组成上看，这种可能性也是存在的。藻类的细胞壁含肽聚糖而没有纤维素，并且藻类缺乏维管组织，几乎不含木质素；但它们的分泌液中均存在类蛋白化合物及类腐殖物质<sup>[52, 71-72]</sup>。

地衣：是子囊真菌与蓝细菌的复合共生体，一直以来均被认为是土壤形成的开路先锋，特别是荒漠地区，对原始HS的形成起重要作用<sup>[73-74]</sup>。近期发现，地衣可分泌漆酶、纤维素酶等多种酶类，对木质纤维素及HS的转化产生直接或间接影响<sup>[75-78]</sup>。更有学者认为，地衣所形成的HS并非酶类直接催化降解植物残体而成，而是在矿物及酶的催化作用下由低分子前体物质如酚类缩合而成<sup>[79-81]</sup>，这与地衣所生长的环境极为契合。苔藓因没有维管组织而被界定为低等植物，生物学家认为它是水生藻类登陆演化的先驱<sup>[82]</sup>。苔藓多生长于沼泽，其残体因特殊的多酚结构难以被微生物降解，经腐殖化过程可转化为泥炭<sup>[83]</sup>。

蕨类植物：是进化水平最高的孢子植物，与种子植物一起总称为维管植物，体内几乎均含有木质素<sup>[84]</sup>。在研究白垩纪早期蕨类植物的化石时发现，其中的酚类组分如木质素、蜡质等发生了重新排列，通过芳构化和交联反应进入干酪根结构中<sup>[85]</sup>。蕨类植物含有大量脂族叶蜡、木质素和单宁，与此类物质含量同样高的被子植物一起对土壤有机质的形成与转化起着至关重要的作用<sup>[86]</sup>。在对16种不同植被覆盖土壤中的腐殖酸进行热解并检测其释放的木质素组分时发现，裸子植物土壤腐殖酸中缺乏紫丁香醇，而被子植物土壤腐殖酸中均可检出愈创木酚<sup>[87]</sup>，可见不同植被的木质素结构对其形成的HS结构有影响<sup>[88]</sup>。植物体内木质素结构差异的背后是植物种类本身的高度多样性，它带来的营养元素平衡及土壤微生物和动物的丰富度对HS结构形式的影响是最重要的<sup>[89-90]</sup>。另有学者从植物进化方面分析土壤中HS的形态，认为：相对粗腐殖质和中等腐殖质而言，细腐殖质极有可能是在开花植物出现之后才形成的<sup>[91-92]</sup>。这些研究与设想对我们从生物多样性的进化角度考虑HS结构特征的想法产生了很多启发与激励作用。

## 5 HS形成机理及HA、FA形成顺序

对HS生成机理认识的差异，也影响到对HA、FA形成顺序的推断，按“木质素理论”是先形成HA，然后由HA裂解成FA；按“多酚理论”则是先形成FA，再由FA聚合成HA（尽管也存在着直接形

成HA的可能性）；也可能不同环境条件有不同的形成顺序。研究表明，玉米秸秆分解期间，无论是土壤还是灼烧土，HA/FA比值均在开始时最低；葡萄糖分解期间，第24小时才出现HA。说明最初FA的形成速度大于HA，即FA形成的时间较HA早，符合“多酚理论”<sup>[93]</sup>。关于Hu相对于HA、FA形成的时间顺序，资料很少，按木质素理论推测，有可能先形成Hu；但也有不同的观点，Hu的芳香度和坚硬度较HA高，说明其更少地来自于新的植物残体，这比较符合多酚理论的推测<sup>[1]</sup>。这样看来，HA可能是居于中间阶段。关于微生物纯培养+黏粒条件下HA、FA形成顺序，国内外均未见报道。

## 6 矿物黏粒催化与HS形成

由于土壤系统太复杂，用微生物纯培养方法来研究HS的形成是十分必要的。存在的问题是，由于未加黏粒矿物催化，形成的类腐殖物质与土壤HA相差很大，因此既肯定微生物纯培养的优点，又考虑引入商品黏粒矿物来模拟土壤环境，是下一步研究的方向之一。Filip等<sup>[94-95]</sup>研究了微生物（*Epicoccum nigrum*和*Stachybotrys chartarum*）参与下，蒙脱石、高岭石以及石英粉末对腐殖酸类聚合物形成的影响，并指出，黏土矿物可缩短暗色物质形成的时间，且可增加碱提取腐殖酸类聚合物的数量。黏土矿物的催化作用主要通过间接影响酚类物质的形成而实现，但所形成的HS结构未见差异。Fukuchi等<sup>[96]</sup>研究了邻苯二酚、甘氨酸与葡萄糖在天然沸石催化作用下的缩聚反应，表明沸石有利于类HA中氮含量及分子量的提高，对醌、酮等羰基碳含量亦有提升作用。Duarte等<sup>[97]</sup>研究发现，矿物表面对土壤HA分子中非晶形亚甲基结构数量的提升具有显著抑制作用，即提高了HA的芳香度。自然界中，土壤矿物通过氧化偶联反应催化酚向腐殖物质聚合物转化，采用三元体系法，通过长绒毛栓菌所分泌的虫漆酶、水钠锰矿（ $\delta$ - $MnO_2$ ）以及邻苯二酚之间的相互作用来模拟土壤过程，表明酶-土壤矿物-有机质间的相互作用对腐殖质形成过程具有重要意义<sup>[98]</sup>。此外，Colombo等<sup>[99]</sup>和Fang等<sup>[100]</sup>研究了铁与HA的相互作用，也受到重视。

## 7 HSQC、TOCSY、FT-ICR-MS和同步辐射等技术的应用

由于腐殖物质的复杂性，其特异性的探索必须使用现代结构分析技术<sup>[4, 101-107]</sup>。

二维NMR：异核单量子相干谱（HSQC）和总相关谱（TOCSY）较一维NMR提供了更多的信息。因为涉及到<sup>1</sup>H-<sup>13</sup>C，一般选用氘代试剂作溶剂，常用DMSO-d<sub>6</sub>，只要确保样品纯化好且处于氢型，一般HA样品可全部溶解<sup>[4]</sup>。

离子回旋共振质谱：随着各种离子化技术的出现，傅里叶变换离子回旋共振质谱（FT-ICR MS）用于研究胡敏酸（HA）大分子已经成为可能，HA中含有较多的羧基、羟基等极性官能团，通过电喷雾电离源（ESI）技术，在负离子模式下，可形成去质子的带一个负电荷的准分子离子峰<sup>[101]</sup>。

同步辐射技术：C1s软X射线近边吸收精细结构（NEXAFS）是基于同步辐射谱学的元素形态表征方法，具有非破坏性、不受样品中水分和其他元素干扰的优点<sup>[102]</sup>，是目前原位测定土壤有机碳官能团组成最有效的方法之一。一般将待测样品粉末（过0.125mm筛）固定于镀金的样品托上即可测定<sup>[103]</sup>。

## 8 结论与展望

HS从形成到结构组成是十分复杂的，但它毕竟是一类天然存在的物质，在其形成过程中可能产生了特殊的化学组合比例、特殊的化学结合方式，甚至是形成了某种特殊的化合物，这就是HS的特异性所在。中国土壤学家应该有责任、有兴趣对其有无“特异性”，“特异性”是什么，“特异性”是如何产生的等科学问题给出确定的答案，这将有助于解释HS的稳定性和土壤固碳机制。

针对上述问题，未来可能的研究思路是：分两条路线研究HS的特异性。第一，选取有代表性的不同来源的HS，与公认的HS前体生物分子单体和基于生物多样性（进化）的天然生物材料进行对比，根据有无差异，便可以回答HS有无“特异性”、“特异性”是什么的科学问题。第二，选择普遍性与可控性兼顾的培养条件，对天然生物材料作为HS前体进行微生物接种培养，用同样的检测方法比较培养前后及过程中前体材料与生成物之间

结构特征的差异性及变化规律，来回答HS特异性是否由腐殖化作用产生。这样的研究思路，无论研究结果如何，都至少可以将“HS有无特异性和特异性是什么”这一科学之谜提高到更接近可知的境地；无论在哪一点上取得突破，均会为HS生物化学（HS的形成、本性、化学组成与结构特征、稳定性机制及其与环境相互关系）提供基础资料；为如何通过环境条件预设来调控HS组成，近而为调控土壤肥力和固碳提供新的理论依据；均会对HS研究及逐步解决土壤学、生物地球化学、农学及环境科学中与HS有关的科学问题起到推动作用。

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## The Structural Distinctiveness of Humic Substances and Its Formation Mechanism in Simulated Incubation

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**Abstract** Humic substances (HS) is the main part of natural organic matter (NOM), which have very complicated composition and structure, the reason called a kind of substances, is based on the common structural characteristics or the “distinctiveness” of their structure, formed in the humification process. But after nearly 200 years of exploration, the “distinctiveness” is still not clear. Faced with this dilemma, in recent years, some research results are deduced unreasonably, and there seems to question “distinctiveness” of HS. This relates to the foundation of HS chemistry and must be extensively studied and answered. In this paper, the existing researches, including the structural “distinctiveness” of humic substances, comparison of biological molecules monomer to the feature of the HS structure, HS formation with natural biological material based on biological diversity (evolution), formation mechanism of HS and formation sequence of humic acid (HA) and fulvic acid (FA), mineral clay catalyst for HS formation, modern techniques application such as heteronuclear single quantum coherence (HSQC), total correlation spectroscopy (TOCSY), Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) and synchrotron radiation soft near-edge X-ray adsorption fine-structure spectroscopy (NEXAFS) are waiting for carrying on review, and the direction for further HS research are put forward.

The “distinctiveness” of HS will be studied in future in two ways: (1) Representative HS which come from different sources is compared with the recognized precursor monomers of biological molecules and bio-based (evolution) diversity of natural biomaterials. According to the differences, we can answer the question of the HS presence of “distinctiveness” and what is the “distinctiveness”. (2) Choosing universal and controllable incubation conditions, the natural biological material is incubated by microbial inoculated incubation as the HS precursors. The differences of structural characteristics before and after incubation between products and precursors, the changes in the incubation process will be investigated by the same test method, to reply the HS “distinctiveness” is whether produced by the humification or not.

The research idea should be that: No matter what the research results were, at least we could make the scientific mystery about whether the HS holding the “distinctiveness” or not and what was the “distinctiveness” seeming closer to the level of known. No matter which aspect was made breakthrough, it could provide the basic data for the HS biochemistry such as the formation, nature, chemical composition and structural characteristics, stability mechanism of HS and its relationship with the environment; It also could solve how to adjust the composition of HS through pre-setting the environmental conditions, which could



provide the new theoretical basis for the regulation of soil fertility and carbon sequestration. In a word, based on the mention above, It could play a promoting role in the HS research and solve the scientific problems gradually associating with the HS from the different fields, such as the soil science, biogeochemistry, agronomy and environmental science.

**Key words** Humic substances; Structural characteristics; Distinctiveness; Simulated humification; Formation mechanism

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