

·研究简报·

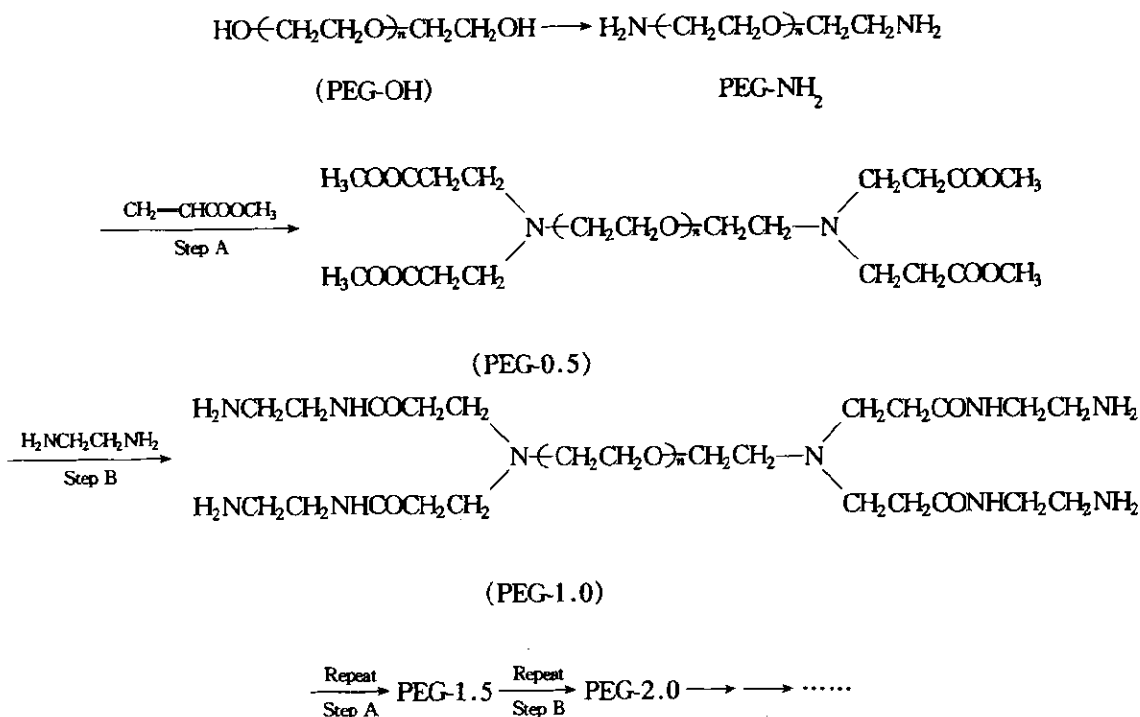
大核树枝状大分子的合成及其凝血、溶血性能研究*

石绍华** 余万桂 张道明 刘立建 吕正荣 卓仁禧
(湖北职工医学院 荆州 434000) (武汉大学化学学院 武汉 430072)

关键词 聚乙二醇, 大核树枝状大分子, 凝血时间, 溶血浓度

从80年代中期开始, Tomalia、Newkome、Frechet 等对树枝状大分子开展了卓有成效的研究, 合成了多种结构的树枝状大分子^[1], 并对它们的应用进行了积极的探讨. 在数枝状大分子的合成方面, 寻找新的多官能团引发核就是一个研究热点, 例如采用大分子核, Frechet 等采用聚乙二醇作为核用收敛法合成了聚芳醚和聚芳酯树枝状大分子^[2,3], 为嵌段共聚增添了新的内容. 而 Tomalia 等以氨和乙二胺等小分子为核合成的聚酰胺-胺类树枝状大分子呈粘稠状^[4], 取样、称量

等操作很麻烦, 其应用也受到限制. 聚乙二醇无毒、无抗原性, 生物相容性好^[5]; 而且有不同分子量的聚乙二醇可供挑选. 因而聚乙二醇是一个优良的药物载体. 但聚乙二醇的端基数有限, 限制了其载药量. 为了得到非粘性的端基数较多的树枝状大分子, 同时利用聚乙二醇的良好生物相容性, 我们采用聚乙二醇作为大分子引发核用 Tomalia 的发散法^[4]合成了一类聚酰胺-胺树枝状大分子, 并对其凝血、溶血性进行了研究. 以聚乙二醇为核的树枝状大分子合成路线如下:



1 仪器与试剂

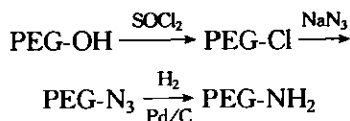
Nicolet 170SX 型 FT-IR 光谱仪, Carlo Erba 1106 元素分析仪.

聚乙二醇, $M_w = 2000$, 市售分析纯试剂; 二氯亚砷、丙烯酸甲酯, 市售化学纯试剂, 用前重蒸; 乙二胺, 市售分析纯试剂, 用前精馏, 收集 116℃

馏分;叠氮酸钠,市售化学纯试剂,用前未经处理;肝素钠,生化试剂;其它试剂均为分析纯,甲醇、正丁醇、吡啶经无水处理。

2 ω, ω' -聚乙二醇二胺的制备^[5]

制备过程的反应式如下:



取 20g PEG 溶于 150mL 甲苯,经共沸蒸馏除水后加入 2mL 无水吡啶,在 30min 内回流滴加 5mL 新蒸 SOCl_2 ,继续回流 4h,冷却至室温,过滤除去白色吡啶盐酸盐沉淀。滤液在旋转蒸发仪上蒸去甲苯,残留物溶于 CH_2Cl_2 后用无水 K_2CO_3 除水,再用 50g 在 120°C 下活化过 2h 的 Al_2O_3 脱硫,过滤。用无水乙醚重沉淀,得到 17.8g PEG-Cl。取 16g PEG-Cl 溶于 100mL DMF,加入 1.04g NaN_3 , 120°C 下搅拌 2h 冷却,抽滤,滤液蒸除 DMF,残留物用 CH_2Cl_2 -无水乙醚重沉淀,得到 15.1g PEG- N_3 。将 14g PEG- N_3 溶于 200mL 无水乙醇,加入 1g 10% Pd/C,通入 H_2 ,反应 12h 后滤除 Pd/C,滤液用无水乙醚重沉淀,得到 PEG- NH_2 12.6g。

3 聚乙二醇为核的树枝状大分子的制备^[4]

3.1 Micheal 加成反应(Step A) 12g 氨基聚乙二醇(PEG- NH_2)溶于 100mL 无水甲醇,加入 4.0mL 丙烯酸甲酯,室温避光搅拌 48h,减压蒸馏除去甲醇和过量的丙烯酸甲酯,得到 11.9g PEG-0.5。

3.2 彻底酰胺化反应(Step B) 上述 PEG-0.5 溶于 100mL 无水甲醇,缓慢加入 30mL 乙二胺,室温搅拌 48h,减压蒸馏除去甲醇和大部分乙二胺,分两次加入 20mL 正丁醇,减压共沸蒸馏除尽乙二胺和正丁醇,得到 11.6g PEG-1.0。

重复 Step A 和 Step B 可依次得到 PEG-1.5 至 PEG-6.0 各代树枝状大分子。随着代数的增大,使用的丙烯酸甲酯和乙二胺依次成倍增多。

4 凝血、溶血实验^[6]

用玻片法测定凝血时间,即取体重 2.5kg 健康白兔的自然流出的耳垂血一滴,加入一滴一定浓度的树枝状大分子溶液,搅匀,每隔 0.5min 用

针头轻挑血滴一次,直至挑出纤维状血丝,停表计时。以聚乙二醇为参照物判断各整数代树枝状大分子的凝血性。

取 1mL 上述白兔的颈动脉血,用肝素抗凝,加入一定浓度的树枝状大分子溶液,颠倒混匀,室温静置 2h,上清液呈透明红色而管底有红细胞者为开始溶血管,管液浓度为溶血浓度。在显微镜下观察红细胞的形态并计数,与聚乙二醇溶血管中红细胞相比较。

5 氨基聚乙二醇及其中间产物的结构表征

聚乙二醇氯代、叠氮代和催化还原的各步产物均经过红外表征。PEG-Cl: 663cm^{-1} (C-Cl), 1100cm^{-1} ($\text{CH}_2\text{-O-CH}_2$), 无 $\sim 3300\text{cm}^{-1}$ (-OH); PEG- N_3 : 2100cm^{-1} (-N_3), 1100cm^{-1} ($\text{CH}_2\text{-O-CH}_2$), 无 663cm^{-1} (C-Cl); PEG- NH_2 : 3300cm^{-1} (-NH_2), 1100cm^{-1} ($\text{CH}_2\text{-O-CH}_2$), 无 2108cm^{-1} (-N_3)。PEG- NH_2 含量通过以甲基红作指示剂用盐酸滴定确定。

6 以聚乙二醇为核的树枝状大分子的结构表征

氨基聚乙二醇的各代树枝状大分子的结构均经过 FT-IR 和元素分析的鉴定,并用中和滴定法测定了各整数代端氨基数。PEG- NH_2 和各整数代于 $\sim 3300\text{cm}^{-1}$ 处有端 -NH_2 红外吸收峰;而各半数代没有该吸收峰。表明各步 Micheal 加成反应进行得彻底。各半数代都有 $1375\sim 1742\text{cm}^{-1}$ 的酯羰基吸收峰,当它们转变成相应的整数代时,该吸收峰消失,表明各步酰胺化反应完全发生。元素分析的结果进一步证实了上述结论。以聚乙二醇为核的树枝状大分子的红外和元素分析数据列于表 1 中。

对各整树代树枝状大分子用盐酸进行电位滴定,以判断端氨基的数目。结果如表 2 所示,端氨基数与理论值基本相符。

元素分析及端氨基数测定结果表明 PEG-5.0 和 PEG-6.0 分析数据与理论值相差较大。这可能是由于这些较大的树枝状大分子挟裹着一些小分子物质难于除去所致。

得到的 PEG-1.0、PEG-2.0 为固体物质,其余各整数代仍呈粘稠状。

Table 1 FT-IR and elemental analysis data of dendrimer with PEG core

Dendrimer	IR(cm^{-1})			C(%)		H(%)		N(%)	
	$\nu(\text{NH}_2)$	$\nu \text{C—O, ester}$	$\nu \text{C—O, anide}$	Calcd	Found	Calcd	Found	Calcd	Found
PEG-NH ₂	~3300			54.86	54.80	9.34	9.36	1.40	1.39
PEG-0.5		1737		54.91	54.88	8.98	9.02	1.19	1.20
PEG-1.0	~3300		1656	54.36	54.32	9.22	9.24	5.69	5.71
PEG-1.5		1736	1662	54.54	54.40	8.71	8.88	4.44	4.40
PEG-2.0	~3300		1657	53.76	54.10	9.07	9.01	10.77	10.88
PEG-2.5		1735	1660	54.18	53.90	8.44	8.70	7.63	7.48
PEG-3.0	~3300		1648	52.20	52.10	8.94	8.98	15.56	15.70
PEG-3.5		1735	1660	53.89	53.40	8.23	8.49	10.14	10.20
PEG-4.0	~3300		1645	52.79	50.81	8.84	9.02	19.19	10.02
PEG-4.5		1736	1666	53.70	51.10	8.09	9.12	11.80	11.86
PEG-5.0	~3300		1646	52.50	50.61	8.78	8.90	21.52	21.80
PEG-5.5		1736	1660	53.59	52.19	8.01	8.20	12.78	12.90
PEG-6.0	~3300		1645	52.35	50.16	8.74	8.44	19.56	20.03

Table 2 Number of terminal aminogroups of the integral generations of the dendrimer determined by potentiometer titration

Dendrimer	PEG-1.0	PEG-2.0	PEG-3.0	PEG-4.0	PEG-5.0	PEG-6.0
Calculated number of —NH_2	4	8	16	32	64	128
Number of —NH_2	3.91	7.75	15.30	30.19	55.45	97.08

Table 3 Results of coagulation and hemolysis experiments of the integral generations of the dendrimer

Dendrimer	PEG	PEG-1.0	PEG-2.0	PEG-3.0	PEG-4.0
Coagulation concentration(mol/L)	0.008	0.007	0.008	0.007	0.010
Coagulation time(min)	6.42	6.53	7.13	6.63	5.80
Hemolytic concentration(mol/L)	0.0483	0.0507	0.0492	0.0477	0.0465

7 树枝状大分子凝血、溶血性能研究

树枝状大分子凝血、溶血试验结果列于表 3。溶液浓度高于表中所列凝血浓度时均出现抗凝现象,各整数代树枝状大分子的凝血性、溶血性与聚

乙二醇相比无显著差异。显微镜下观察处于溶血浓度下的红细胞的凹陷变小,红细胞膨胀;红细胞计数表明树枝状大分子并不比聚乙二醇更强的影响红细胞脆性。

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SYNTHESIS OF DENDRIMERS WITH MACRO CORE AND RESEARCH ON THEIR PROPERTIES OF COAGULATION AND HEMOLYSIS

SHI Shaohua, YU Wangui, ZHANG Daoming

(Hubei Medical Staff College, Jingzhou 434000)

LIU Lijian, LÜ Zhengrong, ZHUO Renxi

(Chemistry College, Wuhan University, Wuhan 430072)

Abstract In order to obtain the inviscid dendrimers with more terminal amino-groups than those of ω, ω' -polyethylene glycol diamine, in the meanwhile to utilize the fine biocompatibility of polyethylene glycol, a series of polyamidoamine dendrimers with polyethylene glycol core were synthesized by Tomalia's divergent method which involved a repeated two-step process: (1) exhaustive Michael addition of ω, ω' -polyethylene glycol diamine as the initiating core, with methyl acrylate and (2) exhaustive amidation of the resulting esters with a large excess of ethylenediamine. The structures of all generations of the dendrimer were identified by FT-IR and elemental analysis, and the numbers of the terminal aminogroups of the integral generations of the dendrimer were determined by potentiometer titration. Although PEG-NH₂ and the integral generations had adsorption at $\sim 3300\text{ cm}^{-1}$ in FT-IR, the half generations did not have. It indicated that all the steps of Michael addition took place thoroughly. On the other hand, the conversion of the half generations into their corresponding integral generations made the adsorption of the ester's carbonyl at $1735 \sim 1740\text{ cm}^{-1}$ disappear, it suggested that the conversion was complete. The results of elemental analysis also affirmed the above discussion. However the data of elemental analysis and potentiometer titration of PEG-5.0 and PEG-6.0 didn't entirely coincide with their theoretical values, that may be due to the residue of the ingredients such as ethylenediamine which were bundled up by larger generations with more branches and difficult to remove. Except that PEG-1.0 and PEG-2.0 are solids, the other integral generations are viscous. Influences of the integral generations on coagulation and hemolysis were examined, and the results showed that the properties of coagulation and hemolysis of the integral generations of the dendrimer were similar to those of polyethylene glycol.

Key words ω, ω' -Polyethylene glycol diamine, Dendrimer with macro core, Coagulation time, Hemolytic concentration