

学校编码: 10384

分类号 _____ 密级 _____

学号: 200425015

UDC _____

厦门大学

硕士学位论文

全内反射荧光法研究蛋白质与壳聚糖
的相互作用

Study of Interaction between Protein and Chitosan by Total
Internal Reflection Fluorescence Spectroscopy

陈喆

指导教师姓名: 李耀群 教授

专业名称: 分析化学

论文提交日期: 2007年10月

论文答辩时间: 2007年10月

学位授予日期: 2007年 月

答辩委员会主席: _____

评阅人: _____

2007年10月

厦门大学学位论文原创性声明

兹提交的学位论文，是本人在导师指导下独立完成的研究成果。本人在论文写作中参考的其他个人或集体的研究成果，均在文中以明确方式标明。本人依法享有和承担由此论文产生的权利和责任。

声明人（签名）：

年 月 日

摘要.....	I
Abstract.....	IV
第一章 文献综述	1
1.1 全内反射荧光法在生物体系中的应用	1
1.1.1 全内反射荧光法原理.....	1
1.1.2 蛋白质在固-液界面的研究.....	2
1.1.2.1 吸附机理的研究.....	3
1.1.2.2 蛋白质在界面上的构象和吸附取向.....	4
1.1.2.3 蛋白质在表面的竞争吸附.....	5
1.1.2.4 固体表面的化学性质对蛋白吸附的影响.....	7
1.1.2.5 生物材料与蛋白质的相互作用.....	8
1.1.3 其它应用.....	10
1.1.3.1 传感器的研究.....	10
1.1.3.2 生物细胞的研究.....	10
1.1.3.3 表面定量与表面物理化学性质研究.....	11
1.1.3.3.1 表面定量技术.....	11
1.1.3.3.2 表面物理化学性质的研究.....	12
1.1.3.4 液-液界面研究.....	12
1.1.3.4.1 荧光物质的研究.....	13
1.1.3.4.2 蛋白质的研究.....	14
1.2 壳聚糖与蛋白质相互作用的研究进展	14
1.2.1 壳聚糖的性质与应用.....	14
1.2.1.1 壳聚糖的简介.....	15

1.2.1.2 壳聚糖的物理和化学性质.....	15
1.2.1.3 壳聚糖的应用.....	16
1.2.2 壳聚糖与蛋白质相互作用的研究进展.....	18
1.3 论文构思	20
参考文献	22
第二章 适合全内反射荧光法研究壳聚糖与蛋白质相互作用的表面固定方法	35
2.1 引言	35
2.2 实验部分	36
2.2.1 试剂.....	36
2.2.2 仪器.....	36
2.2.3 实验方法.....	36
2.3 结果与讨论	39
2.3.1 全内反射样品池的改进.....	39
2.3.2 FITC 偶联壳聚糖.....	41
2.3.3 旋涂法应用于全内反射研究的考察.....	47
2.3.4 化学键合法固定壳聚糖膜.....	51
2.3.4.1 选择合适的化学键合法.....	51
2.3.4.2 化学键合法固定壳聚糖膜的优化.....	52
2.4 小结	59
参考文献	59
第三章 蛋白质与超薄壳聚糖膜相互作用的研究	61
3.1 引言	61
3.2 实验部分	62
3.2.1 试剂.....	62

3.2.2 仪器.....	62
3.2.3 实验方法.....	63
3.3 结果与讨论.....	63
3.3.1 FITC 标记各种模型蛋白质.....	63
3.3.2 牛血清白蛋白与超薄壳聚糖膜的相互作用.....	65
3.3.2.1 牛血清白蛋白在壳聚糖膜上吸附随本体浓度变化的情况.....	65
3.3.2.2 缓冲液 pH 变化对牛血清白蛋白在壳聚糖膜上吸附的影响.....	67
3.3.2.3 离子强度变化对牛血清白蛋白在壳聚糖膜上吸附的影响.....	69
3.3.3 牛血纤维蛋白原在壳聚糖膜上的吸附.....	71
3.3.4 溶菌酶在壳聚糖膜上的吸附.....	74
3.3.5 壳聚糖膜上 BSA 与 Fbn 的竞争吸附.....	77
3.4 小结.....	79
参考文献.....	80
第四章 罗丹明 6G 在石英/水界面上的荧光光谱性质.....	82
4.1 引言.....	82
4.2 实验部分.....	82
4.2.1 试剂.....	83
4.2.2 仪器.....	83
4.2.3 实验方法.....	83
4.3 结果与讨论.....	84
4.3.1 R6G 本体溶液的激发和发射光谱.....	84
4.3.2 本体溶液中同步荧光光谱扫描参数的选择.....	84
4.3.3 R6G 界面荧光的激发、发射及同步荧光光谱.....	85
4.3.4 界面荧光光谱移动.....	87
4.3.5 随浓度变化的 R6G 吸附行为.....	90

4.3.6 酸度效应.....	92
4.3.7 荧光强度的离子强度效应.....	94
4.4 小结.....	97
参考文献.....	97
结语与展望.....	103
附录.....	105
致谢.....	106

厦门大学博硕士学位论文摘要库

CATALOG

Abstract (Chinese)	I
Abstract (English)	IV
Chapter 1 Preface	1
1.1 Applications of Total Internal Reflection Fluorescence in Biological Systems	1
1.1.1 Theory Fundation of Total Internal Reflection Fluorescence.....	1
1.1.2 Study of Protein Adsorption in Solid/Liquid Interface.....	2
1.1.2.1 Mechanism of Protein Adsorption.....	3
1.1.2.2 Configuration and Orientation of Protein at the Interface.....	4
1.1.2.3 Competitive Adsorption on the Surface.....	5
1.1.2.4 Influence of Chemical Property of Solid Surface to Protein Adsorption.....	7
1.1.2.5 Development of Biomaterials Study.....	8
1.1.3 Other Application.....	10
1.1.3.1 Development of Biosensor.....	10
1.1.3.2 Study in Biological Cell.....	10
1.1.3.3 Quantitatiion and Properties of Physical Chemistry on Surface....	11
1.1.3.3.1 Techniques of Surface Quantitatiion.....	11
1.1.3.3.2 Properties of Physical Chemistry on Surface.....	12
1.1.3.4 Study in Liquid/Liquid Interface.....	12
1.1.3.4.1 Study of Fluorescent Substances.....	13
1.1.3.4.2 Study of Protein.....	14
1.2 Review of Study in Interaction between Chitosan and Protein	14

1.2.1 Property and Application of Chitosan.....	14
1.2.1.1 Introduction of Chitosan.....	15
1.2.1.2 Physical and Chemical Property of Chitosan.....	15
1.2.1.3 Application of Chitosan.....	16
1.2.2 Development of Study in Interaction between Chitosan and Protein.....	18
1.3 Conception of Dissertation.....	20
References.....	22
Chapter 2 Surface immobility methods suitable for the study on chitosan and protein interaction by Total Reflection Internal Fluorescence.....	35
2.1 Introduction.....	35
2.2 Experimental.....	36
2.2.1 Materials.....	36
2.2.2 Apparatus.....	36
2.2.3 Methods.....	36
2.3 Results and Discussion.....	39
2.3.1 Improvement for TIRF Cell.....	39
2.3.2 FITC Labeled Chitosan.....	41
2.3.3 Immobilized Chitosan for TIRF Study by Spin-Coating.....	47
2.3.4 Immobilized Chitosan for TIRF Study by Chemical Combining.....	51
2.3.4.1 Comparing of Suitable Protocols for Chemical Grafting.....	51
2.3.4.2 Optimized Chemical Grafting for Chitosan Immobility.....	52
2.4 Conclusions.....	59

References.....	59
Chapter 3 Interaction between Proteins and Ultrathin Chitosan Film.....	61
3.1 Introduction.....	61
3.2 Experimental.....	62
3.2.1 Materials.....	62
3.2.2 Apparatus.....	62
3.2.3 Methods.....	63
3.3 Results and Discussion.....	63
3.3.1 FITC Labeled Model Proteins.....	63
3.3.2 Interaction between BSA and Ultrathin Chitosan Film.....	65
3.3.2.1 BSA Adsorption on Chitosan Film with Variation of Bulk Concentrations.....	65
3.3.2.2 pH Effect on BSA Adsorption on Chitosan Film.....	67
3.3.2.3 Ionic Strength Effect on BSA Adsorption on Chitosan Film.....	69
3.3.3 Adsorption of Fibrinogen on Chitosan Film.....	71
3.3.4 Adsorption of Lysozyme on Chitosan Film.....	74
3.3.5 Competitive Adsorption of BSA and Fibrinogen on Chitosan Film.....	77
3.4 Conclusions.....	79
References.....	80
Chapter 4 Fluorescence Spectral Properties of Rhodamine 6G at the Silica/Water Interface.....	82
4.1 Introduction.....	82

4.2 Experimental	82
4.2.1 Materials.....	83
4.2.2 Apparatus.....	83
4.2.3 Methods.....	83
4.3 Results and Discussion	84
4.3.1 Excitation and Emission Spectra of R6G in Bulk Solution.....	84
4.3.2 Optimized Scanning Parameters of Synchronous Fluorescence Spectra of R6G in Bulk Solution.....	84
4.3.3 Excitation and Emission Spectra of R6G at interface.....	85
4.3.4 Shift of Interface-Originated Fluorescence Spectra.....	87
4.3.5 Adsorption Behaviors of R6G with Concentrations.....	90
4.3.6 pH Dependence of Fluorescence Intensity at the Interface.....	92
4.3.7 Effect of Ionic Strength on Fluorescence Intensity.....	94
4.4 Conclusions	97
References	97
Postlude and Prospect	103
Appendix	105
Acknowledgements	106

厦门大学博硕士学位论文摘要库

摘 要

壳聚糖作为重要的天然聚合物,以良好的生物相容性、生物可降解性,对细胞组织不产生毒性影响,在生物医学等方面得到了广泛的研究应用。壳聚糖对蛋白质有良好的亲和能力,研究蛋白质与壳聚糖在界面上的相互作用、理解蛋白质与壳聚糖分子在界面接触时的吸附过程变化对蛋白药物的开发和生物化学研究具有重要的意义。全内反射荧光法是研究界面蛋白质吸附的一种有效的手段。它利用指数衰减的倏逝波选择性激发介质表面的荧光团分子,产生全内反射荧光,具有高度的界面(表面)特异性,从而可有效排除本体干扰,获取界面信息,而且又具有非破坏性,能够实时、原位检测蛋白质吸附过程。本文致力于改进实验室已有的固/液界面全内反射荧光分析的部件,考察壳聚糖超薄膜的固定方案,继而研究三种模型蛋白在壳聚糖超薄膜上的吸附情况。此外,还对罗丹明6G(R6G)在石英/水界面上的吸附进行研究,进一步认识咕吨染料在界面的情况。论文分为四章:

第一章综述了全内反射荧光法在生物体系中的研究应用,以及壳聚糖与蛋白质相互作用的研究概况。简要介绍全内反射荧光法的原理和应用,对目前全内反射荧光法在生物体系,特别是固/液界面蛋白质吸附的研究作了详尽的概述,同时还介绍了壳聚糖的性质、应用以及它与蛋白质相互作用研究的状况,最后提出整篇论文的设计。

第二章在现有可行的实验条件下,以石英片为载体,改进实验室已有全内反射荧光部件,使其易于进行表面修饰,并且使样品的检测体积减少至200 μL 。考察了旋涂法与化学键合法固定壳聚糖薄膜的情况,同时应用全内反射恒波长同步荧光分析方法,监测壳聚糖表面固定情况。最后确定了以硅烷化试剂自组装在石英载体上引入活性基团进行表面接枝壳聚糖的化学键合法,并对接枝方法进行了优化,获得了表面均一的壳聚糖超薄膜。

第三章用荧光素异硫氰酸酯(FITC)标记常见的模型球型蛋白(牛血清白蛋白、溶菌酶、牛血纤维蛋白原),应用全内反射荧光法分析它们分别与壳聚糖

超薄膜相互作用的情况,以及对牛血纤维蛋白原和牛血清白蛋白的二元竞争吸附体系进行研究。

首先成功用FITC标记各种模型蛋白质,标记率在0.6-3.8之间,观测其吸收、荧光波长均有所红移,荧光各向异性值有6-10倍的增长。

牛血清白蛋白(BSA)在壳聚糖上随着本体浓度增加,出现两个吸附增长区。随浓度增加,吸附在壳聚糖表面上的BSA可能从平躺取向排布向竖直取向变化;在平衡浓度大于400 $\mu\text{g}/\text{mL}$ 以上可能出现多层吸附。pH对BSA在壳聚糖上的吸附作用也有显著的影响,随着pH增加,BSA在pH 6.5附近出现了极值,说明主要以静电相互作用驱动了BSA吸附。离子强度的增加,屏蔽了蛋白质与壳聚糖表面、蛋白质-蛋白质分子之间的静电斥力,使得BSA的吸附量增加;而当盐浓度大于0.1 mol/L之后,由于壳聚糖分子链的刚性变小,容易成团,与蛋白质接触面积减小,导致吸附量下降。

此外,还考察了另外两种模型蛋白吸附随本体溶液浓度变化的影响,以及对模型蛋白的吸附过程进行动力学方程拟合。牛血纤维蛋白原的界面荧光随着蛋白质本体浓度的增加而增加;在低浓度下,双常数速率方程能够得到较好拟合性,而在高浓度下,准二级动力学模型能够得到较好的拟合性。而溶菌酶在考察浓度范围内,界面信号随浓度增长变化曲线与Langmuir吸附等温线形状近似。溶菌酶对壳聚糖的表面亲和能力比BSA更好,这可能由于壳聚糖与溶菌酶的底物结构相似,溶菌酶易吸附于壳聚糖膜上。溶菌酶的吸附动力学过程可用准二级吸附动力学模型描述,随着吸附初始浓度的增加,吸附速率常数k也总体有一个数量级的增长。

比较壳聚糖膜上BSA与牛血纤维蛋白原在生理条件下竞争吸附的情况,在壳聚糖界面上,牛血纤维蛋白原的相对竞争吸附能力要大于BSA。这表明壳聚糖也能够正常驱动牛血纤维蛋白原的吸附,激发血小板和凝血因子而发生凝血。

第四章利用全内反射恒波长同步荧光法直接测量在石英/水界面上的罗丹明6G(R6G)。通过对其本体、界面荧光光谱的分析,观察到R6G分子在亲水石英/水界面上出现5 nm红移,红移的原因主要是染料分子旋转运动受到限制,石英表面的刚性限制了分子的自由度,同时,石英/水界面更低的极性也对红移产生影响。同时还考察了R6G在石英/水界面上的吸附情况与本体溶液的浓度、pH

以及离子强度的关系。

关键词：壳聚糖薄膜；蛋白质；全内反射荧光法

厦门大学博硕士学位论文摘要库

Abstract

As an important natural polymer, chitosan has been applied extensively in biomedicine for its biocompatibility, biodegradation, as well as non-toxicity to cellular tissues. There is good affinity between chitosan and protein. For the development of protein drugs and the research of biochemistry, it is significant to probe the interaction between chitosan and protein, and to observe changes of adsorption when both of them contact at interface. Total internal reflection fluorescence spectroscopy (TIRF) is an effective technique to investigate protein adsorption at interface. The total internal reflection fluorescence generates from interfacial fluorophore, which is selectively excited by evanescent wave. Therefore, there are several characteristics for evanescent wave inducing fluorescence, such as surface specificity, elimination of bulk interference and non-destruction. It has been successful to investigate protein adsorption in situ, real-time by this technique. This dissertation is concerned on the improvement of instrumental device of TIRF for probing solid/liquid interface, the option of suitable method for immobilizing chitosan, and the analysis of interaction between model proteins and ultrathin chitosan film which was immobilized on silica substrate. In addition, the adsorption behavior of rhodamine 6G (R6G) at silica/water interface was also concerned. The dissertation is composed of following parts:

In the first chapter, the application of total internal reflection fluorescence technique in the research of biological systems and the advance on the study of interaction between chitosan and protein were reviewed. The principle and application of TIRF were introduced briefly. The research on biological systems, especially on the study of protein adsorption at solid/water interface, was described in detail. Then the property and application of chitosan were introduced, as well as the hot topics on the study of chitosan-protein interaction. The plan of dissertation was put forward at the last part of this chapter.

In the second chapter, a new TIRF cell was developed based on the present experimental

Degree papers are in the "[Xiamen University Electronic Theses and Dissertations Database](#)". Full texts are available in the following ways:

1. If your library is a CALIS member libraries, please log on <http://etd.calis.edu.cn/> and submit requests online, or consult the interlibrary loan department in your library.
2. For users of non-CALIS member libraries, please mail to etd@xmu.edu.cn for delivery details.

厦门大学博硕士学位论文摘要库