学校编码：10384　　　　　　分类号________密级 ______

学　　号：200225012　　　　　UDC________________________

厦　门　大　学

硕　士　学　位　论　文

全内反射同步荧光法研究界面吸附行为及其应用

Study of Interface Adsorption Behavior and its Application by Total Internal Reflection Synchronous Fluorescence Spectroscopy

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论文提交日期：　2005 年 8 月

论文答辩日期：　2005 年　 月

学位授予日期：　2005 年　 月

答辩委员会主席：________

评　　阅　　人：________

2005 年 **8** 月

Study of Interface Adsorption Behavior and its Application by Total Internal Reflection Synchronous Fluorscence Spectroscopy

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Submitted to the Graduate School of Xiamen University

for the Degree of

MASTER OF SCIENCE

August 2005

Department of Chemistry, Xiamen University

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摘 要

卟啉具有重要的生物物理化学作用，它能与蛋白质相互作用并吸附到细胞膜表面，因此卟啉和蛋白质在界面上相互作用的研究具有重要的意义。全内反射荧光技术是目前在单分子水平上研究界面的一种有效方法，同步荧光技术具有提高选择性、简化光谱等优点；将上述两种技术相结合，可有效地减少界面散射光的干扰，并窄化界面荧光光谱。本文应用全内反射同步荧光技术研究水溶性卟啉与牛血清蛋白以及不同表面活性剂在界面上的吸附及应用。论文分为如下四章：

第一章简要介绍了全内反射荧光法的原理以及特点，并比较了一些目前已有的界面研究方法的优缺点。对目前全内反射荧光法在蛋白质的固-液界面吸附的应用，以及液-液界面吸附研究做了综述，同时介绍了卟啉与蛋白质界面化学的研究状况。

第二章建立了利用蛋白质吸附在液－固界面上的同步荧光信号随本体溶液浓度的增加呈线性关系，定量测定本体蛋白质溶液浓度的新方法。应用全内反射同步荧光法研究了以 meso-四（4－对磺酸基苯基）卟啉（TPPS）标记牛血清蛋白（BSA）在石英亲水界面上的吸附。考察了检测时间、pH 值、离子强度以及 TPPS 浓度等影响蛋白质界面吸附的因素，检测限是 94 μg/L。该法用于实际人血清蛋白样品的测定，得到令人满意的结果。

第三章应用全内反射同步荧光技术研究了不同的表面活性剂对 TPPS 在正己烷/水界面上吸附的影响。比较了 TPPS 在界面上与在水相中荧光性质的差异，研究了全内反射同步荧光强度随表面活性剂种类、表面活性剂浓度及溶液 pH 值的变化情况，探索了 TPPS 的界面吸附行为。在阳离子表面活性剂中，TPPS 在界面上有较强的吸附；而在阴离子表面活性剂、非离子表面活性剂存在或无表面活性剂存在的情况下 TPPS 则在界面的吸附很微弱；并着重考察了阳离子表面活性剂 CTAB 对 TPPS 界面荧光性质的影响。界面上的荧光光谱与未质子化的 TPPS（$TPPS^{4-}$）相近，但红移 8-9nm；结果说明，不论表面活性剂存在与否，$TPPS^{4-}$能够选择性地吸附在正己烷/水界面上，但在阳离子表面活性剂中，$TPPS^{4-}$在液-液界面的吸附能力最大。研究显示静电力在 TPPS 界面吸附过程中应起重要作用。

第四章应用全内反射同步荧光技术研究蛋白质与卟啉在甲苯－水界面的相互作用。

首先考察了 pH 对不同浓度体系中 BSA 与 TPPS 结合的影响，在 BSA 和 TPPS 浓度比较高(1.0×10^{-5}mol/L)的体系中，BSA 与 TPPS 强烈结合，pH 对二者结合不产生影响；而在浓度较低体系 (1.0×10^{-7}mol/L)中，pH 对 BSA 和 TPPS 的结合有着决定性的影响。

其次建立了现场检测油-水双相体系的技术，考察 BSA 与 TPPS 在水相、界面以及油相的分配和型体特征，得到 C_{BSA}，C_{TPPS} 均为 1.0×10^{-6}mol/L 的体系中，界面吸附量为 1.1×10^{-10}mol/cm^2。并对卟啉在不同环境中的荧光光谱进行比较，证明：油-水界面极性与非极性的有机相接近；BSA 与 TPPS 结合后，BSA 为 TPPS 提供了类似非极性的微环境。

进一步考察了不同的 BSA 与 TPPS 浓度比例对本体和界面荧光特征

光谱影响，在一定条件下，不管本体中两者如何结合，BSA 与 TPPS 总是以 1:1 的结合体（BSA-TPPS）吸附到界面上。pH=3.1，根据体系浓度与界面荧光信号强度的曲线关系，得到 BSA 在油-水体系中的临界胶束浓度（cmc）为 1.0×10^{-4}mol/L；并根据 Langmuir 吸附公式，计算得到 BSA-TPPS 在甲苯－水界面的饱和吸附量为 9.3×10^{-10}mol/cm^2，吸附平衡常数是 1.2×10^{7} L/mol。

关键词：牛血清蛋白、卟啉、表面活性剂、液-液界面、液-固界面、全内反射同步荧光

Abstract

Porphyrins play important role on biological chemistry process, since they interact with protein, adsorb to or penetrate membrane. So it is significant to study their character of interfacial behavior. Total Internal Reflection Fluorescence Spectroscopy (TIRF) is an effective method to investigate interface on molecular scale. And synchronous fluorescence spectroscopy can improve selectivity and simplify the spectrum. Combining those two techniques above can successfully decrease the scattering light from interface and simplify spectrum. This dissertation is concerned on the investigation of meso-tetrakis (4-sulphonatophenyl) porphyrin (TPPS) interaction with bovine serum albumin (BSA) and different surfactants, and their adsorption behavior on interface by Total Internal Reflection Synchronous Fluorescence (TIRSF). The dissertation is composed of following parts:

In the first chapter, the principle and advantage of total internal reflected fluorescence was introduced, and other techniques for interfacial study were reviewed. The TIRF applications on water-solid, liquid-liquid interface research were described in detail. Research about interaction between protein and porphyrins was introduced. The plan of dissertation was put forward at the last part of this chapter.

In the second chapter, a new method to determine protein concentrations was established, based on the synchronous fluorescence from water-solid interface

by TIRSF. The interfacial fluorescence, which was emitted from of TPPS binding to BSA and adsorbing onto quartz surface, increased linearly with bulk concentration. The adsorption factors, such as detection time, ion strength, pH and TPPS concentration, were carefully investigated. The detection limitation of this method was 94 μg/L. Real human serum samples were measured with good results.

In the third chapter, influence of surfactants on TPPS adsorption to n-hexane/water interface was studied by TIRSF. A comparison was made of the differences in fluorescence spectra of TPPS at interface and in aqueous solution by using TIRSF. The adsorption behavior of TPPS under different concentrations, pH values and kinds of surfactants were studied. The strong TIRSF intensity emitted from TPPS was observed in the presence of cationic surfactant, while weak signals were found in the presence of anionic, nonionic surfactant and without surfactant. The TIRSF spectrum was similar to that of nonprotonated forms of TPPS ($TPPS^{4-}$) in aqueous solution with or without surfactant, which indicated $TPPS^{4-}$ were adsorbed selectively at interface with or without surfactants. However, cationic surfactant promoted the adsorption of $TPPS^{4-}$. The result showed that electrostatic played important role on the adsorption of TPPS at liquid/liquid interface.

In the forth chapter, the interaction of BSA and TPPS on toluene-water interface was studied by TIRSF.

Firstly, influence of pH on BSA binding with TPPS was discussed. At higher concentration, the strong binding of BSA to TPPS showed low dependence of

pH; while at lower concentration, pH had great effect on their binding and electrostatic played important role.

Secondly, an on-line technique to detect the distribution of BSA-TPPS complex in the oil-water system was established in this chapter. At pH=3.1, adsorption capacity of 1.1×10^{-10}mol/cm^2 was obtained at interface when both C_{BSA} and C_{TPPS} were 1.0×10^{-6}mol/L. Compared spectra of TPPS from different environments, it was concluded that the polarity of oil/water interface was close to that of organic phase, and BSA provided nonpolar microenvironment after binding with TPPS.

And then, the synchronous fluorescence spectra from toluene-water interface and aqueous solution were analyzed. Under the experimental condition, the complex of BSA and TPPS adsorbed onto the interface by concentration proportion of 1:1. According to the relationship between the interfacial fluorescence intensity and total concentration, the critical micelle concentration (cmc) of BSA in oil/water system was obtained with a value of 1.0×10^{-4}mol/L. At pH=3.1, an adsorption equilibrium constant of 1.2×10^{7} mol/L, and maximum adsorption capacity of 9.3×10^{-10}mol/cm^2 were obtained, according to Langmuir formula.

Keywords: BSA, TPPS, surfactant, liquid-liquid interface, solid-liquid interface, total internal reflection synchronous fluorescence