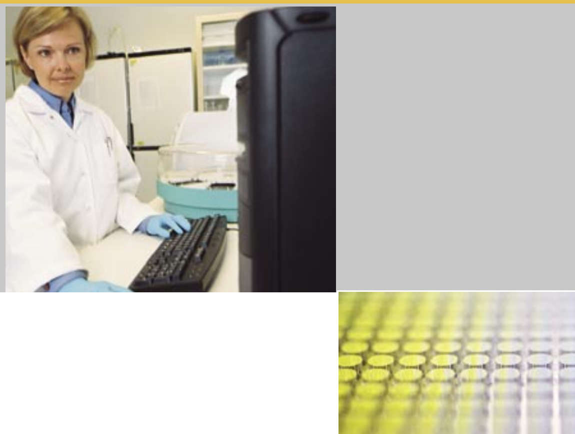
Application Note

Microplate Instrumentation



Fluorescence based ligand-binding studies of ß-lactoglobulin

This application note describes the advantage of Thermo's Varioskan in ligand binding studies of ß-lactoglobulin. The experiments are based on tryptophan fluorescence quenching in consequence of ligand-binding. The method can be easily applied to other respective protein-ligand studies as well.

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Abstract

The protein ß-lactoglobulin (ß-LG) is the major whey protein in bovine milk. It has been recognized that ß-LG may play a role in the transport of retinol and fatty acids (Sawyer et al. 1998). ß-LG usually exists as a dimer in which the molecular weight of the subunit is about 18000 Da (Papitz et al. 1986). The ß-LG molecule consists of an anti-parallel ß-sheet, formed by 9 strands wrapped around to form a flattened cone or calyx. The core of the ß-LG is an eight-stranded, antiparallel ß-barrel. The extra ß-strand forms the dimer interface (Sawyer & Kantopidis 2000). There is also an α-helix lying on the surface. ß-LG binds ligands of a diverse nature. ß-LG can bind hydrophobic ligands as retinol, retinoic acid, long-chain fatty acids and aromatic compounds. The retinol binding site is inside the ß-barrel (Naryan & Berliner, 1997).

There are many analyses which can be appropriate if the ligand binds to ß-LG. The most commonly used methods are based on fluorescence. ß-LG contains two tryptophan residues, Trp19 and Trp61 (Cho et al. 1994). The binding of retinoids and palmitate to ß-LG causes a quenching of the fluorescence of Trp19, which is present at the bottom of calyx (Wang et al. 1997).

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Experimental

Emission spectra of ß-LG were recorded in the absence and in the presence of the added retinol. All experiments were performed using Thermo Varioskan and Thermo Microtiter® UV 96 plates (cat. no. 8404). Before measurement plates were shaken for 20 s. Excitation wavelength was 280 nm and the emission measurement range was 300-450 nm. The sample volume was 200 µl per well. All samples had eight replicates. The value of blank solution (PBS) was subtracted from the value of the sample. The measurement was made 15 minutes after pipetting the samples. The apparent dissociation constant for ligands was calculated from the fluorescence intensity results using the method of Cogan et al. 1976. Before all experiments the concentration of ß-LG was measured using the wavelength 280 nm and corrected using the pathlength correction option (data not shown).

Results and Discussion

The fluorescence emission maximum of ß-LG was at 330 nm. The fluorescence emission spectra of ß-LG with and without retinol are shown in Figure 1. The fluorescence of ß-LG tryptophanyl residues were quenched in the presence of retinol indicating retinol-ß-LG binding. Figure 2 shows relative fluorescence intensities of ß-LG tryptophanyl residues at 330 nm. The fluorescence was quenched from 100 % to 68 % in the presence of increasing concentration of retinol. The calculated apparent dissociation constant was 0.14 µM. These results show that this fluorescence based method is a valuable tool in the investigation of ligand-binding studies of ß-LG and also for other proteins and can easily be measured by Thermo Varioskan.

References

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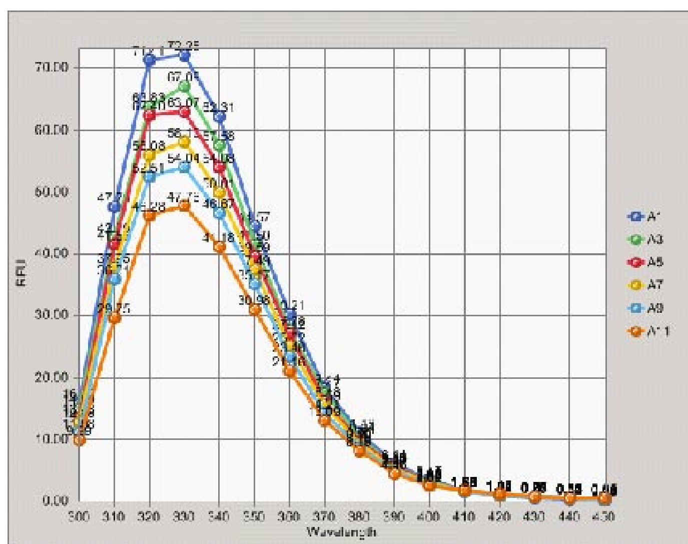

Figure 1 - The fluorescence emission spectrum of ßLG (A1) and ßLG with different concentration of retinol (A3-A11). The emission maximum was at 330 nm, excitation wavelength was 280 nm.

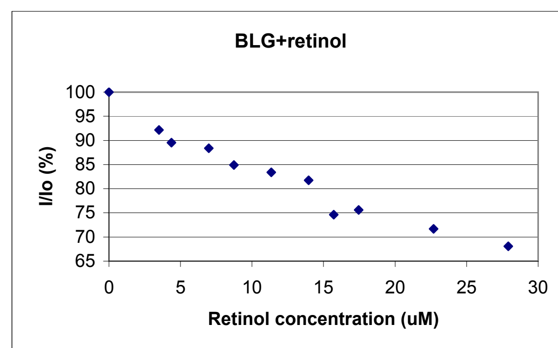

Figure 2 - Relative fluorescence emission intensity of ßLG at 330 nm as a function of added retinol.

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