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Title of the doctoral dissertation: „*Complex formation of small ligands with nucleic acids*”

Abstract

Progress in molecular biology depends on discoveries of new specific dyes that stain cellular structures. Observing intracellular components, studying their interactions, and quantifying them will improve our understanding of cellular life processes. This knowledge will enable the development of new therapeutic methods that directly target cellular structures and improve existing ones.

Today, fluorescent dyes are mainly used to investigate cellular targets. They are safer and easier to use than the radioactive tracers used in the past, and they offer higher selectivity. The application of fluorescent dyes enables the study of nucleic acid interactions, determination of protein concentration, structure, and interactions, determination of ion concentrations in the cell, and observation of ion channel function, as well as of whole organelles. Many disorders or abnormalities in the concentrations, structures, or interactions of these biomolecules are markers of numerous diseases. Fluorescent dyes also allow the researcher to observe, with good resolution, many different cell components of interest.

In my studies, I used fluorescent dyes and anthracyclines to investigate their reactions with nucleic acids. The experimental part of my dissertation is divided into four sections: determination of cyanine dye equilibrium constants; determination of anthracycline equilibrium constants; photobleaching of the YO-PRO-1 dye complexed to immobilised DNA; and determination of YO-PRO-1 dye's cellular targets. In the next paragraphs, I briefly describe sections of my experimental work.

First, I have focused on cyanine dyes that stain nucleic acids. Using this group, I studied the interactions between the YO-PRO-1 and YOYO-1 dyes at the single-molecule level. In my work, I carried out a quantitative analysis of their interactions with nucleic acids. I determined the equilibrium constant and investigated the mechanism of interaction between the YOYO-1 dye and DNA.

The second area of my interest was anthracyclines, as they are also intercalators. They are a family of antibiotics initially isolated from *Streptomyces* bacteria with a broad spectrum of activity in oncological treatment. These drugs intercalate into the DNA in the cell's nucleus, destabilising its structure and interacting with Topoisomerase II. These changes prevent

transcription, leading the cell to enter the apoptotic cycle. Due to the weakened DNA repair mechanisms in cancer cells, they are more sensitive to the effects of anthracyclines.

However, these chemotherapeutics are characterised by severe cardiotoxic effects. Numerous studies are underway to develop new anthracycline derivatives with high therapeutic efficacy while reducing cardiotoxicity. Numerous clinical trials using anthracyclines are reported in the literature. Despite extensive attempts to modify this group of antibiotics, anthracyclines remain a highly toxic group of highly effective anticancer drugs.

In my work, I investigated the binding of anthracyclines to DNA and determined the equilibrium constants for the complex-formation reactions.

I also utilised a new method for characterising biochemical reactions by determining their equilibrium constants, which involves analysing changes in molecular brightness. This technique, using fluorescence, allows the equilibrium constant for the formation of non-covalent complexes at nanomolar concentrations, like those found in cells, to be accurately determined. The method is based on the change in intrinsic molecular brightness that results from the complexation reaction. It also enables research into the reaction mechanism.

In my work, I use this technique to determine the equilibrium constants for the complexation of the dyes YOYO-1 and YO-PRO-1, and of anthracyclines with DNA. Moreover, applying this method has also allowed me to determine the fraction of dyes bound to immobilised DNA that do not undergo excitation due to the orientation of their dipole moments.

Also, I identified the cellular targets of YO-PRO-1 by testing various nucleic acid probes in buffer. The targets were further confirmed in HeLa cells by my colleague.

