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Summary

Understanding in detail how proteins bind ligands is a formidable and fascinating challenge. Within this context, the work described in this thesis is dedicated to the spectroscopic characterization of three H_1 antihistamines —TRP, MEP, and BPA— and their binding mechanism to the human serum protein HSA. The suitability of luminescence —in particular fluorescence— in combination with UVRR spectroscopy to elucidate molecular structures and investigate aspects of protein–ligand interactions is explored for the first time with the abovementioned molecules.

H_1 antihistamines are agents used against a wide variety of human pathologies. They exert their action by interacting with the H_1 GPCR. Nevertheless, the important question on how binding occurs at the receptor site still remains unsolved. These antihistamines also act in the central nervous system, causing sedation due to their weak binding ability to HSA, the main transporter of H_1 antihistamines to the receptor, which is able to penetrate the blood–brain barrier. There is a general lack of information on the kind of interactions occurring with serum proteins. To shed light onto those fundamental topics, the aim of the research was the evaluation of the feasibility of the use of H_1 antihistamines as intrinsic optical probes in protein binding studies. Structure elucidation of the binding mechanism has obvious relevance for the development of new drugs

and therapies.

At the time the research started little information was available about the spectroscopic behavior of these H_1 antihistamines. In work dating from the 1970's anomalies concerning the luminescence behavior of TRP and MEP were reported without, however, an explanation or possible cause of those effects. In order to test the feasibility of the ligands as intrinsic optical probes detailed knowledge of their spectroscopic behavior in solution is of central importance. That is why, in the first part of this thesis, much attention is devoted to the spectroscopic characterization and elucidation of solution structures of TRP and MEP. Here the relation between the spectroscopic properties and the structures of H_1 antihistamines is derived as a function of pH. It is known from literature that ligand binding to GPCR's implicates ionic interactions with key amino acids of the receptor, leading to a strong pH dependence.

These H_1 antihistamines possess multiple (de)–protonable groups and we conclude that this property makes them suitable intrinsic optical probes since the protonation state of a particular functional group will determine the charge and the possibility of intermolecular hydrogen bond formation with the protein. At physiological pH, by the combination of fluorescence and UVRR spectroscopy, the relation between spectral properties and the solution structures was elucidated. Both TRP and MEP show complex pH–dependent effects, of which, according to our results, the reported abberations may now be interpreted in terms of an intramolecular hydrogen bond occurring at physiological pH between the protonated ethylamine side chain and the intracyclic nitrogen of the AP chromophore. Therefore those ligands exist in solution at physiological condition in two conformers: stretched, and bent with an internal hydrogen bond. This structural behavior, responsible for the anomalous spectroscopic effects and anomalous pK_a value, depends on the extent of protonation of the ethylamino side chain. The two conformers of TRP and MEP were now characterized with distinctive absorption, emission, and fluorescence maxima as well as fluorescence lifetimes, which means that by selective excitation of the ligands with respect to protein, and by monitoring their emission, it should

be in principle possible derive which of the conformers is bound to the target protein.

A very important consequence of the conformational isomerism of TRP and MEP is that ligand conformers show different activity towards the target proteins, because they cannot be equally complementary to the three dimensional structure of the protein; hence the characterization of the various conformational forms of these H_1 antihistamines represents a fundamental step in elucidating the molecular background to the biological versatility. Indeed, binding of TRP and MEP to HSA, as reported in the second part of the thesis, gives evidence that that the structural versatility of the ligands accounts for their weak binding to HSA. At physiological pH only the small percentage of the non–hydrogen bonded stretched conformers bind to HSA. In contrast BPA, which is not affected by intramolecular hydrogen bond and it is therefore supposed to bind in a stretched conformation, has a weak affinity towards the protein when the ethylamino side chain is protonated. The affinity strongly increases when the molecule is fully de–protonated.

Obviously the structural versatility of H_1 antihistamines also opens new questions on how those ligands bind to the H_1 GPCR, since, as it is known from literature, the protonated ethylamino side chain is implicated in binding with this receptor as well.

The human H_1 receptor is a protein extremely difficult to obtain in a sufficiently purified form, and impurities generally mask the intrinsic fluorescence of the receptor. For this reason exploratory binding studies with H_1 antihistamines were conducted in the presence of HSA. Not just because it is their transport protein, but also because it has only a single tryptophan, which simplifies study of specific ligand–protein interaction by means of fluorescence and UVRR spectroscopy considerably. The experience and results obtained will be helpful for the understanding of H_1 receptor–H_1 antihistamine interactions, since it is the tryptophan residue of HSA and H_1 receptor that is implicated in H_1 antihistamine binding as well. Therefore, knowledge gained from the probe system presented in this thesis may eventually represent a reference study for

future characterization of H_1 antihistamine–GPCR interactions by means of fluorescence and UVRR spectroscopy.