1P153**Effects of SNPs on the Drug Binding and Metabolizing Activity of CYP1A2**Tadayuki Uno¹, OMasafumi Egawa^{1,2}, Shin-ichi Murata², Akihide Tsujimoto¹, Yoshikazu Tomisugi¹, Hatsuo Maeda¹¹Osaka Univ., ²Kumamoto Univ.

Cytochromes P450 (CYPs) are membrane-associated heme proteins that metabolize physiologically important compounds in living cells. CYPs have been reported to show some SNPs that originate low metabolic activity, posing significant problems to the clinical use of drugs. The effects of SNPs on the drug metabolism, however, have not been fully understood at molecular level. CYP1A2 is one of the major isoforms of human hepatic CYPs, mainly metabolizing aromatic amines and polycyclic aromatic hydrocarbons. In this paper, we focused on two SNP mutants (E168Q and F186L) and investigated the drug binding affinities and metabolizing activities from structural point of view. Resonance Raman spectra of WT, E168Q and F186L proteins in ferric and CO-bound states were essentially the same, and hence E168Q and F186L mutations do not affect heme vicinities in CYP1A2. Although the activity of E168Q was similar to that of WT, F186L mutant showed larger K_m value than WT protein. To clarify the origin of the large K_m , we determined drug dissociation constant (K_d), and the K_d values were found to be larger for F186L mutant than that for WT protein. These results strongly suggest that the phenyl ring of Phe186 in CYP1A2 greatly contributes to the plausible drug binding thereby enhancing the drug metabolism. Since the K_m and K_d values were variable among the substrate drugs, the effects of SNPs are now established to be determined by the combination of SNP mutation and chemical structure of drugs.

1P155**Spectroscopic Studies of Neuronal PAS Protein 2 (NPAS2), a Transcription Activator Protein Associated with Circadian Rhythms**O Naoki Hikage¹, Jotaro Igarashi¹, Emiko Sato¹, Ikuko Sagami², Toru Shimizu¹¹Institute of Multidisciplinary Research for Advanced Materials, Tohoku University,²Grad. Sch. of Agr., Kyoto Pref. Univ.

In animals, biological rhythms are coordinated in adaptive synchrony by the brain, specifically by the suprachiasmatic nucleus of the hypothalamus. In its simplest form, the molecular clockwork consists of auto-regulatory transcriptional and translational feedback loops that have both positive and negative elements. The positive components are two transcription factors, CLOCK and BMAL1, both of which contain a basic helix-loop-helix (bHLH) domain and two PAS domains. These two transcription factors form a heterodimer that binds to the E-box sequence, which drives the transcription of three Period genes and two cryptochrome genes. The mammalian forebrain expresses NPAS2, which is homologous to CLOCK. NPAS2 plays an important role in maintaining circadian behaviors in normal light/dark and feeding conditions. NPAS2 also forms a heterodimer with BMAL1, and the heterodimer activates the transcription of *per* and *cry*. NPAS2 has bHLH, PAS-A, and PAS-B domains in its N-terminal region, and both the PAS-A and PAS-B domains of NPAS2 contain a heme binding site, and CO binding to the heme inhibits the DNA-binding activity of the NPAS2-BMAL1 heterodimer. Because CO binding to the heme causes dissociation of NPAS2 from BMAL1 and the E-box sequence, the bound heme itself may affect the DNA-binding properties of NPAS2 by interacting with the bHLH region. In the present study, we investigated the role of the heme in transcription by characterizing the isolated heme-bound bHLH-PAS-A domain of NPAS2 using optical absorption spectroscopy.

1P154**Resonance Raman Studies of CYP2C19 and Its Complexes with Substrates**Sayed M. S. Ali^{1,2}, Yukiko Oyama², Yoshinobu Ishikawa², Yoshikazu Tomisugi¹, Hatsuo Maeda¹, OTadayuki Uno¹¹Graduate School of Pharmaceutical Sciences, Osaka University, ²Graduate School of Pharmaceutical Sciences, Kumamoto University

Cytochrome P450 (CYP) is a class of heme-containing enzymes which mainly catalyze a monooxygenation reaction of various chemicals, and hence plays a key role in the drug metabolism in our body. Amino acid mutations originated from single nucleotide polymorphisms (SNPs) at genetic level often enhance or impair enzyme activity of CYP, raising a serious problem in clinical use of drugs. Although CYP2C19 isoform is a minor hepatic CYP, it mainly metabolizes basic drugs such as omeprazole and propranolol. In this paper, we focused on WT and SNP mutants (E92D, R120W, R132Q, and I331V) of CYP2C19, and structural change in the heme vicinity in the absence and presence of substrates was studied by the use of resonance Raman spectroscopy. Resonance Raman spectra of drug-free WT and mutants were essentially the same in both ferric and ferrous CO-bound states, indicating that the effect of SNP mutations on the heme structure is minimal. The ferric enzyme was revealed to be in the six-coordinated high-spin state, supporting the water coordination at the iron sixth site. Addition of a substrate, however, resulted in the appearance of five-coordinated species, the degree of which being dependent on the mutations. On the other hand, the Fe-CO stretching frequency in the CO-bound state was dependent on the chemical structure of drugs. These results indicate that the SNP mutations and the drug binding induce structural change in the heme environments, which may result in the change in metabolic activity of CYP2C19.

1P156**Thermodynamic analysis of cofactor binding to the reductase domain of neuronal nitric oxide synthase**

O Ikuko Sagami, Ryuhei Sanae, Fumiaki Kurokawa, Yusuke Izutani, Masayuk Oda

Dept. of Biofunction Chemistry, Grad. Sch. of Agri, Kyoto Pref. Univ.

Neuronal NOS (nNOS) is regulated by the reversible binding of Ca^{2+} /calmodulin (CaM) to the site located between the N-terminal oxygenase domain and the C-terminal reductase domain. Ca^{2+} /CaM binding activates electron transfer from NADPH to FAD, FMN in the reductase domain and also electron transfer between the reductase domain and the oxygenase domain. Ca^{2+} /CaM also activate the reduction of external electron acceptors such as cytochrome *c* in the reductase domain. It has been suggested that NADPH binding induces a conformational change in the nNOS reductase domain, preventing electron transfer to the oxygenase domain and cytochrome *c*, and CaM binding reverses this effect. To understand the conformational change caused by cofactor binding, we analyzed the interaction between 2', 5'-ADP or NADP^+ and the isolated reductase domain of nNOS with (RedCaM) or without (Red) CaM binding site by using isothermal titration calorimetry. Interaction between 2', 5'-ADP and Red or RedCaM was thermodynamically observed as an exothermic reaction with the calculated binding stoichiometry of 1. The K_a value of 2', 5'-ADP for Red was larger than that for RedCaM, suggesting the CaM binding site itself might interfere the binding. In 2', 5'-ADP binding to RedCaM, Ca^{2+} /CaM addition did not affect the thermodynamic parameters. On the other hand, the binding affinity of NADP^+ to RedCaM is significantly smaller than that of 2', 5'-ADP. We will discuss the results in comparison with those of cytochrome P450 reductase recently reported by other group.