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Comparative study of specific and non-specific interactions between bio-macromolecules and ligands

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Abstract. Interactions between inhalation anesthetics and three kinds of bio-macromolecules, firefly luciferase (FFL), bovine serum albumin (BSA) and dipalmitoylphosphatidylcholine (DPPC) bilayer membrane were compared with the corresponding ones between long-chain fatty acids and the bio-macromolecules. The influence of the anesthetics on FFL and DPPC bilayer membrane antagonized against that of the fatty acids. On the contrary, the influences of both ligands on BSA synergized, however, that of the anesthetics was remarkably smaller than that of the fatty acids. The results are attributable to the difference in ligand receptivities, that is, molecular-recognition abilities of the bio-macromolecules. The present study showed the importance of non-specific interactions in anesthetic mechanism.

Keywords: Bovine serum albumin; Dipalmitoylphosphatidylcholine; Firefly luciferase; Inhalation anesthetic; Long-chain fatty acid

1. Introduction

Anesthetics affect biological membranes and induce the action. However, whether the anesthetic action originated from specific interactions between anesthetic molecules and membrane-constituting bio-macromolecules such as channel proteins and phospholipids

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bilayers or from non-specific interactions between them has not yet been determined and the argument continues. For a better understanding of the anesthetic action, it is useful to show the fundamental difference in specific and non-specific interactions between small ligands and the bio-macromolecules by comparing both interactions. Two kinds of small ligands, inhalation anesthetics such as halothane and isoflurane and long-chain fatty acids such as myristic acid and palmitic acid, are selected for this purpose. Comparative studies of the ligand effects on three model systems of bio-macromolecules, two proteins, firefly luciferase (FFL) and bovine serum albumin (BSA) and one membrane lipid, dipalmitoyphosphatidylcholine (DPPC) bilayer membrane, are described.

2. Ligand effects on FFL

FFL is a hydrophobic protein and it emits a light through a reaction with substrate luciferin. Ueda and Suzuki [1,2] have performed comparative studies of anesthetics and fatty acids on FFL. Both ligands inhibited light emission of FFL dose-dependently, however, their inhibition modes were significantly different. The anesthetics inhibited in mM order while the fatty acids inhibited in μM order. The Hill numbers for the ligands, which were calculated from their dose–response curves, were greater than 1 for the anesthetics while they were smaller than 1 for the fatty acids, that is, anesthetics inhibit FFL with positive cooperativity while fatty acids inhibit with negative cooperativity.

Proteins denature with increasing temperature. Anesthetics and fatty acids were found to cause different changes in the denaturation temperature of FFL [1,2]. The anesthetics decreased the denaturation temperature at mM ranges dose-dependently. Whereas the fatty acids conversely increased the denaturation temperature at μM ranges, also dose-dependently. Similar elevation of the denaturation temperature at μM ranges was observed for a strong luciferin competitor oxyluciferin in the presence of ATP [1].

One of the characteristics of anesthetic phenomenon is the pressure reversal of anesthesia. We investigated the high-pressure effect on light intensity of ligand-doped FFL [3]. High pressure increases the light intensity of halothane-doped FFL; on the contrary, it decreases the intensity of myristate-doped FFL. The volume changes of FFL by binding of two ligands were thermodynamically calculated from the Johnson and Eyring plots. The volume-change value was positive for halothane but negative for myristic acid, respectively. The contrasting result indicates that the effect of anesthetics is antagonized by pressure but that of fatty acids is synergized by pressure.

3. Ligand effects on BSA

BSA is a very hydrophilic protein in contrast to hydrophobic FFL although they have similar molecular weights to each other. BSA is also well known as a unique protein because BSA induces the structural transitions by changing the solution pH: the N (normal)–F (fast) transition occurs at acidic pH and the N–B (basic) transition occurs at alkaline pH. We measured the thermal denaturation temperature of BSA in the presence of anesthetic halothane by a method of differential scanning calorimetry (DSC) [4]. Halothane elevated the denaturation temperature of N state BSA at pH 7.0 [4,5]. At pH 8.0, although the thermal peak of pure BSA became small and shifted to the lower temperature region, the peak recovered by adding halothane. The increase in pH reduces