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John Edsall's influence

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1. Encounters with John Edsall

My first meeting with John Edsall was in Cambridge when I was a postdoctoral fellow with Max Perutz working on the structure determination of hemoglobin at 5.5-Å resolution. However, as John Kendrew and his colleagues had solved the structure of myoglobin not long before and were currently extending the resolution of the map to 2.0 Å, their results were the major topic of conversation. These discussions barely involved me because I was still very much an outsider in the world of biochemistry. My interests were in solving challenging and interesting crystal structures using my knowledge of physics, mathematics, and early electronic computers. Nevertheless, I was aware of the importance my more knowledgeable colleagues placed on the visit by John Edsall.

Another 2 years or so passed before I would see John Edsall again. By this time, the low-resolution structure of hemoglobin had been determined and I had started to become fascinated by its biological implications. I think it was the evolutionarily significant discovery that the α and β chains of hemoglobin and myoglobin had similar structures that stimulated me to attend undergraduate biochemistry classes. But even without that stimulation, it was difficult to escape the scientific excitement that always accompanied Francis Crick in the confined space of the MRC 'hut' where we worked and congregated. In addition, Max had made numerous, kindly attempts to educate me. Thus, when I had the opportunity to visit Harvard to see the Colonel (Bill Lipscomb), my first postdoctoral mentor, John very kindly invited me to give a seminar on the recent hemoglobin discoveries. Unfortunately, my fascination with the crystallographic technology was still dominant in my thinking, causing me to give a seminar that was surely of little interest to the assembled biochemists. But, in spite of my lack of biochemical knowledge, John was, as always, a perfect host and never hinted that he might have expected to be given greater insight into the complex functions of hemoglobin.

In 1971, my wife and I spent half a year in Uppsala while I was on sabbatical leave. I had decided to learn something about virus crystallography by spending my sabbatical leave in Bror Strandberg's laboratory, housed in the Wallenberg building, a kind of research hotel run by Uppsala University, for unclassifiable biological research groups. Bror had been part of the myoglobin team with John Kendrew at the time that I was working with Max in Cambridge. Bror, Dick Dickerson (also part of the Kendrew team), and I had been office mates in the MRC 'hut'. Subsequently, Dick returned to the US and Bror to Sweden to establish their own protein structure laboratories, while I remained in Cambridge for another 5 years before starting my laboratory at Purdue University. Although Bror and

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I had both wanted to study viruses, neither of us felt the time was ripe in our initial independent studies. Bror decided to work on carbonic anhydrase and I selected dehydrogenases utilized in glycolysis. By the time I arrived in Uppsala, I had gained some experience and knowledge of protein structure. At that time, Anders Liljas was finishing his Ph.D. studies of carbonic anhydrase while working with Bror. This was the first protein structure in Sweden and (together with Carl Brändén's work on alcohol dehydrogenase at the nearby Agricultural University) established the great Swedish Structural Biology Center in Uppsala. I was honored to be asked to be a member of Anders' Ph.D. examining committee, which also included John Edsall. In those days, a Swedish Ph.D. was not only a major event, but also a major accomplishment requiring many years of study. This required that both John and I had to study carefully all components of Anders' work. For me this was a significant learning experience, but I think for John it was just a little extension of his already existing deep appreciation of how enzymes work and why their activity is required in specific cells.

I have chronicled my gradual awakening to an appreciation of biological sciences in terms of my early encounters with John Edsall. Thus, it would seem fitting to dedicate to John this account of one of the interests of his occasional student.

2. Dengue virus

The flaviviruses (Latin *flavus* meaning yellow because of the jaundice induced by yellow fever virus) comprise a large genus of medically-important, arthropod-transmitted, enveloped viruses with members that include yellow fever, dengue, West Nile, tick-borne encephalitis (TBEV), and Japanese encephalitis viruses [1,2]. Dengue virus is one of the most significant human viral pathogens transmitted by mosquitoes and causes 50 million or more cases of infection worldwide each year, resulting in approximately 24 000 deaths [3]. Infection is usually characterized by fever and severe joint pain, but more serious syndromes, dengue hemorrhagic fever or dengue shock syndrome, occur sometimes following dengue infection. Dengue hemorrhagic fever was mostly confined to Southeast Asia until the 1960s, when it also became endemic in Central America and, more recently, in South America. There are four distinct serotypes of dengue virus, and it has been postulated that hemorrhagic fever, or shock syndrome, is usually the result of sequential infection with multiple serotypes. Although vaccines have been developed for several flaviviruses, control of dengue virus through the use of vaccination has proved to be elusive [1].

Electron micrographs showed that dengue virions are characterized by a relatively smooth surface, with a diameter of approximately 500 Å, and an electron-dense core surrounded by a lipid bilayer. In addition to the plus-sense RNA genome of ~10 700 nucleotides, there are three structural proteins that occur in stoichiometric amounts in the particle: core (C, 100 amino acids), membrane (M, 75 amino acids), and envelope (E, 495 amino acids). The atomic structure for the homologous E protein of TBEV has an elongated shape consisting of a central domain (I) that connects an Ig-like domain (III) to a dimerization domain (II) [4]. Based on the shape of the molecule and the location of antibody epitopes, Rey et al. [4] postulated that the E protein would lie flat along the surface of the virus lipid bilayer.

Alphaviruses (including Sindbis, Semliki Forest, and Ross River viruses) and flaviviruses were formerly considered genera within the togavirus family because of their similar structural organization. However, they differ in gene order and replication strategy and are now classified into separate families [5]. In contrast to the smooth surface of the flaviviruses, the alphaviruses have prominent spikes that project from the bilayer membrane [6]. Despite these differences, recent studies [7,8] have shown that there is a close structural and functional relationship between the flavi- and alpha-virus E and E1 glycoproteins, respectively. These glycoproteins participate in the process of membrane fusion [9,10] and form icosahedral scaffolds.

In collaboration with Richard Kuhn, Jim Strauss, and Tim Baker [11], we determined the structure of dengue virus type 2 S1 strain, a vaccine candidate derived from the PR-159 isolate [12]. A three-dimensional image reconstruction at 24-Å resolution obtained by using cryo-electron micros-

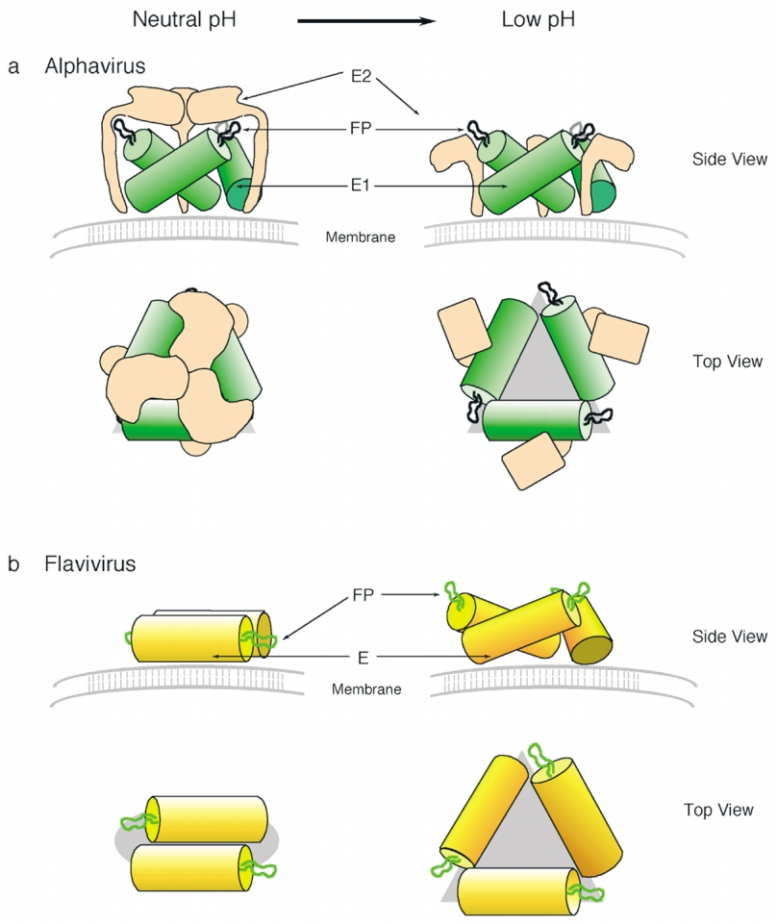

Fig. 1. Configuration of glycoproteins of alphaviruses (a) and flaviviruses (b) on the surface of virions at neutral pH (left column) and the proposed configuration at acid pH (right column). In (a), E1 glycoproteins are shown as green cylinders, E2 glycoproteins as tan shapes, and the fusion peptide as a black curved line. In (b), the E glycoproteins are shown as yellow cylinders with the fusion peptide as a green curve. In both cases, the membrane is shown in gray.

copy (cryoEM) and image reconstruction showed that the virion has a well-organized outer protein shell, a lipid bilayer membrane, and a less-well-defined inner nucleocapsid core. The known atomic structure of the homologous E protein dimer of TBEV [4] has been fitted into the outer layer of density in cryoEM reconstruction. The icosahedral scaffold consists of 90 such dimers with three monomers in the icosahedral asymmetric unit, but lacking $T=3$ quasi-equivalent environments [13]. The glycoprotein organization suggested a common, class II fusion mechanism for alpha- and flaviviruses based on the insertion of a β-barrel-type structure into the host cell membrane. Class I and II fusion proteins were first differentiated by Lescar et al. [7] based on a description of a variety of properties related to the activation and position of the fusogenic peptide. In contrast, our results point to structural and mechanistic differences between these fusion classes (Fig. 1).

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