Graduate Studies

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Biophysics of Complementary Molecules

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The research in my laboratory focuses on the physical properties of molecules that bind as complementary pairs, and the physiological consequences of that binding. We use a wide range of different techniques to analyze complementary interactions, including capillary electrophoresis, muscle mechanics, enzyme kinetics, nuclear magnetic resonance and differential UV spectroscopy. Our analytical techniques also extend to theoretical calculations using diffusion models as well as catastrophe theory, used to assess state changes in biological systems. Our initial interest in molecular complementarity arose when we found that pyruvate kinase and creatine kinase directly couple and exchange ATP, defining a new pathway of muscle energy utilization. Subsequent studies discovered a novel method for measuring dissociation constants using electric fields; a highly sensitive method for measuring tissue metabolites using nanoliters of extract; quantitation of a wide range of molecular pair interactions; demonstration that membrane electric field dissociation of complementary complexes is molecular size dependent; measurement of the ascorbate dependence of catecholamine activity, including the underlying mechanism for the cardiovascular consequences of sympathomimetics such as ephedrine; and developing patents for new treatments for circulatory shock.

The figures on the right demonstrate a few of the different results that have led to above advances. Figure 1 shows a series of sigmoidal binding curves of norepinephrine and ascorbate at different electric fields using capillary electrophoresis (CE). CE has always been used to separate molecules. We theorized that if two molecules are bound at zero electric field and separated at high electric field, there must be an electric field where they are half-bound and half-free, defining the dissociation constant in an electric field. We showed that this supposition is correct, and by extrapolating the dissociation constants to zero electric field, we produced the true dissociation constant for the molecular pair.

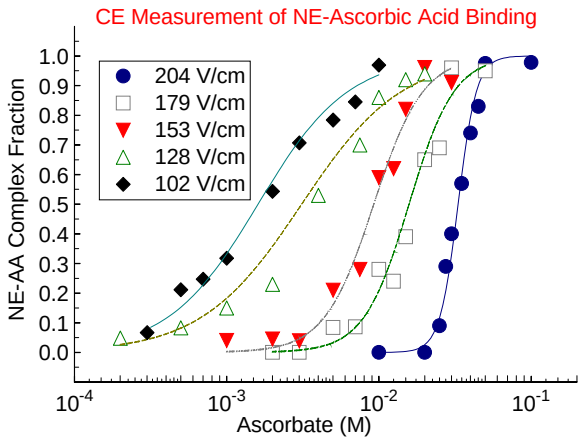

Figure 1.

Our measurement of the binding constant for ascorbate and NE showed that most NE will be ascorbate bound *in vivo*. This should have physiological consequences, and we showed ascorbate enhances the sensitivity of catecholamines by a mechanism in which ascorbate binds to the adrenergic receptor. Figure 2 shows that at a concentration of NE that is barely sufficient to produce any force in vascular smooth muscle, there is a significant increase in NE-induced force in the presence of ascorbate. We found similar results using the sympathomimetics PPA and ephedrine, demonstrating an underlying basis for the severe cardiovascular consequences of these now-banned substances. The addition of ascorbate to treatments that use catecholamines, such as the infusion of epinephrine during circulatory shock, holds the promise of increased tissue responsiveness with reduced pharmacological side effects.

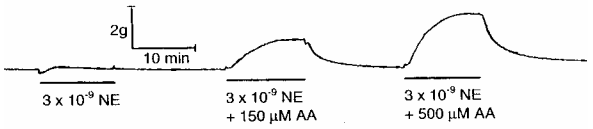

Figure 2.

It is known that the cell membrane produces an electric field within 10 nm of the membrane surface that is far greater than the electric field we showed is sufficient to separate complementary pairs. Molecules that move through the body bound together will separate as they approach a membrane, freeing the molecules to bind to cell surface receptors, while being protected from oxidation or enzymatic degradation as they circulate in a bound form. Figure 3 shows the relationship between the electric field/dissociation slope dependence and the radius of the paired molecules, including two small molecule pairs (NE-ascorbate, NE-morphine sulfate), two small proteins (Insulin/Glucagon) and a larger protein/small molecule pair (Epinephrine/Bovine Serum Albumin). The plotted relation shows that larger molecules shield their pair binding site from the electric field, and defines the molecular shielding constant.

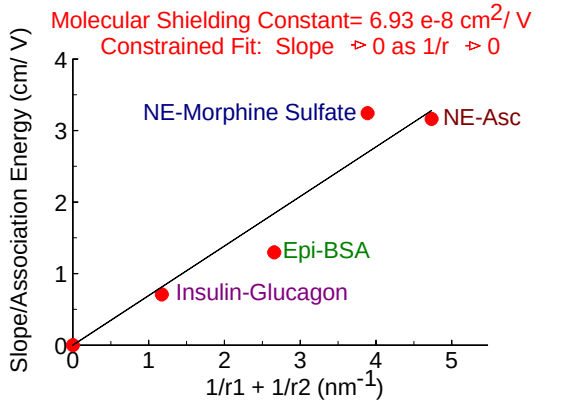

Figure 3.

The wealth of novel results generated by our lab makes every subsequent project exciting and highly anticipated. Our current work focuses on the binding, membrane electric field dissociation and physiological effects of smooth muscle agonists in cardiovascular and pulmonary tissues.