Molecular Shielding of Electric Field Complex Dissociation

Patrick F. Dillon, Robert S. Root-Bernstein, and Charles M. Lieder
Department of Physiology, Michigan State University, East Lansing, Michigan 48824

ABSTRACT We have previously demonstrated the ability of electric fields to dissociate ascorbate and catecholamines and shown that the electric field generated by cell membranes is sufficient to produce dissociation of these complexes up to 8 nm from the cell membrane. We show here that this process is applicable to a wide range of biological complexes including small molecules (norepinephrine-morphine sulfate), protein-protein complexes (insulin-glucagon), and small molecule-protein complexes (epinephrine-bovine serum albumin). The extrapolation of the slope of the electric field dependence to zero electric field can be used to estimate the log of the dissociation constant (K_D) of a complex and, by multiplying the log(K_D) by $-2.303RT$, the association energy (E) of the complex. The slope of the electric field dependence is inversely related to the molecular radii, with the best fit of the slope related to $E^*(1/r_1 + 1/r_2)$, where r is the estimated radius of each molecule in the complementary pair. This indicates that the binding site of the pair is shielded by the remaining parts of the molecules, and the larger the molecule the greater the shielding. When the slope of the electric field dependence goes to 0 as r goes to infinity and $1/r$ goes to 0, the molecular shielding constant is 7.04×10^{-8} cm^2/V. Very large complexes will be minimally affected by the electric field due to molecular shielding and reduced electric field as their radius restricts approach to the membrane. Large protein receptors will deflect the membrane electric field and allow agonist binding.

INTRODUCTION

In a previous work we have shown that an electric field can be used to measure the dissociation constant between two molecules in solution and, further, that the electric field produced by a membrane is more than sufficient to produce dissociation of molecular complexes as they approach within 8 nm of the membrane (1). These findings were based on experiments using norepinephrine (NE) and related catecholamines binding to ascorbate (Asc) and related compounds. These findings left several areas unexplored. First, was this a general phenomenon, available to affect other small molecule complexes, complexes of small molecules and proteins, and protein-protein complexes? Second, what quantitative differences might appear between complexes having these different compositions? Third, were there general rules that might be derived based on the interaction of these different complexes with an electric field? Fourth, how would the membrane electric field affect the binding of membrane receptors to agonists carried into their vicinity by carrier molecules in complex with the agonist?

In testing these questions we ascertained the effect of electric fields on the small molecule-small molecule complex NE-morphine sulfate (MS), the small molecule-protein complex epinephrine-bovine serum albumin (Epi-BSA), and the protein-protein complex insulin-glucagon (IG). The known binding of NE and MS (2) may play a role in both the therapeutic (3) and abusive (4) effects of morphine. The known binding of Epi to albumin (5) protects Epi from oxidation and enzymatic degradation as it passes through the circulatory system. Yet, there still must be dissociation of the complex for Epi to have its hormonal effects. The data show that for the measured dissociation constant of NE-Asc binding, a significant fraction of NE will circulate bound to Asc (1). In contrast, it has been shown that insulin and glucagon can bind to one another (6) but with a dissociation constant determined here that is well above both their physiological concentrations. Thus, although this is a useful model system for measuring protein-protein binding, it can also preclude the binding of insulin and glucagon under physiological conditions.

The positive results of the experiments using these complexes allowed us to make several unique conclusions. These include the quantitative expression of the molecular shielding constant, the degree to which a molecular complex shields its binding sites from an electric field based on the association energy of the complex and the molecular radius of the complex components. Also, having ascertained the behavior of different sized molecules in the electric field, we were able to conclude that complexes of very large proteins have such a large amount of shielding and limited approach to the membrane that they are not substantially affected by the membrane electric field. The consequences of our results are discussed below.

METHODS

The methods employed in measuring the dissociations of NE-Asc, IG, NE-MS, and Epi-BSA have been described previously for NE-Asc (1).

Solutions

All solutions were prepared in the buffer which was used in the particular capillary electrophoresis (CE) experiment, 25 mM sodium borate 10-hydrate ($Na_2B_4O_7{\cdot}10\ H_2O$) at pH 9.4. NE, Asc, MS, Epi, BSA, insulin, and

Submitted August 3, 2005, and accepted for publication October 27, 2005.

Address reprint requests to Patrick F. Dillon, Tel.: 517-355-6475, ext. 1102; Fax: 517-355-5125; E-mail: dillon@msu.edu.

0006-3495/06/02/1432/07 $2.00

doi: 10.1529/biophysj.105.071969

glucagon were purchased from Sigma-Aldrich Chem. Co. (St Louis, MO). The BSA is Sigma A7409, Initial Fractionation by Heat Shock, 35% in 0.85% sodium chloride. All chemicals were made as stock solutions and diluted to the required concentration in the CE injection vials. The NE-Asc dissociation constants were determined using 1 mM NE and altering the concentration of Asc between 0 and 100 mM. The NE-MS dissociation constants were determined using 1 mM NE at 204 and 255 V/cm and 0.1 mM at 153 V/cm and altering the concentration of MS between 0 and 40 mM. The IG dissociation constants were determined using a constant concentration of 20 μM glucagon and altering the concentration of insulin between 0 and 1.6 mM. The reciprocity of the IG binding was demonstrated using 20 μM insulin and 0, 19, and 190 μM glucagon. The Epi-BSA dissociation constants were determined using a constant concentration of 40 μM Epi and altering the concentration of BSA between 0 and 2.32 mM.

CE procedures

Samples were vacuum injected into a 100-μm diameter, 98-cm length capillary tube (volume 7.7 μl) for 2 s (injection volume 8.6 nl/s). The capillary tube had a detection window 66 cm from the injection site in an ISCO (Lincoln, NE) model 3850 electropherograph with an absorbance detector at 195 nm, absorbance maximum from 0.02–0.2 absorbance units, and a rise time of 3.2 s. The driving voltage was varied between 5 and 25 kV, corresponding to electric fields of 51 and 255 V/cm. The peaks were recorded on a chart recorder (The Recorder Company, Houston, TX) at 1 cm/min and 1 V full scale.

Molecular radius

Estimates of molecular radius are based on the model of the NE-Asc complex (7) and the CPK molecular model system (8) of 1.25 cm/Å. For the 345 g/mol NE-Asc complex, the complex volume is estimated to be 0.63 nm^3. The relationship between molecular weight (MW) and volume is therefore 0.00183 (MW) = nm^3. For a sphere of volume $4\pi r^3/3$, this corresponds to a radius of 0.53 nm for the NE-Asc complex. The radii for the other molecules and complexes are calculated in the same way.

Data analysis

The constant molecule (NE, Epi, or glucagon) was always prepared and run as a standard. For each complex, the initial electric field dependent dissociation constant was calculated by plotting the log of the varied molecule concentration against the log of the constant-varied complex/free constant ratio and applying a least-squares best fit for the linearization and 95% confidence interval using the Axum data processing system. The initial dissociation constant at a particular electric field, the K_E, was estimated from the linearization at the 0.5 complex-0.5 free point. The initial K_E = [free constant][free varied]/[constant-varied complex]. The [free constant] equals the [constant-varied complex] at the K_E. Thus, the true K_E is the [free varied], which is equal to the [total varied] – 0.5[total constant], and this was the value used for the K_E. The estimate of the dissociation constant at zero electric field, the K_D, was made by extrapolating the log of the K_E values at a given electric field to zero electric field. The binding energy of the complexes was calculated by multiplying the log(K_D) by −2.303RT for the determination of J/mol at 298 K.

Membrane dissociation

The membrane potential of a membrane decreases exponentially with a Debye-Hückel length constant of 1 nm and a voltage change of 30 mV (9–11). The electric field is the length derivative of the voltage decay (10,11). Having ascertained the relationship of slope (σ) (converting V/cm into mV/nm) and K_D between electric field and dissociation constants for a complex pair, the dissociation constant at a given distance (x) from a membrane with a change in voltage (V_0) and a length constant (λ) (1) is

$$\log(K_E)_x = \sigma(1/\lambda)V_0\exp(-x/\lambda) - \log(K_D).$$

This equation can be rearranged to calculate the distance at which different changes in K_D occur. This is done in Table 1 for 10-, 100-, and 1000-fold changes in the dissociation constant as the four different complexes approach the membrane.

RESULTS

Fig. 1 shows the electropherograms of NE, Asc, and the combination of NE and Asc from Dillon et al. (1). Both NE and Asc run as a single peak, with Asc passing the detection window later due to its more negative charge/mass ratio than NE. When present together at pH 9.4, a third peak appears to the left of the original NE peak position. This peak has been shown to vary its size relative to the Asc concentration, increasing as the Asc concentration increases, with a concomitant decrease in the free NE peak (1). It is a complex of NE and Asc. At pH 7.4, both the complex and free NE run at the same time, with the height of the peak changing with the concentration of Asc. The dissociation constants for the two pH values are very similar (1). The dissociation constant for NE-Asc varies with the applied voltage, shown in the lower panel of Fig. 1. As the voltage increases, an increasing amount of Asc is needed for the NE-Asc complex to form, countering the dissociative effects of the electric field.

The reciprocal nature of the electric field dissociation of molecular complexes is shown in Fig. 2. Free glucagon and free insulin appear as single peaks when run alone. When run together at the same concentration, there is a decrease in the size of the glucagon peak and an increase in the size of the insulin peak. A 10-fold increase in the glucagon concentration produces a larger increase in the size of the insulin peak, and the 10-fold increase in the insulin concentration produces a larger decrease in the glucagon peak. The size of the glucagon peak is plotted against the insulin concentration at two different electric fields in the lower panel. Note that despite having a greater range of electric fields than the NE-Asc peak titration curves in Fig. 1, the IG curves are much closer together. The electric field has a smaller effect on the dissociation of IG than it had on NE-Asc.

Electropherograms and individual dissociation curves for NE-MS and Epi-BSA are shown in Figs. 3 and 4. In the NE-MS titration in Fig. 3, the NE peak decreases as the MS

TABLE 1 Membrane dissociation of molecular complexes

Complex	Radius nm	Increased dissociation distance (nm)		
		10-fold	100-fold	1000-fold
NE-Asc	0.53	8.37	7.68	7.28
NE-MS	0.71	8.53	7.83	7.43
Ins-Glu	2.15	6.89	6.20	5.79
Epi-BSA	2.97	7.29	6.60	6.19

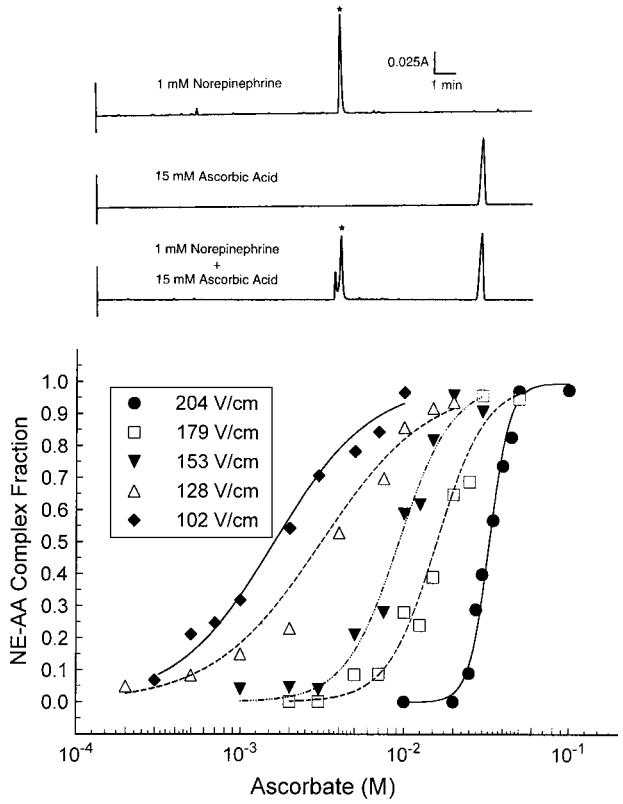

FIGURE 1 (*Top panel*) NE-Asc complex formation. Separate injections of NE and Asc produce single peaks. A new peak appears when they are mixed and injected together. The * indicates free NE. (*Bottom panel*) Electric field dependence of NE-Asc binding. Data points and best fit lines for the titration of 1.0 mM NE by Asc at different electric fields. At greater electric fields, more Asc is needed to saturate NE. The K_Es for 102, 128, 153, 179, and 204 V/cm are 1.1, 2.6, 8.3, 14.8, and 32.4 mM, respectively.

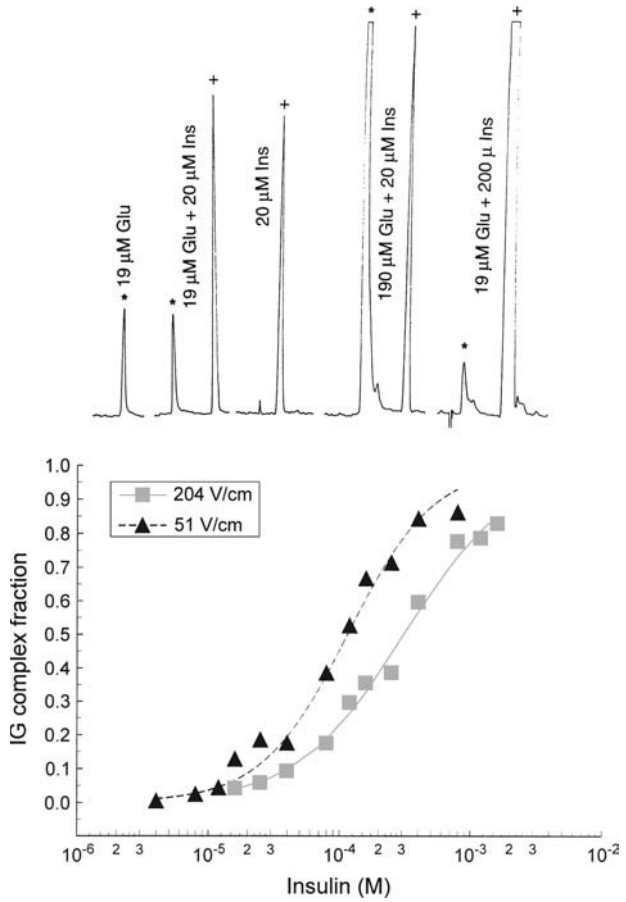

FIGURE 2 (*Top panel*) Electropherograms of insulin and glucagon. The * indicates the glucagon peak. The + indicates the insulin peak. When present together, the molecules bind with a reduction in the glucagon peak and increase in the insulin peak. (*Bottom panel*) Titration of 20 μM glucagon with insulin in electric fields of 204 and 51 V/cm. The squares indicate the 204 V/cm data and the triangles indicate the 51 V/cm data. The K_Es for 51 and 204 V/cm are 95 and 295 μM, respectively. At higher electric fields more insulin is needed to bind glucagon to overcome the dissociating effect of the greater electric field.

increases. The effect of NE on MS can also be seen by comparing the rightmost peak of the MS triplet at zero NE, on the right of the electropherogram stack, to the same rightmost peak in the presence of NE. When NE is present, the rightmost peak is smaller. In Fig. 4, the Epi decreases in height and broadens as the concentration of BSA increases. It is well known that Epi binds to BSA (5). This CE procedure allows a novel way to measure the dissociation constant for that binding.

Fig. 5 shows the calculated K_Es for the different paired complexes at different electric fields and the linear intercepts at zero electric field, the K_Ds. The complex pairs having two small molecules, NE-Asc and NE-MS, have much greater slopes than those complex pairs with proteins, insulin-glucagon, and Epi-BSA. Note that the two complexes with protein and the NE-Asc complex have very similar K_Ds, whereas the K_D for NE-MS is much lower. The second ordinate shows the association energy for the complexes. There are five binding sites for the NE-Asc complex, four hydrogen bonds and one π-π bond (7), each with ~5 kJ/mol (12,13). The calculated association energy of 25.2 kJ/mol from the intercept closely approximates this. Despite the complex nature of the proteins in the IG and Epi-BSA complexes, the similar dissociation constants can be inferred to indicate similar binding energies.

It has been shown that the electric field generated by a membrane is sufficient to cause dissociation of molecular complexes as they approach the membrane (1). Table 1 compares the size of the four molecular complexes and the distances from the membrane at which there would be 10-, 100-, and 1000-fold increases in the dissociation constant, leading to easier dissociation. In all cases, the molecular radius of the complex pair is much less than the distance from the membrane that produces significant changes in the dissociation constant resulting in enhanced dissociation of the complex.

As the change in dissociation produced by the membrane requires that the complex approach the membrane, very large molecular complexes will not be able to approach the

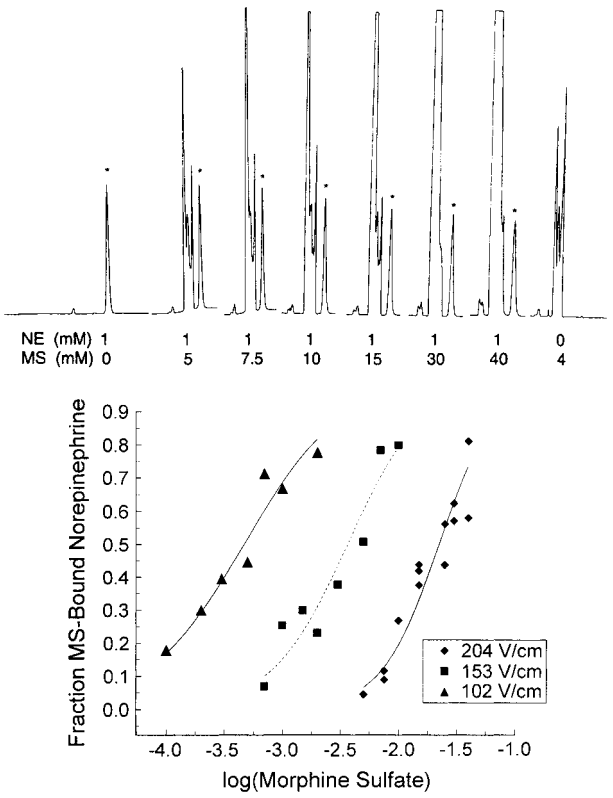

FIGURE 3 (*Top panel*) Electropherograms of NE and MS. The * indicates the NE peak. When present together, the molecules bind with a reduction in the NE peak and a decrease in the rightmost of the three MS peaks. (*Bottom panel*) Titration of 1 mM NE with MS in electric fields of 102 (▲), 153 (■), and 204 (♦) V/cm. The K_Es for 102, 153, and 204 V/cm are 0.42, 3.02, and 21.9 mM, respectively.

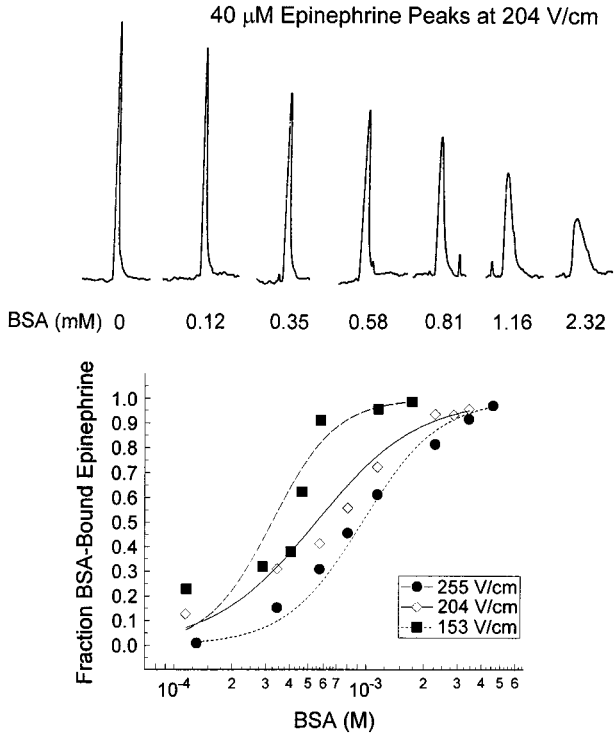

FIGURE 4 (*Top panel*) Electropherograms of Epi in the presence of different concentrations of BSA. There is a progressive decrease in the Epi height as the BSA increases. (*Bottom panel*) Titration of Epi with BSA at 153 (■), 204 (◇), and 255 (●) V/cm. The K_Es for 153, 204, and 255 V/cm are 302, 550, and 955 μM, respectively.

membrane sufficiently closely to increase dissociation significantly. Fig. 6 shows the log of the complex radius plotted against the log of the distance necessary for a 10-fold decrease in the dissociation constant. At the x intercept, the radius, calculated from the log(radius), will be the largest size a complex can be and still have a 10-fold change in dissociation. This size corresponds approximately to an 800,000 MW complex.

Table 2 shows the relation between the different elements of these experiments and the slope of the electric field dissociation. The association energy alone and the total MW alone of the two molecules of the complex are not good indicators of the slope. The best fit, with a correlation coefficient of 0.90, relates the sum of the inverse of the radius of each molecule multiplied by the association energy to the slope.

The molecular shielding constant is calculated in Fig. 7. The best fit equation in Table 2 has been rearranged, dividing each side by the association energy, and the four complexes plotted as a function of their electric field dependent slope, association energies, and inverse radii. It is clear from the equation that the slope will become zero as $1/r$ approaches zero, or as the radius becomes infinite. This is also intuitively satisfying, as a very large radius will shield the complex binding sites from the electric field. Constraining, therefore, the line to go through zero ($C = 0$ in the model), the molecular shielding constant (the slope in Fig. 7) is 7.04×10^{-8} cm^2/V.

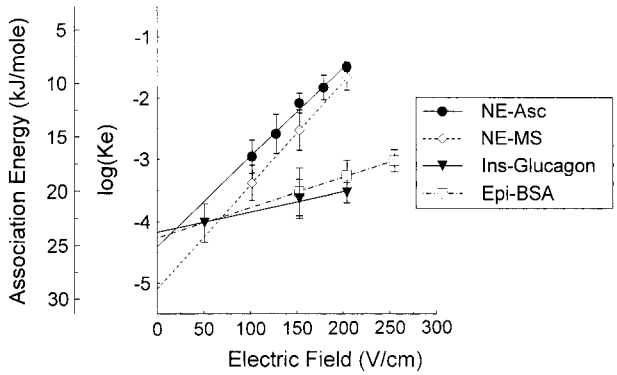

FIGURE 5 Comparative electric field dependence of dissociation constants and association energy of the complementary pairs NE-Asc, NE-MS, insulin-glucagon, and Epi-BSA. Their K_Ds are 39, 8, 68, and 55 μM, respectively. Different pairs show different electric field dependent slopes.

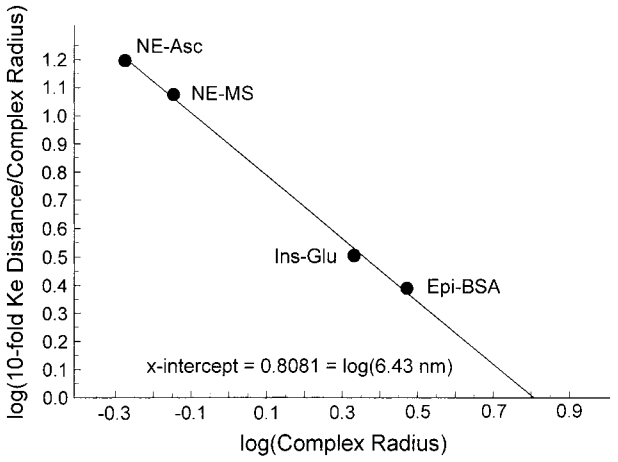

FIGURE 6 Maximum complex size for 10-fold increase in K_D by membrane electric field. As the complex radius increases, the binding site cannot approach the membrane (distance/radius = 1, $\log(d/r) = 0$). Complexes of large protein pairs (MW 800,000 ~7.0 nm radius) will have negligible membrane electric field dissociation due to high molecular shielding and restricted approach to the membrane.

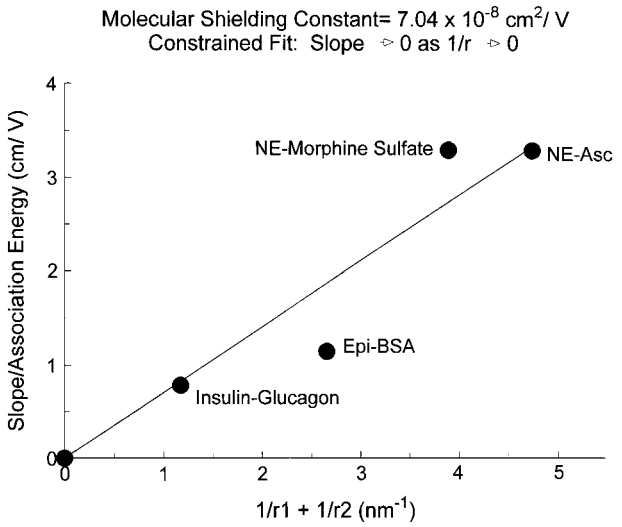

FIGURE 7 Calculation of the molecular shielding constant in an electric field (E). The best fit linear model was constrained such that as $1/r$ approaches zero (radius goes to infinity), the electric field dependent slope (σ) goes to zero as the binding sites are completely shielded from the electric field. The molecular shielding constant is $\sigma/E/(1/r_1 + 1/r_2) = 7.04 \times 10^{-8}$ cm^2/V.

DISCUSSION

In originally using capillary electrophoresis to measure dissociation constants, all the molecules tested were catecholamines and related compounds binding to Asc and related compounds (1). These experiments were designed to test the range of molecules over which this method could be used (Figs. 1–4). NE-MS (2), insulin-glucagon (6), and Epi-BSA (5) have all been known to form complementary complexes. These complexes were chosen because they represented a small molecule-small molecule complex (similar to the catecholamine-Asc system), a small molecule-protein complex, and a protein-protein complex. All demonstrate that CE can be used for more than just separating molecules based on their charge/mass ratio: because CE does not denature molecules, intermolecular interactions can also be measured.

The multiple examples of molecular coupling show that the CE method of measuring dissociation constants is general. When holding one molecule concentration constant, changes in the concentration of its complementary molecule result in peak changes to the first molecule. CE causes molecules to separate in an applied electric field. If two molecules are bound at zero electric field, and separated at high electric field, there must be an electric field at which they are half-bound and half-free. Further, as the electric field increases, increasing the force of separation, then the concentration of complementary molecule needed to maintain half of the first molecule in the complexed form must also increase. Fig. 5 shows that this is the case for all these combinations of molecules tested here. Extrapolating to zero electric field defines the dissociation constant, K_D, in free solution.

TABLE 2 Models of electric field slope dependence on association energy (E), molecular weight (MW), and molecular radius (r)

$\text{Slope} = k \times 1/\text{MW}_{\text{total}} + \text{C}$	$R^2 = 0.60$
$\text{Slope} = k \times (1/\text{MW}_1 + 1/\text{MW}_2) + \text{C}$	$R^2 = 0.63$
$\text{Slope} = k \times E + \text{C}$	$R^2 = 0.68$
$\text{Slope} = k \times 1/r_{\text{total}} + \text{C}$	$R^2 = 0.73$
$\text{Slope} = k \times (1/r_1 + 1/r_2) + \text{C}$	$R^2 = 0.79$
$\text{Slope} = k \times (1/r_1 + 1/r_2) \times E + \text{C}$	$R^2 = 0.90$

The insulin-glucagon and NE-MS electropherograms in Figs. 2 and 3 show that peak changes can be reciprocal. Just as insulin causes the glucagon peak to decrease, so too does glucagon cause the insulin peak to increase. In the case of NE-MS, however, just as the NE peak decreases with an increase in MS, the rightmost peak of the MS complex decreases in the presence of NE. Thus, one cannot make a priori judgments on the direction a peak change will take when its complement is present. Molecular coupling would normally be expected to produce changes in the peak characteristics, although there could be circumstances in which this does not occur.

We have shown that NE and Asc are connected by four hydrogen bonds and one π-π interaction (7). Hydrogen bonds and π-π bonds have similar energy (13). In aqueous solutions, hydrogen bonds must compete with water molecules, and hydrogen bonds between nonwater molecules have a strength of 5 kJ/mol (12). The binding energy we calculate for the NE-Asc binding of 25.2 kJ/mol is consistent with the five interactions previously shown (7). The IG and Epi-BSA bindings may have a different number or type of bonds but produce a similar binding energy. The NE-MS

association energy is consistent with having stronger bonds than the NE-Asc binding. NE-MS binding involves a stronger π-π bond than NE-Asc, hydrogen bonds and an ionic bond (2), resulting in a stronger association energy.

Even a cursory analysis of Fig. 5 shows that those complexes containing a protein have much shallower electric field dependence than those with only small molecules. Since increasing electric field strength increases dissociation, proteins must reduce the amount of dissociation as the electric field increases. This cannot be due to association energy differences, as the protein association energies are in the same range as the NE-Asc small molecule pair association energy. Therefore, qualitatively, increasing the size of molecules must protect their binding sites from the effects of the electric field. The molecular shielding constant provides a starting point for estimates of the effect of an electric field on a complex. Since the radii for most molecules can be estimated fairly well, the slope/association energy can be predicted. The units of the molecular shielding constant, cm^2/V, are also intuitively satisfying. An applied voltage is spread over an area, and the greater the area the more the voltage is distributed, lessening its effects. Larger molecules will have greater areas, lessening the dissociative effects of the electric field.

Given the assumptions made regarding the spherical shapes of the different molecules and their subunits (insulin is a dimer (14) and glucagon a trimer (15)), it is remarkable that this model of the data, using only the radii and association energies, should give such a good correlation. The radii used in these calculations are based solely on the MW of the molecules, assuming a spherical shape. No estimate of the nonspherical shape of particular molecules or of molecular complexes was included. That using the simplest assumptions produced such robust results leads us to conclude that although nonspherical shapes may alter the quantitation of the molecular shielding constant, this alteration will be small, as will changes in the estimates of the dissociation distance as a complex approaches the membrane. The spherical assumption may work, in part, because molecules are constantly rotating in the electric field. Calculations of the effect of the electric field on the rotational energy of a molecule show that this effect is minimal, with thermal forces causing random orientations far exceeding any orientation of the complex produced by the field (16). The change in dipole orientation equilibrium will be $<1\%$ at 10^4 V/cm. Thus, any quantitative effects produced by the nonspherical nature of a molecule or complex will be minimized by the randomization of its orientation within the electric field.

Because the membrane potential, in millivolts, exponentially decays over a distance of nanometers in the surrounding fluid, the electric field near the membrane, in mV/nm, or 10^4 V/cm, will be much greater than the field needed to produce dissociation, which is in the 10^2 V/cm range (see Fig. 5). The exponential decay of the field means this field will only exist within 10 nm or less from the membrane. But within this range, as shown in Table 1, the membrane electric field is more than sufficient to produce dissociation of molecular complexes. Very large molecular complexes of proteins may be unable to approach the membrane sufficiently close to cause dissociation, as shown in Fig. 6. Large protein complexes will have both molecular shielding and limited membrane approach, minimizing the effect of the electric field on these complexes. This will not be the case for small molecule complexes.

The distance over which the membrane electric field could affect agonist binding covers the range of membrane protein projection into the interstitial fluid. The electric field (E) is the length derivative of the voltage. As previously cited, the dielectric constant of water produces a Debye-Hückel length constant (λ) of 1 nm (9–11) at physiological ionic strength. The equation governing the determination of the electric field is

$$E = (-1/\lambda)V_o\exp(-x/\lambda),$$

where V_o is the voltage at the membrane and x is the distance from the membrane. For the extreme electric fields used in these experiments, given a membrane voltage of 30 mV, $E =$ 255 V/cm at 7.07 nm and $E = 51$ V/cm at 8.68 nm. Given that the usual distance between adjacent cell membranes is 20 nm, complexes within the extracellular space will always be exposed to an electric field sufficient to alter their binding. Membrane proteins may project over several nanometers into the extracellular fluid, having an electric field at their farthest point that could alter binding. For example, the electric field at the far points of 2.7 and 5.9 nm for two mycobacterial porins (17) are 20,000 and 800 V/cm, respectively, 6.4 nm (500 V/cm) for the transferrin receptor (18), and 9.7 nm (18 V/cm, and therefore a much lower electric field effect) for the insulin receptor (19), spanning the distance where electric field dissociation will occur. As these are proteins they will partially shield their immediate surroundings from the full electric field. The effect of the electric field on the binding of an agonist will be a function of both the distance of the binding from the membrane and the shielding of the binding site by the receptor. In relative terms, however, mobile complexes of small molecules will be more affected by the electric field of the membrane than will an agonist-receptor complex, with the large radius of the receptor providing preferred binding of the agonist to the receptor over that of the agonist to the carrier molecule. Further, the fixed orientation of the receptor relative to the electric field of the membrane should provide even more molecular shielding than the average molecule shielding provided by a complex in solution able to have unrestricted orientations relative to the membrane electric field. As complexes containing an agonist approach the membrane, the field may cause dissociation in areas adjacent to the receptor to a greater degree than at the receptor itself. This may produce a gradient of the free agonist toward the receptor, where the agonist can now bind. Thus molecules like Epi can

circulate in the blood bound to albumin or Asc, preventing oxidation. They then diffuse into the interstitial fluid still bound to Asc (but not albumin, which cannot pass through a capillary pore) and dissociate as they near a membrane, available now to bind to a receptor. Based on the broad range of different molecular pairs presented here, this appears to be a general phenomenon.

This phenomenon will not be limited to the interstitial space. The electric field will also be significant for several nanometers on the intracellular side of the membrane, where the electric field will mirror the electric field on the outside of the membrane (20). So too, molecular complexes circulating in the plasma, such as Epi-BSA, will dissociate as they near the capillary membrane. Other factors not addressed here experimentally should also be considered. The glycocalyx extends the membrane potential by several nanometers on the outside (but not the inside) of the cell membrane (21). The membrane potential has its greatest change (and largest electric field) just beyond the glycocalyx. Thus, in cells with a large glycocalyx, the effect of the electric field on agonist binding will have this additional factor. This will be a further consideration when considering the degree to which the water shell around an ion such as Na^+ is stripped by the electric field as it approaches an ion channel. If the extent of the pore is within the range of the glycocalyx, the stripping effect of the electric field will be reduced.

It may be possible to apply electric dissociation in a specific manner for practical purposes such as drug delivery. Bonds with energies in this range absorb energy in the infrared spectrum (13). Capillary electrophoresis uses DC voltage to effect dissociation. Within the DC field are all the AC frequencies with wavelengths shorter than the length of the DC field, in this case 98 cm. The power input in our system for the 204 V/cm field is 1.58 J/s. Application of one or several frequencies specific to the complementary pair will split the pair with a lower total energy input. Differences in the number of H-bonds, π-π bonds, and frequency differences produced by bond stretching (13) may produce unique frequency patterns that allow selective dissociation of a specific complex, if these frequencies can be determined and applied. Thus, it should be possible to separate molecular complexes with much lower energy input than using conventional CE. If these frequencies can penetrate skin sufficiently, they can be used to dissociate complexes in situ.

In summary, capillary electrophoresis can be used to measure the dissociation constants of many complementary pairs; the large size of proteins will reduce the effect of the electric field on the dissociation of bound molecules; membrane electric fields will cause dissociation of all complexes except the largest protein-protein complexes within 10 nm of the membrane; and agonists can be delivered to membrane receptor sites bound to carrier molecules and dissociate in the vicinity of the receptor.

This work was funded in part by a grant from Acorn Technologies.

REFERENCES

1. Dillon, P. F., R. S. Root-Bernstein, P. R. Sears, and L. K. Olson. 2000. Natural electrophoresis of norepinephrine and ascorbic acid. *Biophys. J.* 79:370–376.
2. Root-Bernstein, R. S. 1987. Catecholamine bind to enkephalins, morphiceptin, and morphine. *Brain Res. Bull.* 18:509–532.
3. Motsch, J., and M. Kamler. 1997. Alpha 2-adrenergic agonists. Use in chronic pain—a meta analysis. *Schmerz.* 11:339–344.
4. Georges, F., and G. Aston-Jones. 2003. Prolonged activation of mesolimbic dopaminergic neurons by morphine withdrawal following clonidine: participation of imidazoline and norepinephrine receptors. *Neuropsychopharmacology.* 28:1140–1149.
5. Patil, N., P. Fraundorfer, and P. K. Dutta. 1996. Stereo selective modification of circular dichroism spectra of rat lung β-adrenoceptor protein preparation by enantiomers of epinephrine. *Chirality.* 8:4363–4365.
6. Root-Bernstein, R. S., and C. Dobbelstein. 2001. Insulin binds to glucagon forming a complex relationship. *Autoimmunity.* 33:153–169.
7. Root-Bernstein, R. S., and P. F. Dillon. 2002. Fostering venture research: a case study of the discovery that ascorbate enhances adrenergic drug activity. *Drug Dev. Res.* 57:58–74.
8. Harte, R. A. 1969. Molecules in Three Dimensions: A Guide to the Construction of Models of Biochemically Interesting Compounds with CPK Models. American Society of Biological Chemists, Bethesda, MD.
9. Delahay, P. 1966. Double Layer and Electrode Kinetics. John Wiley and Sons, New York.
10. Moore, W. J. 1972. Physical Chemistry, 4th ed. Prentice-Hall, Englewood Cliffs, NJ.
11. Tien, H. T. 1974. Bilayer Lipid Membranes (BLM): Theory and Practice. Marcel Dekker, New York.
12. Tinoco, I., K. Sauer, and J. C. Wang. 1995. Physical Chemistry: Principles and Applications in Biological Sciences. Prentice Hall, Upper Saddle River, NJ.
13. Chang, R. 1977. Physical Chemistry with Application to Biological Systems. MacMillan, New York.
14. Falconi, M., M. T. Cambria, A. Cambria, and A. Desideri. 2001. Structure and stability of the insulin dimer investigated by molecular dynamics simulation. *J. Biomol. Struct. Dyn.* 18:761–772.
15. Oomen, R. P., and H. Kaplan. 1990. Binding of glucagon to lipid bilayers. *Biochem. Cell Biol.* 68:284–291.
16. Dörr, F. 1983. Structure determination of biomolecules by physical methods. *In* Biophysics. W. Hoppe, W. Lohmann, H. Markl, and H. Ziegler, editors. Springer Verlag, Berlin. 168–169.
17. Faller, M., M. Niederweis, and G. E. Schulz. 2004. The structure of mycobacterial outer membrane channel. *Science.* 303:1189–1192.
18. Fuchs, H., U. Lüchen, R. Tauben, A. Engel, and R. Gessner. 1998. Structure model of phospholipids-reconstitutes human transferring derived by electron microscopy. *Structure.* 6:1235–1243.
19. Woldin, C. N., F. S. Hing, L. Jongsoon, P. F. Pilch, and G. G. Shipley. 1999. Structural studies of the detergent-solubilized and vesicle-reconstituted insulin receptor. *J. Biol. Chem.* 274:34981–34992.
20. McLaughlin, S., and H. Harahy. 1974. Phospholipid flip-flop and the distribution of surface charges in excitable membranes. *Biophys. J.* 14:200–208.
21. Glaser, R. 2001. Biophysics. Springer-Verlag, Berlin.