The Electrode Kinetics of Cyanide Hemichrome*

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ABSTRACT: Apparent exchange current densities for the *trans*-dicyanoprotoporphyrinferrate(III)–(II) redox couple at a platinum electrode have been measured using the charge-step relaxation (coulostatic) method. The intrinsic rate of oxidation–reduction for this system in a nonbiological environment was found to be among the fastest of electrode reactions, having an apparent heterogeneous rate constant of 4.0 ± 2.0 cm $\sec^{-1}$. Results are shown to compare favorably with nonelectrochemical work on the electron transport rates of cytochromes.

The hexacyanoferrate(III)–(II) couple was used as a model system to test the method and a new approach to analysis of relaxation data.

Oxygen transport by hemoglobin and electron transfer in the mitochondrial respiratory chain by cytochromes are among the most extensively studied biochemical phenomena in which iron porphyrins are the active reaction sites. Recent electrochemical work has been carried out on iron–protoporphyrin complexes[1] on the premise that a more complete understanding of the electron donor–acceptor properties of model systems will yield insight into the remarkable behavior of the more complex systems *in vivo*. In work directed toward elucidating the electron transfer behavior of hemoglobin, the controversy over involvement of polymeric hemin species in aqueous alkaline solution has been resolved (Jordan and Bednarski, 1964; Bednarski and Jordan, 1967). Our work has focused on hemichromes with particular emphasis on processes related to respiratory chain activity. A survey of several hemin complexes using a variety of electrochemical techniques has provided basic information about the conditions under which the systems are amenable to electrochemical study and about the complexes themselves (Davis and Martin, 1966).

The rate of electron transport in the cytochrome section of the respiratory chain has been the subject of several nonelectrochemical investigations and considerable discussion (Chance *et al.*, 1965; Gibson *et al.*, 1965). The rates of individual electron transfer reactions are fast; for instance, the second-order rate constant for the reaction of oxygen with cytochrome oxidase is on the order of 10^7 M^{-1} $\sec^{-1}$. However, it has been shown that the over-all turnover number of cytochrome *c* during oxidative phosphorylation is only about 5 $\sec^{-1}$ under many conditions (Chance, 1955). We have previously demonstrated that ligand substitution reactions of some iron porphyrins (Davis and Orgeron, 1966) occur at about the same rate as the turnover of cytochrome *c* and thus may be a necessary step in the over-all process. Steric effects and the accumulation of intermediates may also influence the reaction rate (Chance *et al.*, 1965).

The work reported here was conducted in an attempt to show whether or not electron transfer reactions of iron–porphyrin complexes are inherently fast in a nonbiological environment or whether special factors, such as physical arrangement of the active centers (iron ion) are the major reasons for high rates of electron transport in the cytochrome section of the respiratory chain. The dicyano complex was selected in preference to other possibilities primarily because both oxidant and reductant are sufficiently soluble under the solution conditions employed to permit investigation of solutions containing equal concentrations of both oxidation states (*vide infra*).

The Coulostatic Method

The coulostatic impulse experiment consists of rapidly injecting a small quantity of charge into a microelectrode (working electrode) which is immersed in a quiescent solution containing both halves of the redox couple under investigation as well as excess supporting electrolyte. (Note: the reduced species may be soluble in the working electrode, *e.g.*, a mercury amalgam, or may be the electrode itself, *e.g.*, mercury.) The effect is to abruptly charge the electrical double layer, changing the potential of the working electrode by a few millivolts. Charge and mass transfer parameters are determined by observing the potential decay back to its equilibrium value. The method has been applied to the study of the rates of oxidoreduction of inorganic ions at mercury surfaces (Delahay and Aramata, 1962; Hamelin,

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[1] The nomenclature of iron–prophyrin complexes in solution has been discussed in detail elsewhere (Davis and Martin, 1966). Briefly, the common name cyanide hemichrome refers to a monomeric, six-coordinate, essentially octahedral species more properly named the *trans*-dicyanoprotoporphyrinferrate(III) ion. Four coordination sites of the iron(III) are occupied by the pyrrolic nitrogens of the planar protoporphyrin IX, while the out-of-plane (*z* axis) sites are occupied by two cyanide ions. Cyanide hemochrome refers to a structurally similar complex, but with the iron in a formal oxidation state of two.

1963a,b, 1964, 1966; Weir and Enke, 1967; Kooijman and Sluyters, 1967).

The simplest experimental arrangement yet devised was suggested and used by Delahay and Aramata (1962) and is shown in Figure 1. With the switch, SW, in position 1, the injection capactior, C_1, is fully charged to an accurately known battery voltage, B. Break-before-make action of the switch shunts the capacitor through the cell which typically is composed of two electrodes (working and auxiliary) immersed in the test solution. The series combination of working and auxiliary electrode double-layer cpacities is essentially that of the working electrode due to the relatively vast surface area of the auxiliary electrode. Thus, the working electrode acquires an overpotential in a time vanishingly small (<0.1 μsec) compared with times during which relaxation is observed (a few microseconds). Since the potential of the auxiliary electrode is virtually unperturbed during and after charge injection, overpotential relaxation is monitored with respect to it by means of an oscilloscope. A particularly disenchanting feature of this apparatus stems from the fact that at the instant of contact between the switch armature and position 2, the full battery voltage (*ca.* 10 V) is applied to input of the oscilloscope. This results in a rather drastic overdrive of the preamplifier since the sensitivity is set to record potentials on the order of a few millivolts. Recovery from the voltage spike depends upon the particular characteristics of the scope and preamp but the point to be emphasized is that there is an interval of about 0.4–1.0 μsec (from the instant charge injection has begun until the time the scope has fully recovered) before overpotential measurements can be made. The consequences of this are noted below.

Before presenting the theoretical relationships which describe relaxation behavior, it is worthwhile to briefly consider the physical processes taking place on the solution side of the metal–solution interface. Prior to charge injection the system is in a state of dynamic equilibrium. No difference in electrochemical potential exists across the interface and the cathodic reaction (reduction of oxidant) is occurring at exactly the same rate as the anodic reaction. The rate of these reactions (either one) is given by the exchange current density, I_a^0 (A cm^{-2}), which, when oxidant and reductant are initially present in equal concentrations, is related to the heterogeneous rate constant, k_a^0 (cm sec^{-1}), by

$$I_a^0 = nFk_a^0C^0 \quad (1)$$

where n is the number of equivalents per mole of reactant, F is the Faraday constant, and C^0 is the common bulk concentration. The subscript a of I_a^0 and k_a^0 denotes that these are apparent quantities, *i.e.*, uncorrected for double-layer effects (see Delahay, 1965). The working electrode is suddenly perturbed by an abrupt change in its charge density creating a charge transfer overpotential, $\eta_{t=0}$ (V). That is, it is assumed that the charge is delivered sufficiently fast that no reactant is consumed during injection. Even for the most rapid of electrode reactions, this assumption is valid provided the double layer is charged in times on the order of 10–40 nsec. To minimize the difference in electrochemical potential across the interface, a charge transfer reaction takes place, *e.g.*, a reduction of oxidant in response to a cathodic charge step ($\eta_{t=0}$ negative). One may envision the relaxation process as the discharge of a capacitor, the double-layer capacity, C_{dl}, through a charge transfer impedance. As relaxation proceeds, reactant is consumed creating concentration gradients which are minimized by diffusion from the bulk solution. It should be clear that the initial response of the system is capacitive discharge through a constant charge transfer resistance while later, a time-dependent contribution from mass transfer enters in. Which processes (charge transfer, mass transfer, or both) control relaxation depends upon the time interval during which relaxation is observed, the earliest times being richest in electrode kinetics information.

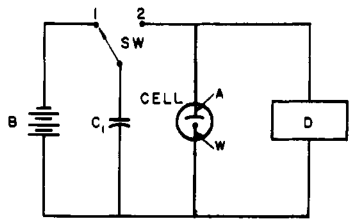

FIGURE 1: The principle of the coulostat. B, battery; SW, break before make relay; C_1, capacitor; A, auxiliary electrode; W, working electrode; D, detector.

The theoretical relationships embodying these features were worked out independently by Delahay (1962) and by Reinmuth (1962). For a simple charge transfer reaction (neglecting double-layer effects and contributions from mass transfer) the result is

$$\eta = \eta_{t=0} \exp\left[-\frac{I_a^0 nF}{C_{dl}RT}t\right] \quad (2)$$

The double-layer capacity needed to evaluate I_a^0 from the slope of the linear dependence of ln η *vs.* t can be evaluated from the intercept, *i.e.*

$$C_{dl} = \frac{C_1B}{\eta_{t=0}A} \quad (3)$$

where A is the electrode area and other symbols have been defined.

Including mass transfer results in a considerably more complicated relationship

$$\eta = \frac{\eta_{t=0}}{b-a}[b \exp(a^2t)erfc(at^{1/2}) - a \exp(b^2t)erfc(bt^{1/2})] \quad (4)$$

where $a = [P + (P^2 - 4NQ)^{1/2}]/2N$, $b = [P - (P^2 - 4NQ)^{1/2}]/2N$, $N = nFD_O$, $P = I_a^0D_O[(1)/(C_O^0D_O^{1/2}) + (1)/C_R^0D_R^{1/2})]$, and, $Q = I_a^0D_O(nF)^2/C_{dl}RT$. D_O and D_R are the diffusion coefficients of oxidant and reductant, respectively; C_O^0 and C_R^0 are the respective bulk concentrations. From the viewpoint of data analysis, it is certainly desirable to assure that mass transfer effects are negligible, for then eq 2 is applicable and things are simple. For very fast reactions this is not possible due to practical limitations in reliable measurements at extremely short times. Early in our work we found that the cyanide hemichrome couple was characteristically a highly reversible system and that analysis of coulostatic relaxation data would require application of eq 4. To this end we developed a computer program which uses a nonlinear least-squares curve-fitting technique to determine the best I_a^0 and C_{dl} for the given set of input data (Martin, 1967).

Experimental Section

All solutions used in this work were made with triply distilled water. Regular distilled water was passed through a column of prepurified activated charcoal and subsequently distilled twice from alkaline permanganate. The distillate had a specific resistance greater than 1.5 Mohms cm. The electrolyte solution used for the hexacyanoferrate(III)–(II) studies (0.5 M potassium sulfate) was prepared from reagent grade K_2SO_4 which was recrystallized from water, dried at 100°, and baked 12 hr at 450°. Solutions of equal concentrations of potassium hexacyanoferrate(III) and potassium hexacyanoferrate(II) in 0.5 M potassium sulfate were prepared either by weight or by controlled potential coulometry. For the dicyanide hemihemochrome studies, solutions were prepared by dissolving the appropriate quantity of freshly recrystallized chlorohemin (Nutritional Biochemical Co.) in triply distilled water containing the appropriate reagent grade chemicals such that the final solution was 0.5 M NaCN, 0.5 M $NaNO_3$, and 30% by volume ethanol. The pH of such a solution was 12.0 ± 0.1. Precisely 50% of the iron–porphyrin complex was reduced to the iron(II) state by controlled potential coulometry under nitrogen.

Deaeration was accomplished with nitrogen which was pretreated by being passed through activated charcoal maintained at Dry Ice–acetone temperature, two chromous sulfate scrubbers, and two scrubbers containing portions of the test solutions as appropriate.

Transfer of solutions from the controlled potential cell to the coulostatic cell was accomplished in a closed Pyrex–Teflon system which prevented air from contacting the solution after preparation.

Construction of the spherical working electrode was accomplished according to the following procedure. Pyrex capillary (1 in.) (1-mm i.d., 7-mm o.d.) was fused to 1.5 in. of 7-mm (o.d.) Pyrex tubing. The capillary end was tapered on a carborundum grinding wheel and a 0.75-in. length of 18-gauge platinum wire (with copper wire soldered to it) was secured in the unit such that about 3 mm of platinum wire extended beyond the tapered seal. The very tip of the exposed wire was melted in a fine hydrogen–oxygen flame and the platinum beaded up into a shiny, pitless sphere. A coxial cable whose center conductor was removed was slipped over the copper wire. The cable ended in a BNC coaxial connector. Lead construction of a similar nature was used for the all electrodes. The capillary tubing was used not only to permit the tapered seal but also to retain insulating epoxy resin at a sufficient distance from the sphere that the platinum could be heated to redness (part of a pretreatment procedure). During each such heating, the glass at the top of the sphere receded slightly so the electrode area was not constant from one run to the next. Consequently, the area had to be recalculated after each heating. The diameter and height of the spherical segment were measured with a Gaetner measuring microscope. During the course of the experiments, the area varied from 0.078 to 0.095 cm^2. The auxiliary electrode which complemented the spherical working electrode was prepared by cutting a cylindrical platinum gauze electrode in half, folding the cut edge toward the center of the cylinder, and sealing the stem (only 0.5 in. was used) into 11-mm Pyrex tubing and preparing a lead as before. The net effect was a basket arrangement in the center of which was positioned the working electrode. This disposition of electrodes, coupled with the tapered feature of the working electrode, provided uniform current distribution over the exposed spherical surface. Cell resistance, in 0.5 M potassium sulfate, was 6.0 ohms at 1 kHz and 6.5 ohms at 10 kHz.

Coulostats. Construction of the coulostat followed the basic design depicted in Figure 1. A 7.5-V (nominal) Mallory-M-9 battery, B, was used to charge capacitor, C_1. The exact voltage across B was determined with a Leeds and Northrup type K-3 potentiometer and a 120-kohm voltage divider, which consisted of several 0.05% General Radio precision resistors. The voltage of several similar batteries ranged from 7.744 to 8.071 V. A given battery was checked before and after each series of experiments and was found to change not more than 3 mV. Polystyrene or Mylar capacitors of the required size were not commercially available but several high-quality condensors with mica dielectric were generously supplied by the General Instrument Corp., Darlington, S. C., and served the purpose well. A General Radio Capacitance Bridge, type 1610, was used to calibrate the condensor, C_1. The capacitance of each capacitor used was invariant in the frequency range 1–100 kHz; they ranged from 357.4 to 371.5 ± 0.2 pfd.

The switch used in these experiments was a shielded, break-before-make, latching, mercury-wetted-contact relay (BABCOCK BW2-3002 F). Its characterisics were: time from break until make, 50 μsec; full-make response, less than 0.1 μsec; unconnected contact resistance, greater than 9000 Mohms. The switch was operated by a triangular voltage wave form obtained from an Exact Function Generator, Model 250.

Various detector systems were constructed and used during portions of this work. All configurations gave essentially equivalent results but after various trials it was concluded that the use of a single commerically available preamplifier was most convenient. The detector settled upon was a Model 535A Tektronix oscilloscope equipped with a Tektronix type 1A7 preamplifier.

The frequency response of this detector is sufficient for these experiments (flat from direct current to 350 kHz) but was slower than other systems tried. This resulted in extension of the time required for the amplifier to recover from the voltage spike and overpotential measurements were delayed until about 1.5 μsec after impulse. The advantages of the 1A7 lie in its high sensi-

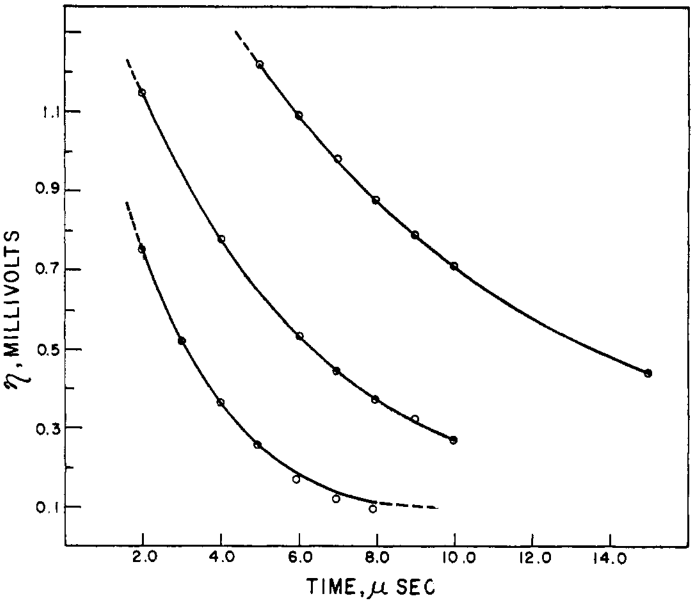

FIGURE 2: Coulostatic relaxation curves for the hexacyanoferrate(III)-(II) couple in 0.5 M K_2SO_4 with a spherical platinum electrode. Circles represent experimental points and lines the best computer fit of data in Table III. The topmost curve is for $C_O = C_R$ = 1155 mM, etc.

tivity (10 μV/cm) and in the convenience of having to calibrate and balance only one amplifier. The sacrifice of speed could be costly if only simple data treatment based on eq 2 is utilized. However, if the more complete data treatment based on eq 4 is used, the sacrifice is of little practical consequence. Electrode reactions which are so fast that little or no kinetic control exists after 3 or 4 μsec are best studied by other methods anyway; since, for such reactions, k_a^0 must be well above 5 cm sec^{-1}.

Calibration of the 535A time base was checked with a properly adjusted Tektronix time mark generator type 180A, and for the sweep rates of interest (1, 2, and 5 μsec/cm) was found to be within the 1% tolerance specified by the manufacturer. The 535A square-wave amplitude calibrator signal, which was used to calibrate the vertical deflection factor of the preamplifier, was checked in the following way. A voltage divider, consisting of a 1.5-V battery, two variable resistors, and several low-resistance General Radio precision (0.025%) resistors was set up such that voltages of 0.100, 0.500, 1.000, and 5.000 mV were available across various combinations of the precision resistors. This was accomplished with the aid of a Leeds and Northrup type K-3 potentiometer. These voltages were then applied to the input of the 1A7 preamplifier at appropriate sensitivities and photographed. Amplitude calibrator signals of the same magnitude were then applied to the same preamplifier (at the same sensitivity settings) and photographed on the same film. It was determined that the calibrator signal was well within the 3% tolerance allowed by the manufacturer (more like 1.0%).

Photographs of the overpotential–time transients were taken with a Tektronix Oscilloscope Camera, C-12, on Polaroid Land film, type 47.

Electrode Pretreatment. There is little doubt that the results of heterogeneous rate constant measurements at platinum electrodes depend a great deal upon the purity of solutions used and upon the method of pretreating the working electrode. The platinum electrodes used first in this work were subjected to a variety of chemical and electrochemical pretreatments. Quite variable results were obtained. The final approach decided upon was to heat the elctrode to redness in a hydrogen–oxygen flame and then to immerse the electrode while still hot into a nitrogen atmosphere. The electrode was kept in nitrogen until the predeaerated test solution was introduced. The pretreatment was repeated for each new solution. Although some inconvenience resulted from the fact that the electrode area had to be redetermined after each treatment, the results were satisfactory.

Results and Discussion

In order to test the apparatus, data treatment programs, and experimental techniques, several series of experiments were run on the hexacyanoferrate couple in 0.5 M potassium sulfate. The greater portion of this work had value only in allowing decisions to be made in regard the electrode geometry and pretreatment and will not be reported here. The results of a series of experiments performed under the conditions finally adopted is shown in Table I and Figure 2. The circles in Figure 2 represent experimental data points and the lines the theoretical curves calculated by the computer program, the best fit giving the k_a^0's and C_{dl}'s of Table I. The results

TABLE I: Values of the Heterogeneous Rate Constant of the Hexacyanoferrate(III)-(II) Couple at Platinum.

$C_O = C_R$ (mM)	k_a^0 (cm/sec)	C_{dl} (μF/cm^2)
11.0	0.045	17.3
20.5	0.064	20.7
30.7	0.088	21.7
	Av 0.07	

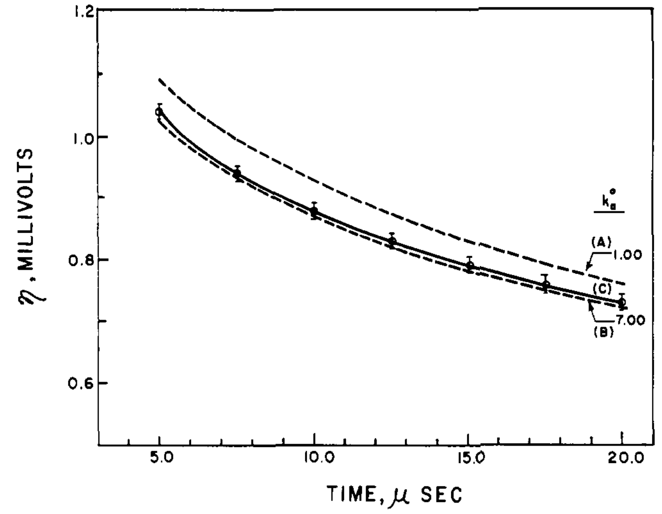

FIGURE 3: Experimental and synthetic overpotential–time curves. Cyanide hemichrome, 1.55 mM. Solid line connects experimental points. Maximum accuracy indicated by vertical lines. (C) $C_O = C_R = 1.55$ mmoles/l. Dashed lines are synthetically generated relaxations. (A) $C_O = C_R = 1.55$ mmoles/l.; $D_O = D_R = 1.85 \times 10^{-6}$ cm²/sec. $C_{dl} = 20.0$ μF/cm²; $k_a{}^1 = 1.00$ cm/sec. (B) Same as A but, $k_a{}^0 = 7.00$ cm/sec.

are satisfactory and compare well with values from the literature as shown in Table II.

Although the results are good and reasonably consistent for work of this type, it can be noticed in Table I that there appears to be an increase in $k_a{}^0$ and C_{dl} with total concentration. This may be due either to an increase in the "activity" of the electrode when it is brought more rapidly to the equilibrium state by a more concentrated solution or to an effect of ionic strength which was not held entirely constant here.

Cyanide Hemi-Hemochrome Couple. Before each coulostatic experiment involving cyanide hemochrome, it was necessary to wait about 10 min for the system to come to equilibrium. That is, after stirring was stopped, the potential difference between the two electrodes gradually increased (quite rapidly at first) to about 2 mV. Although both electrodes were of platinum, differences in the nature of their surfaces (gauze *vs.* shiny bead) undoubtedly affect both rate and extent of adsorption or the activity of the electrode.

Experimental overpotential–time data from the relaxations shown by the solid line in Figure 3 were first treated by a least-squares program based on eq 2 ("kinetic control only" treatment). Results are summarized in Table III. These results were reasonably encouraging and were used as the initial guesses at C_{dl} and $I_a{}^0$ for the curve-fitting data treatment. After five iterations, it became clear that the program, as written, could not handle this particular situation for a very good reason. The relaxation, *even in the 3–10-μsec region*, was primarily governed by mass transfer. There being very little kinetic control, the program "blows up," *i.e.*, it gives unrealistic values for the exchange current density (even negative). Although this is apparently a very undesirable circumstance, it rather dramatically demonstrates an important advantage of the curve-fitting treatment over simple "kinetic control" treatment. Simple data treatment gives no indication of its applicability.

This weakness of the program is not easily remedied, but, to get approximate values for the rate constant, it is a simple matter to guess values of C_{dl}, $I_a{}^0$, D_O, and D_R (the diffusion coefficients of the oxidizing and reduced species) and to generate synthetic curves which bracket the experimental ones. This procedure gave the results shown in Figure 3. The synthetic relaxations are designated by the dashed lines; solid lines represent the

TABLE II: Comparison of Results for $k_a{}^0$'s; Hexacyanoferrate.

$k_a{}^0$ (cm/sec)	C_{dl} (μF/cm²)	Method	References
0.13		Faradaic impedance	Randles and Somerton (1952)
0.07		Rotating disk	Jahn and Vielstick (1962)
0.06$_6$[a]		Faradaic rectification	Agarwal (1963)
	22.0[b]	Bridge	McMullen and Hackerman (1959)
0.07	20.0	Coulostatic	

[a] In 1 M potassium nitrate. [b] In 1 M sodium sulfate with no electroactive species.

TABLE III: Results for Cyanide Hemichrome. "Kinetic Control Only" Data Treatment.

$C_O = C_R$ (mM)	$k_a{}^{0a}$ (cm/sec)	$C_{dl}{}^a$ (μF/cm²)
1.55	0.13	31.5
2.25	0.14	28.8
3.02	0.16	29.4

[a] Each entry is an average of at least three values, each of which was calculated from an independent relaxation curve.

experimental curves. In Figure 3, the experimental curve is bracketed by relaxations corresponding to $k_a{}^0 = 1.00$ and 7.00 cm/sec, all other parameters held constant. Variation of each parameter changes either the shape or over-all position of the curve in a characteristic way so that there are very few *reasonable* combinations of the four which generate a curve which follows the experimental curve throughout the observed time interval. For the relaxation curves obtained, it was possible to select values for each of the variables which lead to a synthetic relaxation which was superimposable on the experimental one. It is not possible to say, however, that this pinpoints the rate constant because of experimental inaccuracies (indicated by the vertical lines in the figures). We must be content to report a "probable range" for the rate constant. The results are summarized in Table IV and can be seen to be self-consistent. The diffusion coefficients are eminently reasonable and although the double-layer capacities are somewhat below what might be expected in the potential and pH ranges encountered, the solution conditions could easily explain this divergence. From the data obtained, the apparent heterogeneous rate constant appears to be: $k_a{}^0 = 4.0 \pm 2.0$ cm sec^{-1}.

TABLE IV: Coulostatic Results for Cyanide Hemichrome. "Kinetic–Diffusion Control" Data Treatment.

$C_O = C_R{}^a$ (moles/cm^3)	$D_O = D_R{}^b$ (cm^2/sec)	C_{dl} (μF/cm^2)	Probable Range of $k_a{}^0$ (cm/sec)
1.55	1.85	20.0	$2 < k_a{}^0 < 7$
2.25	1.60	20.0	$2 < k_a{}^0 < 5$
3.02	1.80	19.0	$2 < k_a{}^0 < 6$

[a] $C \times 10^6$ moles/cc. [b] $D \times 10^6$ cm^2/sec.

Conclusion

A theoretical treatment (Marcus, 1963) has been formulated which should allow comparison of the heterogeneous rate constant determined here with the typical second-order rate constant of 10^7 M^{-1} sec^{-1} for a simple one-step cytochrome reaction (Chance *et al.*, 1965; Gibson *et al.*, 1965). The relationship derived by Marcus for the relationship between homogeneous electron-exchange reactions and heterogeneous (electrode) reactions is

$$\sqrt{\frac{k_{ex}}{10^{11}}} = \frac{k_{el}}{10^4} \tag{5}$$

Putting the value of 10^7 M^{-1} sec^{-1} into the above equation yields a value of $k_{el} = 100$ cm sec^{-1} rather than the 4 cm sec^{-1} found here. This difference may be due to the fact that electrode double-layer effects and electrode surface inactivity may influence our results. On the other hand, Malachesky *et al.* (1965) have noted very large discrepancies when applying eq 5 to systems involving aromatic molecules and their radical anions. They point out that, as Marcus indicates, eq 5 becomes

$$\frac{k_{ex}}{10^{11}} \geq \frac{k_{el}}{10^4} \tag{6}$$

if electroactive species penetrates the solvent layer at the electrode surface. Since metalloporphyrin complexes quite likely resemble aromatic molecules more than the simple metal ions for which eq 5 is usually tested, this modification of theory may well apply here. If eq 6 is used (with the equal sign), a value of 1 cm sec^{-1} is calculated which is in reasonable agreement with the 4 ± 2 cm sec^{-1} found here. The question of whether or not such a calculation is justified will have to await further theoretical and experimental refinements. While these results must be considered as qualitative, there is little doubt that the rate of electron transfer of dicyanide hemichrome is unusually fast. The hemichromes of the cytochrome system are, unlike our model compound, attached to protein in such a way that they are in the proper physical position for electron transfer is achieved. We believe we have shown that the basic unit of electron transfer (hemichromes) in the cytochrome system is inherently capable of rapid electron transfer and thus the physical arrangements of cytochrome units exists primarily to allow "continuous" flow of electrons rather that to assure rapidity of transfer. Further work into the chemical details of such systems especially from the point of view of ligand-transfer reactions and the influence of ligand structure on rates of electron transfer is underway.

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Rotational Frictional Coefficients of Macroscopic Models of T2 Bacteriophage*

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ABSTRACT: We have measured rotational frictional coefficients of plastic scale models of T2 bacteriophage in order to elucidate the structures of the "fast" and "slow" forms of this virus. Results have been compared with the recent electric birefringence data of Maestre (Maestre, M. F. (1968), *Biopolymers 6*, 415). It is concluded that tail fibers cannot be extended in the fast form of T2, which has $s_{20,w} = 1000$ S and rotational diffusion coefficient $D^{\theta\theta} = 555\ \text{sec}^{-1}$, and that the phage head may be somewhat larger than observed in electron micrographs. The slow form of T2, with $s_{20,w} = 700$ S and $D^{\theta\theta} = 111\ \text{sec}^{-1}$, is probably formed from the fast form by extension of the tail fibers. Differences in head porosity are shown to have no effect on frictional properties, and kinking of tail fibers has little effect except when the fibers are close to the head. It is demonstrated that when the tail fibers are extended, the solvent within their domain is effectively immobilized with respect to the phage.

The existence of two distinct forms of T2 bacteriophage in solution is well documented. Fast- and slow-sedimenting forms, with sedimentation coefficients of 1000 and 700 S, respectively, were first observed by Hook *et al.* (1946) and Sharp *et al.* (1946). Recent electric birefringence studies by Maestre (1968) gave rotational diffusion coefficients of 555 ± 54 and 111 ± 22 sec^{-1} for the "fast" and "slow" forms, respectively.

The structural differences giving rise to these differences in hydrodynamic properties have been the subject of some debate. Bendet *et al.* (1957), Lauffer and Bendet (1962), and Maestre (1968) have attributed the greater frictional resistance of the slow form to a greater extension of the virus tail fibers. Cummings and Kozloff (1960, 1962) and Cummings (1963) have attributed the change to a large increase in head porosity, attendant upon a modest increase in head length, on going from the fast to the slow form. Calculations of translational (Bloomfield *et al.*, 1967b) and rotational (Filson and Bloomfield, 1967) frictional coefficients of T2-shaped structures, using the "shell model" theory developed by Bloomfield *et al.* (1967a), seemed to fix the blame for the difference in frictional properties on a large change in head size rather than tail fiber extension or head porosity.

To gain further insight into the structural changes associated with the observed hydrodynamic changes, we have measured rotational frictional coefficients of macroscopic, plastic scale models of the phage. This approach to a structural understanding of the hydrodynamic properties of viruses has precedents in the work of Haltner and Zimm (1959) and Broersma (1960). In this paper we report the effects of head size, head porosity, tail fiber orientation, and tail fiber kinks on the rotational diffusion coefficient.

Experimental Section

Models. Scale models of virus particles were machined from Plexiglass. The scale factor was 3.190×10^{-3} cm/Å. Figure 1 shows the shape and characteristic dimensions of the models. The head is a cylinder of length A and diameter B, capped at both ends by cones of height 230 Å. Two sets of head dimensions were used:

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