Conformational Studies of Nucleoprotein. Circular Dichroism of Deoxyribonucleic Acid Base Pairs Bound by Polylysine†

Catherine Chang, Manuel Weiskopf, and Hsueh Jei Li*

ABSTRACT: The circular dichroism (CD) of DNA complexed with polylysine in 2.5×10^{-4} M EDTA (pH 8.0) by a slow and direct mixing shows red shifts for the positive band near 276 nm, and the crossover. The amplitude of the positive band is also reduced. These resemble the CD changes of DNA in the presence of high NaCl concentrations. The results can be explained as due to the existence of two groups of base pairs in the complex. Base pairs in free DNA regions have the B-form conformation while those in polylysine-bound regions have their characteristic conformation somewhere between B and C forms. The calculated $\Delta\epsilon_b$ is close to that of pure DNA in 6.0 M NaCl. The effect of polylysine binding on the DNA structure can be interpreted as due to dehydration in the vicinity of the DNA molecule and charge neutralization on phosphate lattice of DNA by polylysine.

Optical properties of DNA in solution depend upon salt concentration (Tunis and Hearst, 1968a; Tunis-Schneider and Maestre, 1970; Li *et al.*, 1971), ethylene glycol (Nelson and Johnson, 1970; Green and Mahler, 1970, 1971), humidity (Tunis-Schneider and Maestre, 1970), and temperature (Gennis and Cantor, 1972; Studdert *et al.*, 1972). It is believed that dehydration of DNA leads to its destabilization (Tunis and Hearst, 1968b) and base tilting of its structure from B to C form (Tunis-Schneider and Maestre, 1970; Nelson and Johnson, 1970; Green and Mahler, 1970; Gennis and Cantor, 1972; Studdert *et al.*, 1972).

If dehydration is important to DNA structure, it is expected that tight protein–DNA binding would substantially change the DNA structure since water molecules would be excluded from the vicinity of DNA in the complex. Severe structural changes of DNA have been reported for reconstituted basic polypeptide–DNA complexes (Cohen and Kidson, 1968; Shapiro *et al.*, 1969; Haynes *et al.*, 1970; Carroll, 1972) and histone I–DNA complexes (Fasman *et al.*, 1970, 1971) by using optical rotatory dispersion (ORD) or circular dichroism (CD). Because of strong light scattering and a nonlinear dependence of the optical changes on the ratio of protein to DNA in the above complexes, the interpretation of optical changes is not definitive. We report here that the CD changes in polylysine–DNA complexes prepared by a direct mixing method at very low salt depend linearly upon the input ratio of polylysine to DNA. Our results agree with the assumption that the CD of the complexes are decomposed into two components, that of free and bound base pairs. The calculated CD spectrum of polylysine-bound base pairs is in agreement with that of DNA in high salt and is between the B and C forms.

Materials and Methods

Poly(L-lysine) hydrochlorides (mol wt 170,000 and 15,500) were purchased from Schwarz/Mann. Calf thymus DNA was purchased from Sigma Chemical Co.

The preparation of polylysine–DNA complexes by the method of direct and slow mixing at low salt and the determination of the fraction of base pairs either free or bound by polylysine by thermal denaturation method have been described previously (Li *et al.*, 1973). The CD spectra of DNA and polylysine–DNA complexes were taken on a Durrum-Jasco spectropolarimeter Model J-20 at room temperature in 2.5×10^{-4} M EDTA (pH 8.0), the buffer used for thermal denaturation.

The CD results are reported as $\Delta\epsilon = \epsilon_l - \epsilon_r$, where ϵ_l and ϵ_r are respectively molar extinction coefficients for the left- and the right-handed circularly polarized light. The units of $\Delta\epsilon$ are M^{-1} cm^{-1} in terms of nucleotide. Nucleotide concentration in DNA and polylysine–DNA complexes was determined spectrophotometrically by using $\epsilon = 6500$ M^{-1} cm^{-1} at 260 nm. As reported earlier (Li *et al.*, 1973) light scattering in these complexes is negligible with A_{320}/A_{260} lower than 0.04 where A_{320} and A_{260} are respectively the absorbances of the complex at 320 and 260 nm.

Results

CD Spectrum of Base Pairs Bound By Polylysine. The typical CD spectra of polylysine–DNA complexes are given in Figure 1. The molecular weight of the poly(L-lysine) used is 170,000. As more DNA base pairs are bound by polylysine, the positive band is shifted to the red from 276 to 280 nm as the lysine/nucleotide ratio is increased from 0 to 0.81. The amplitude is also reduced. Red shifts are also observed for the crossover. However, the CD spectra below 240 nm, in which proteins such as polylysine have a significant contribution, are essentially independent of polylysine content in the complexes.

The difference CD spectra, $\Delta\epsilon_m - \Delta\epsilon_0$, of three complexes are shown in Figure 2. The subscripts m and 0, respectively, refer to measured spectra of the complex and that of free DNA. Quantitatively the difference CD spectra are proportional to the ratio of lysine to nucleotide. Qualitatively the shapes are identical. These results lead to the assumption that DNA base pairs in the complexes be classified into two groups, free and bound by polylysine which have characteristic CD, namely, $\Delta\epsilon_0$ and $\Delta\epsilon_b$, respectively. If f is the fraction of base pairs bound by polylysine, we obtain

$$\Delta\epsilon_m = (1 - f)\Delta\epsilon_0 + f\Delta\epsilon_b \quad (1)$$

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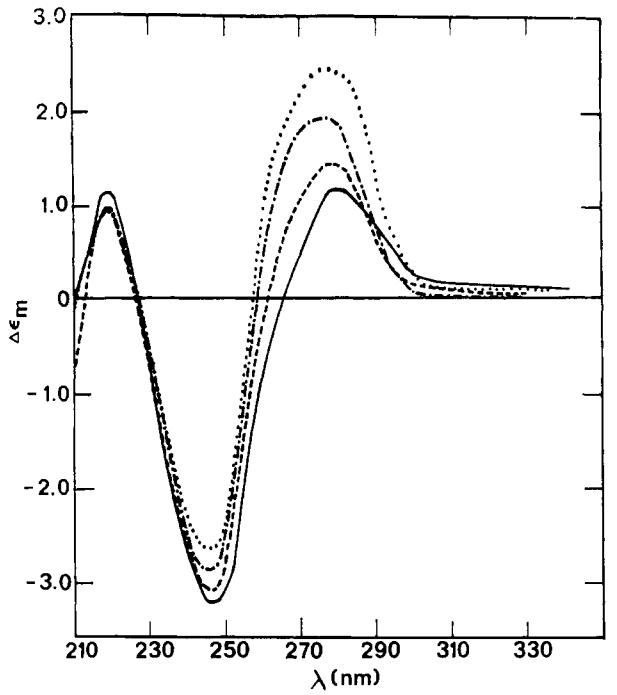

FIGURE 1: CD spectra of polylysine–DNA complexes. The lysine/nucleotide ratio is 0 (· · ·), 0.27 (— · —), 0.54 (- - -), and 0.81 (——). The molecular weight of polylysine is 170,000.

$$\Delta\epsilon_m - \Delta\epsilon_0 = f(\Delta\epsilon_b - \Delta\epsilon_0) \quad (2)$$

Since both $\Delta\epsilon_m$ and $\Delta\epsilon_0$ are determined experimentally, $\Delta\epsilon_b$ can be calculated from eq 2 if f is measured by another method such as thermal denaturation (Li, 1973; Li *et al.*, 1973). Since the average number of lysine per nucleotide in the polylysine-bound region, as determined by the thermal denaturation method, is 1.05, the fraction of base pairs bound by polylysine (f) is equal to the ratio of lysine to nucleotide divided by this number. Using eq 2 $\Delta\epsilon_b$ values were calculated and the results are shown in Figure 3. Within experimental error we

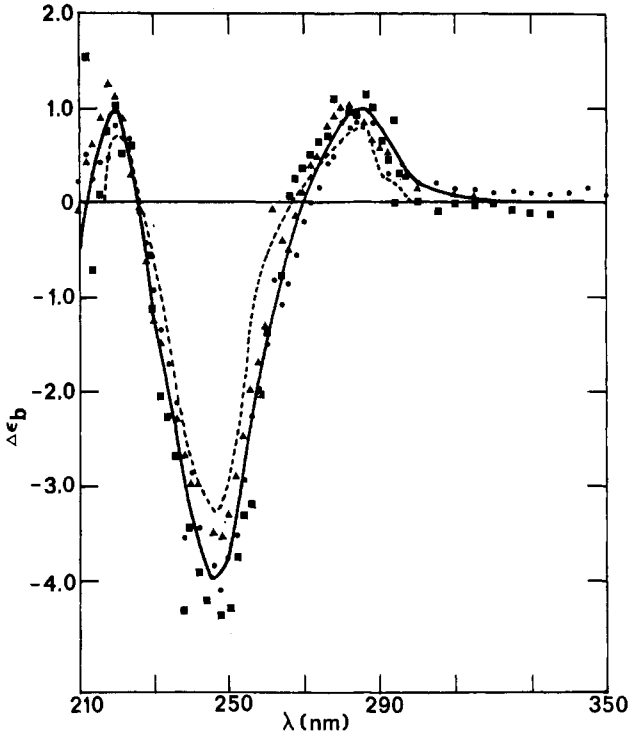

FIGURE 3: Calculated CD spectrum ($\Delta\epsilon_b$) of DNA base pairs bound by polylysine. The lysine/nucleotide ratio is 0.27 (■), 0.54 (●), and 0.81 (▲). $\Delta\epsilon_b$ (——) and $\Delta\epsilon$ of DNA in 6.0 M NaCl (- - -) from Tunis-Schneider and Maestre (1970). The molecular weight of polylysine is 170,000.

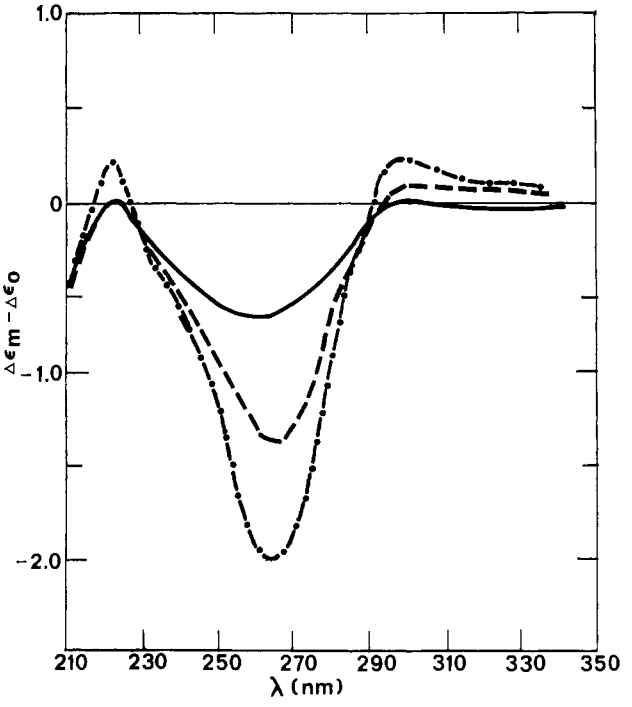

FIGURE 2: Difference CD spectra of DNA ($\Delta\epsilon_0$) and polylysine–DNA complexes ($\Delta\epsilon_m$) obtained from Figure 1. The lysine/nucleotide ratio of the complex is 0.27 (——), 0.54 (- - -), and 0.81 (— · —).

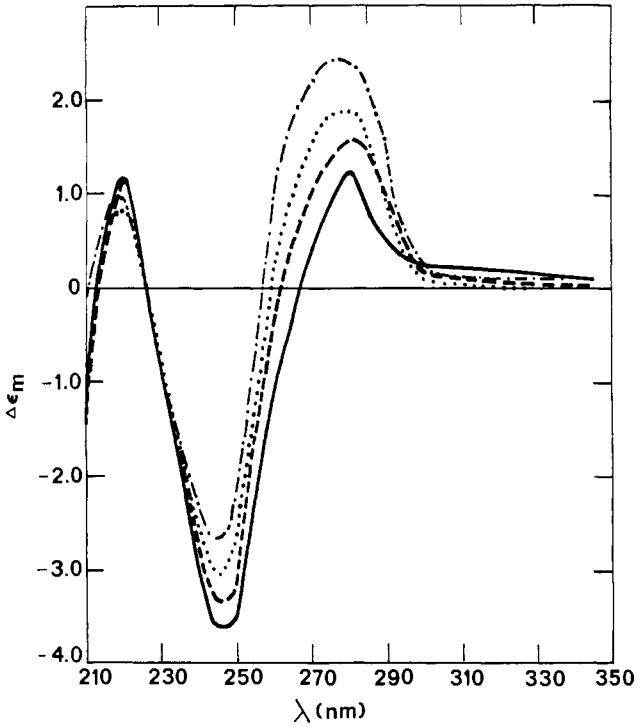

FIGURE 4: CD spectra of polylysine–DNA complexes. The lysine/nucleotide ratio is 0 (— · —), 0.27 (· · ·), 0.54 (- - -), and 0.81 (——). The molecular weight of polylysine is 15,500.

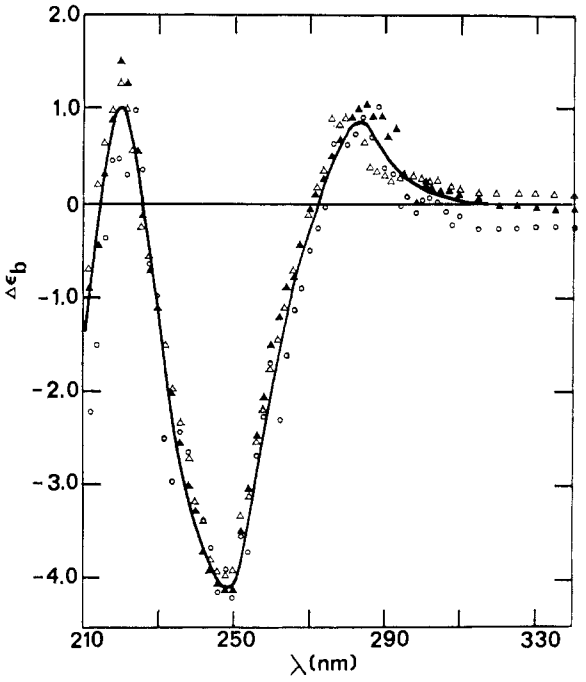

FIGURE 5: Calculated CD spectrum ($\Delta\epsilon_b$) of DNA base pairs bound by polylysine. The lysine/nucleotide ratio is 0.27 (○), 0.54 (▲), and 0.81 (△). The molecular weight of polylysine is 15,500.

can conclude that the CD spectra of base pairs bound by polylysine are independent of the coverage of polylysine on DNA. This implies that our assumption of two characteristic CD spectra of base pairs either free or bound by polylysine is correct.

Effect of the Polylysine Chain Length on the CD of Bound Base Pairs. It has been reported that the CD spectra of reconstituted polylysine–DNA complexes depend upon the chain length of polylysine (Carroll, 1972). In order to see whether

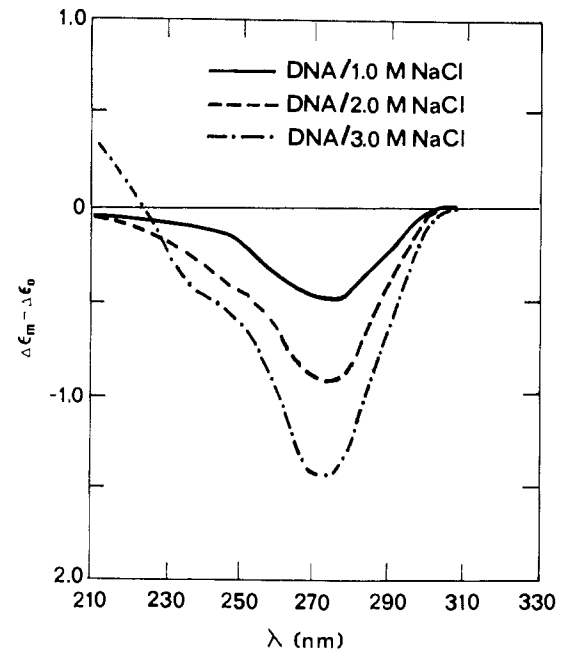

FIGURE 6: Difference CD spectra of DNA in EDTA buffer ($\Delta\epsilon_0$) and in EDTA buffer and NaCl ($\Delta\epsilon_m$). NaCl concentration is 1.0 M (——), 2.0 M (– – –), and 3.0 M (—·—).

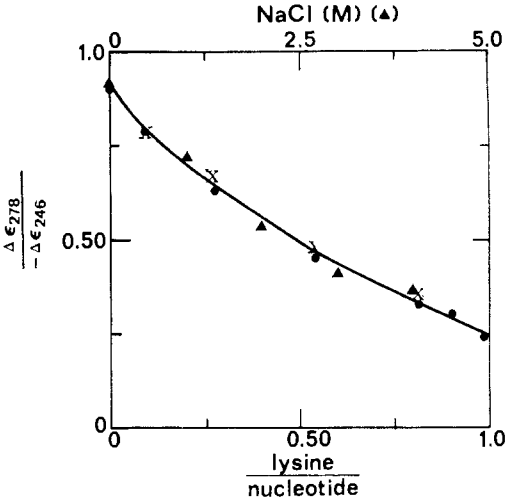

FIGURE 7: Comparison of the effect of NaCl and polylysine binding on the CD of DNA. NaCl (▲), polylysine of molecular weight 170,000 (×), and 15,500 (●).

there is also such a dependence in the CD of the complexes prepared by direct mixing, polylysine of mol wt 15,500 was used. Some typical results are given in Figure 4 which are similar to those in Figure 1 of long polylysine. The precipitation titration curve and thermal denaturation of these complexes are also the same as those reported earlier when polylysine of mol wt 170,000 was used (Li *et al.*, 1973). From eq 2 $\Delta\epsilon_b$ was calculated. The results are shown in Figure 5. They are identical with one another which again support the assumptions behind eq 2. $\Delta\epsilon_b$ in Figures 3 and 5 are also the same, indicating that the CD of base pairs bound by polylysine is independent of the chain length of the latter, at least in the range from 100 to 1000 residues.

Comparison of Polylysine Binding to Salt Effect on DNA Structure. The characteristic CD changes in polylysine–DNA complexes (Figures 1 and 4) are similar to those found in DNA in the presence of high NaCl concentrations, namely a red shift and a reduction of the amplitude of the positive band and a red shift of the crossover. Since polylysine is a polycation, its binding to DNA conceivably excludes water molecules from phosphates and perhaps also from other moieties of DNA. We expect to see a dehydration effect on the secondary structure of DNA in the complexes similar to that found in NaCl. The CD spectra of DNA in EDTA buffer at various NaCl concentrations were also taken. They are essentially identical to those reported earlier (Tunis-Schneider and Maestre, 1970; Li *et al.*, 1971). Figure 6 shows some difference CD spectra, $\Delta\epsilon_m - \Delta\epsilon_0$, where $\Delta\epsilon_0$ is the CD or DNA in EDTA buffer and $\Delta\epsilon_m$ is that in high NaCl. There is a striking similarity between these difference CD spectra in NaCl and those in polylysine–DNA complexes (Figures 2 and 6). Another way of comparing the CD spectra of DNA base pairs under these two conditions, in NaCl solutions or bound by polylysine, is to compare the ratio of the CD of the peak to the trough. In this case $\Delta\epsilon_{278}/-\Delta\epsilon_{246}$ was used. Figure 7 shows the results. As expected from the previous conclusion, the results of short polylysine (mol wt 15,500) and of long polylysine (mol wt 170,000) are identical. The dependence of this ratio ($\Delta\epsilon_{278}/-\Delta\epsilon_{246}$) on NaCl is coincidentally identical with that on the lysine/nucleotide ratio. This comparison is further supported by the results in Figure 3. The CD spectrum of DNA in 6.0 M NaCl is almost identical to $\Delta\epsilon_b$, the CD of base pairs bound by polylysine.

Discussion

Biphasic melting of polylysine–DNA complexes is a well known phenomenon (Tsuboi *et al.*, 1966; Leng and Felsenfeld, 1966; Olins *et al.*, 1967; Shih and Bonner, 1970). Recently we proposed a model that base pairs bound by proteins with varied thermal stabilities are melted at different temperatures (Li and Bonner, 1971; Li, 1973). In polylysine–DNA complexes base pairs can be classified into two groups, free and bound, which melt independently at two completely different temperatures. Here it is further shown that, as far as the secondary structure is concerned, base pairs in polylysine–DNA complexes can also be classified into two groups, free and bound. Based upon this assumption it is deduced that the CD of the base pairs bound by polylysine is similar to that of dehydrated DNA in high NaCl concentration. In other words the conformation of base pairs bound by polylysine is neither B nor C form, but a conformation between them.

It has been reported that the CD spectrum of chromatin above 250 nm is similar to that of DNA in high NaCl concentration (Permogorov *et al.*, 1970; Simpson and Sober, 1970; Shih and Fasman, 1970). Our results imply that the base pairs in histone-bound regions may have a conformation between the B and C forms. This suggestion is in contrast to the recent proposal presented by Hanlon *et al.* (1972) who assumed the existence of base pairs in two extreme conformations, B or C form.

The binding of NaCl to DNA neutralizes charges on phosphate lattice and excludes water molecules from phosphate groups and also possibly from the other moieties of DNA (Tunis and Hearst, 1968b; Tunis-Schneider and Maestre, 1970). Since the binding of NaCl to DNA is not localized as the case in polylysine, the transformation of the DNA conformation from the B to C form in higher salt can be regarded as a result of gradual charge neutralization and dehydration of the whole molecule of DNA. Polylysine binding neutralizes charges on DNA and can also lead to dehydration of DNA. So far as dehydration is concerned, the binding of lysine side chain to phosphate on DNA would be expected to displace water molecules from the DNA. The methyl and the amide groups of the polypeptide which also have close contacts on the DNA helix should also exclude water molecules from the vicinity of DNA. Therefore, it is not surprising to observe that the CD of base pairs bound by polylysine reflects a conformational change of dehydrated DNA. To what extent the dehydration of DNA is due to polylysine binding is hard to estimate. Nevertheless, the results in Figure 3 indicate that it is approximately equivalent to the effect of 6 M NaCl.

Although polylysine binding to DNA has a stoichiometric ratio of one lysine per nucleotide whether the complex is prepared by the method of direct mixing or by reconstitution (Tsuboi *et al.*, 1966; Leng and Felsenfeld, 1966; Olins *et al.*, 1967; Shih and Bonner, 1970), the difference between them is great. The reconstituted complex in general shows a strong light scattering. The binding is cooperative and there is a big CD change which is not a linear function of the ratio of lysine to nucleotide. On the other hand, the complex made by direct mixing has negligible light scattering when the input ratio of lysine to nucleotide is below 0.9 (Li *et al.*, 1973). More experimental data concerning polylysine–DNA complexes have recently been accumulated (Haynes *et al.*, 1970; Carroll, 1972; Li *et al.*, 1973). However, the details of the binding, as reflected in the physical properties of the final complex, is still not clear.

It is interesting to notice that essentially polylysine in the complex has little contribution to the CD near 220 nm. A tight binding of polylysine to DNA somehow reduces the CD of the amide groups in this region. A similar conclusion has been obtained for histone IV when it is bound to DNA (Shih and Fasman, 1971; Li *et al.*, 1971). There are two possible explanations for this fact. Perhaps the binding of polylysine to DNA blue shifts the $n\pi^*$ transition of the amide groups that their CD near 220 nm is reduced. If this binding does not blue shift the $n\pi^*$ transition it may be concluded that the amide groups of polylysine in the complex have a conformation which is none of the three common conformations of proteins in solution, namely, α helix, β sheet, and random coil. If, in the complex, polylysine were in a random coil conformation we would expect its CD to be the same order of magnitude as that of DNA in this region (Holzwarth and Doty, 1965; Greenfield and Fasman, 1969). An even bigger contribution would be expected if it were in the α helix or β sheet conformation. This conclusion is not surprising because polylysine is tightly bound to DNA. Its backbone may conceivably be distorted in the bound than in the free state.

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The Determination of Local Reaction and Diffusion Parameters of Enzyme Membranes from Global Measurements†

John A. DeSimone*‡ and S. Roy Caplan

ABSTRACT: We present a study of simultaneous reaction and transport of benzoyl-L-argininamide in collodion membranes containing cross-linked papain. Two types of symmetrical membranes are investigated: homogeneous membranes and three-layer membranes in which the enzyme layers are separated by an inert region of membrane. In the case where the local substrate concentration within the pores of the membrane is much less than the Michaelis constant (K_m), the local reaction kinetics approach pseudo-first-order. In this case the ratio of the maximum reaction rate to the Michaelis constant (V_m/K_m) and the diffusion coefficient d_s of the substrate can be found from measurements of boundary flow rates and substrate concentrations. In the present case d_s can also be obtained directly by studying diffusion in reaction inhibited membranes. Consequently in the case of homogeneous membranes a self-consistent test of the data obtained with reaction can be made by comparing d_s values found with and without reaction. In the case of three-layer membranes the independent value of d_s enables one to estimate the thickness of the catalytic regions relative to the inert regions. The methods presented are nondestructive. It is shown that reaction alters the diffusion coefficient of the substrate by causing it to increase and introduces cross-diffusion coefficients between substrate and product. These membranes serve as models of facilitated transport systems.

Binding an enzyme within the pores of a membrane can markedly alter its catalytic properties (Silman and Katchalski, 1966; Goldman *et al.*, 1965; Mosbach and Mattiasson, 1970). In addition to possible changes in the intrinsic chemical rate parameters, one must also consider additional influences due to physical transport processes. The effects of the latter on reaction rate have been treated at length by chemical engineers in the analysis of porous catalysts (Thiele, 1939). In previous papers (DeSimone and Caplan, 1970, 1973) we have shown the circumstances under which enzyme membranes may serve as models for biological facilitated and active transport. In this paper we present an experimental study of symmetrical papain–collodion membranes. Our goal is to evaluate, from global measurements of substrate and product flow rates and concentrations, the intrinsic local reaction and diffusion parameters of the system. As we shall show the methods of analysis, because of their kinetic character, have the advantage of yielding results while leaving the membrane intact. By restricting the analysis to the pseudo-first-order kinetic regime we can obtain, in the case of homogeneous membranes, the substrate diffusion coefficient, d_s, as well as the pseudo-first-order rate constant, V_m/K_m, where V_m is the maximum reaction rate and K_m is the Michaelis constant for the substrate.

The membrane used in this study is the papain–collodion membrane developed by Goldman *et al.* (1965). Some of the kinetic properties of this system were examined by Goldman *et al.* (1968b). Work on related systems includes that of Sélégny *et al.* (1969) on glucose oxidase membranes, and of Meyer *et al.* (1970) who developed an alternative method to that presented here for the treatment of first-order reactions in membranes. We treat here two types of symmetrical membranes: the one-layer homogeneous membrane and the three-layer membrane. The former consists of a collodion matrix with a uniform cross-linked layer of papain throughout. The latter is a symmetrical sandwich structure of two papain–collodion layers separated by an internal section of matrix devoid of enzyme. Experimentally each membrane is treated as a "black box" even though in each case the distribution of enzyme is *a priori* known. Nevertheless in the case of three-layer membranes it is possible to establish a lower bound on the relative thickness of the enzyme layer from the results of global measurements on the intact membrane.

An important aspect of our analysis is the recognition of the useful concept of "transport" on a global scale for reacting species. The global membrane can then be characterized dynamically by both transport and reaction processes, just as each elemental membrane volume is so characterized. One's ability to formalize both processes on the local and global levels is essential for a complete characterization of the membrane.

Experimental Section

Preparation of Matrices. Collodion membranes were prepared by the method of Carr and Sollner (1944) and Gregor

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‡ Present address: Central Research Laboratories, 3M Co., St. Paul, Minn. 55119.