DISSOCIATION RATE OF SERUM ALBUMIN-FATTY ACID COMPLEX FROM STOP-FLOW DIELECTRIC STUDY OF LIGAND EXCHANGE

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The magnitude of the dipole vector of the serum albumin molecule is a sensitive indicator of the number of moles of fatty acid (FA) bound by the albumin (1). Other than chemical analysis, this is the most accurate indicator of such binding, and, in the case of human serum albumin (HSA), which lacks a fluorescent chromophore near a principal binding site, the dipole vector is probably the only measure suitable for rapid reaction techniques.

Moreover, it is known (1) that the change in dipole vector upon binding fatty acid is considerably different in bovine serum albumin (BSA) from that in HSA. The equilibrium constants of fatty acid binding are similar in the two proteins (2, 3), and it is therefore to be expected that ligand exchange will occur between the two species of protein if they are mixed. This, together with the differential dielectric effect, thus provides a means of measuring the dissociation rate constant, k_d, of the binding reaction,

$$\text{HSA} + \text{FA} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \text{HSA—FA}. \qquad (1)$$

Svenson et al. (4) measured the k_d of this binding by ligand exchange with matrix-bound albumin, and obtained a rate constant of $4.2 \times 10^{-2}\text{s}^{-1}$ for palmitic acid at 25°C. Certain assumptions about the mechanisms of the exchange and the behavior of the matrix-bound protein are avoided in the single-phase system reported here.

In our experiment, a rapid-flow device with a conventional mixer is used. The solution from syringe I contains HSA defatted by the method of Chen (5), and subsequently re-lipidated with 1 M/M oleate added as the sodium salt. Solution II is a defatted BSA solution. Both solutions are deionized by passage over mixed-bed ion exchange resin (MB-1, Mallinckrodt Inc., St. Louis, Mo.).

The mixture is passed into an electrode chamber in which the dielectric constant is measured at intervals after mixing, by recording and analyzing the response to a step function in applied potential at the electrodes.

The stop-flow device used and the theory and technique of the time domain dielectric measurement have been recently reported (6). The dielectric measurement is made in real-time, rather than by the conventional scan of the frequency domain, but its relation to the molecular dipole vector is based on the classical analysis of Debye (7) and Perrin (8).

The use of this technique has already showed that the forward reaction of Eq. 1 takes place in at least two steps, the first too fast to measure by stop-flow methods, and

the second, a first-order process of $k_a^{II} \cong 3\ s^{-1}$ at 0°C, presumed to be a rearrangement of the protein molecule with the ligand.

Likewise, in studying the ligand exchange reaction, it is not a priori obvious whether the reaction proceeds by way of a (very low) free fatty acid concentration:

$$\text{HSA—FA} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{HSA} + \text{FA} \tag{2}$$

$$\text{FA} + \text{BSA} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \text{BSA—FA},$$

or whether it proceeds by ligand transfer during protein-protein encounter,

$$\text{HSA—FA} + \text{BSA} \underset{k_2'}{\overset{k_1'}{\rightleftharpoons}} \text{HSA} + \text{BSA—FA}. \tag{3}$$

In either case, what is monitored is the amount of apo-HSA and BSA-FA formed, as the increase in dipole vector of the HSA upon losing ligand is greater than the decrease in dipole vector of the BSA upon binding ligand.

The end products of Eqs. 2 and 3 are identical, but the kinetics are different. If, for simplicity, the assumptions are made that $k_1 = k_4$, $k_2 = k_3$, and $k_1' = k_2'$ (in fact very nearly valid assumptions), then

$$\begin{aligned} \text{Eq. 2} &\rightarrow \tau^{-1} = k_1(1 + [\text{HSA}]_{\text{total}}/[\text{BSA}]_{\text{total}}), \\ \text{Eq. 3} &\rightarrow \tau^{-1} = k_1'([\text{HSA}]_{\text{total}} + [\text{BSA}]_{\text{total}}). \end{aligned} \tag{4}$$

In these equations, τ is a time constant measured from the relation, $\Delta\epsilon/\Delta\epsilon_\infty = 1 - e^{-t/\tau}$, in which $\Delta\epsilon$ is the change in dielectric constant of the mixture at time t after mixing, and $\Delta\epsilon_\infty$ is the change after the mixture has reached equilibrium.

Fig. 1 shows the result of mixing a 0.135 mM solution of defatted HSA to which 1 M/M oleic acid had been added, with a 0.223 mM solution of defatted BSA, at 1°C. Each point in this graph is calculated from the response curve to a square pulse in the electric field applied to the mixture.

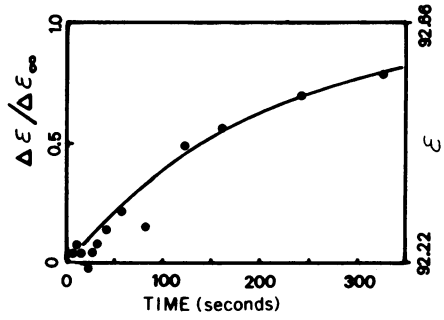

FIGURE 1 Change in dielectric constant as a function of time after mixing of a solution of HSA-FA with a solution of BSA.

The data are consistent with either Eq. 3, $k_1' = k_2' = 14\ s^{-1}\ mol^{-1}$; or with Eq. 2, $k_1 = k_4 = 3 \times 10^{-3}\ s^{-1}$. The two mechanisms have different implications for the physiology of the albumin-fatty acid complexation, but the experiments with a protein concentration series which can distinguish the two have not yet been done.

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TIME-RESOLVED RESONANCE RAMAN CHARACTERIZATION OF THE INTERMEDIATES OF BACTERIORHODOPSIN

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In an attempt to determine eventually the structure of the chromophore of the intermediates involved in the primary process of the photosynthetic proton-pumping cycle of bacteriorhodopsin, new simple techniques have been developed (1–4) to obtain time-resolved resonance Raman spectra of this system. The techniques used in this report involve chopping continuous wave (CW) laser light to produce pulses of variable width and separation to obtain temporal information on the microsecond and millisecond time scales. An optical multichannel analyzer (Princeton Applied Research Corp., Princeton, N.J.) with a Dry Ice-cooled silicon-intensified vidicon is used for detection. By using these techniques as well as different laser frequencies to take advantage of differing resonance enhancements of the intermediates, flow techniques (5–7), and computer subtraction methods, the resonance Raman spectra of the retinal chromophore of the individual intermediates are extracted.