nential behavior principally due to static and dynamic KI quenching effects and time-dependent depolarization effects arising from molecular rotation due to Brownian motion. Also, systematic errors were introduced because of the wavelength dependence of the PM tube temporal response (excitation and fluorescence profiles were determined at different wavelengths) and convolution by the Ludox scattering solution compared to the clear sample solution of the excitation profile. In conclusion, we obtained fluorescence excitation pulse widths of 225 ps FWHM using a static crossed-field PM tube.[1] Because the transit time dispersion of a static crossed-field PM tube is <30 ps[2] it should be possible to display excitation pulse widths limited by the timing resolution of the photon-counting electronics, and with refined experimental and deconvolution methods, to determine relaxation times of the order of 10 ps with better than 20% accuracy.

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ALLOSTERY IN AN IMMUNOGLOBULIN LIGHT-CHAIN DIMER

A CHEMICAL RELAXATION STUDY

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The light chain dimer of the murine immunoglobulin MOPC 315 (L_2 315) has been previously shown to bind the same nitroaromatic haptens as the parent molecules (HL), with similar fine specificity (1, 2). 2 mol of hapten were found to bind per 1 mol of the dimer (1). This finding may be explained in terms of a local twofold rotation axis, with both "original" light chain residues and "new" heavy-chain-homologous residues forming two distinct symmetry-related binding sites for the hapten (2). Recently we reported (3) that hapten binding to L_2 315 involves positive cooperativity, most probably mediated by an allosteric transition of the protein. Two haptens, ϵ-*N*-2-4-dinitrophenyl-L-lysine (DNPL) and 4-(α-*N*-L-alanine)-7-nitro-benz-2-oxa-1,3-

diazole (NBDA) were found to reveal such behavior. The changes in the added haptens' light absorption yielded a sigmoidal saturation curve, the shape of the difference spectrum being constant throughout the titration. This meant that the two binding sites were identical, and that the observed sigmoidity should be accounted for by a symmetric allosteric model. Analysis in terms of such a model, that of Monod, Wyman, and Changeux (MWC), with exclusive binding to the R conformation, was performed, yielding $K_R = 1.6 \times 10^4\ M^{-1}$ with $L = 3$ for DNPL and $K_R = 5.6 \times 10^4\ M^{-1}$, with $L = 110$ for NBDA. Analysis of the DNPL system using the mathematically equivalent nonexclusive model (with binding to both the T and R forms) enabled us to obtain a fit with the same value of $L = 110$ as for DNBA, with $K_t = 2.9 \times 10^3$ and $K_R = 6.7 \times 10^4\ M^{-1}$. We concluded (3) that the first hapten binding to L_2 315 possibly brings about a concerted, symmetry-conserving transition in the dimer, which involves changes in the relative chain position, and which results in a higher binding constant for the second hapten. Such a process in L_2 may resemble antigen-induced changes in the conformation of the structurally homologous intact immunoglobulin.

To establish our mechanistic interpretation further, we also carried out a chemical relaxation study of this system. For L_2 315 with DNPL at 4°C and pH 7.4 only one relaxation time of 2–20 ms was observed. The concentration dependence of this relaxation time is shown in Fig. 1. $1/\tau$ is plotted vs. the sum of total L_2 (A_0) and total hapten (H_0) concentrations. The three almost parallel lines are obtained for a roughly identical range of H_0 (1×10^{-5}–2×10^{-4} M), each line representing a different (constant) A_0 value (0, 17; Δ, 38; +, 120 μM). For a simple association mechanism $1/\tau$ depends equally on the free concentration of both protein (A) and hapten (H), as $1/\tau = k_{on}(A + H) + k_{off}$. Since in our experiment A_0 is comparable to H_0, and the concentration of the complex is relatively low, $1/\tau$ value is expected to be similar for all points with the same $A_0 + H_0$, even when they have different A_0. This is clearly not the case here, and actually, when $1/\tau$ is plotted vs. H_0 alone, all three lines almost coincide, implying that $1/\tau$ is practically independent of A_0. The simplest mechanism compatible with such behavior is $T_0 \rightleftharpoons R_0$ ($K_0 = k_0/k_{-0} = R_0/T_0 \ll 1$); $H + R_0 \rightleftharpoons R_1$ ($K_R = k_R/k_{-R} = R_1/(R_0H)$). Here the pro-

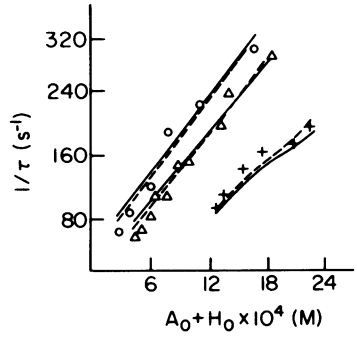

FIGURE 1

tein exists in two conformations T and R, of which only R, which comprises only a small fraction of the free protein, binds the hapten. For this system the relaxation time for the association is $1/\tau = K_R (R_0 + H) + k_{-R}$, and since $R_0 \ll H$ always, $1/\tau \cong k_R H + k_{-R}$. The broken lines in the figure represent the best fit to this mechanism, with $K_R = 1.9 \times 10^6\ M^{-1}\ s^{-1}$, $k_{-R} = 45\ s^{-1}$, and $K_0 = 0.14$. An additional relaxation time representing the monomolecular $R_0 \rightleftharpoons T_0$ equilibrium is also expected. We suspect that this time is very slow and lies outside the time range of the temperature-jump method. This mechanism is not, however, in accord with the static measurements, since it predicts a hyperbolic saturation curve. We then proceeded to analyze the kinetic data using the MWC mechanism. For an allosteric dimer this model is redundant, i.e., many different K_T, K_R, and L sets may conform with a particular binding curve, as far as they all arise from the same pair of Adair constants. For the titrations of DNPL these were found to be $K_1 = 3.5 \times 10^3$ and $K_2 = 1.4 \times 10^4\ M^{-1}$. A fit to the nonexclusive MWC model using the formulas of Kirschner et al. (4) and assuming that only an intermediate (R association) relaxation time is observed, gave a good agreement (full curve in the figure) the best fit parameters being $k_R = 1.9 \times 10^6\ M^{-1}\ s^{-1}$, $k_{-R} = 52\ s^{-1}$, $K_T = 1.9 \times 10^3\ M^{-1}$, $K_R = 3.7 \times 10^4$, and $L = 36$. These represent $K_1 = 2.8 \times 10^3$, $K_2 = 1.5 \times 10^4\ M^{-1}$, in good agreement with the titrations. The value of L obtained is intermediate between those found when exclusive MWC, or when equality of L for both haptens, was assumed. An analysis of relaxation amplitudes gave only a moderate agreement with the MWC model, implying that the actual mechanism may be somewhat more complicated. However, the very good fit of the relaxation time data suggests that the MWC model is a good first-approximation description. Relaxation measurements with NBDA are now underway. A complex spectrum, containing at least two relaxation times, is observed, which serves as a preliminary indication for the complexity of the binding mechanism.

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THE STRUCTURE OF THE RETINYLIDENE CHROMOPHORE IN BATHORHODOPSIN

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ABSTRACT Resonance Raman data on bathorhodopsin (bovine and squid) at 95, 77, and 4°K support a mechanism of excitation proposed by Lewis in which both a