Characteristics of the Binding of Dapsone and Monoacetyldapsone by Serum Albumin[1] (37200)

ROBERT W. RILEY[2] AND LOUIS LEVY

Leprosy Research Unit, Public Health Service Hospital, San Francisco, California 94118

Recent studies of the disposition of dapsone (4,4′-diaminodiphenylsulfone, DDS), the most important agent for the antimicrobial treatment of leprosy, have suggested the importance of binding of both this drug and its monoacetylated derivative (4-amino-4′-acetamidodiphenylsulfone, MADDS) to plasma proteins. Studies in man (1), the mouse (2), the dog (Biggs *et al.*, unpublished data), and other species (3, 4) have shown that plasma protein binding of both compounds occurs to a considerable extent in all species studied. DDS was 70 to 80% bound, and MADDS was 98 to 100% bound in human plasma. In mouse plasma, DDS was 50 to 70% bound, and MADDS was 80% bound. In human plasma diluted to a protein concentration of 1 gm %, MADDS binding was reduced only moderately, compared to the binding of these compounds in undiluted plasma. When mouse plasma was similarly diluted, MADDS binding was moderately reduced, but DDS was only 7% bound.

Because studies by Linderstrom-Lang (5) and by Glazko and co-workers (6) have demonstrated extensive binding of DDS by bovine and human serum albumin, we have examined the characteristics of the binding of DDS and MADDS by human and mouse plasma albumin, in order to find explanations for the difference between DDS and MADDS binding in human plasma, and for the difference in DDS binding between human and mouse plasma.

Materials and Methods. Human serum albumin (HSA) was purchased from Hyland Laboratories, Costa Mesa, CA. Mouse plasma albumin (MPA) was prepared by $(NH_4)_2SO_4$ fractionation of a pool of heparinized BALB/c mouse plasma (7). The fraction of mouse plasma proteins precipitating between 50 and 62% saturation with $(NH_4)_2SO_4$ was purified by solution in 0.075 *M* phosphate buffer (pH 7.4), repeated precipitation between 50 and 62% saturation with $(NH_4)_2SO_4$, and dialysis against the phosphate buffer. Purity of the MPA was demonstrated electrophoretically. Protein concentrations were measured by a spectrophotometric method (8).

Albumin binding of DDS (K and K Laboratories, Inc., Hollywood, CA) and MADDS (Parke, Davis and Co., Ann Arbor, MI) was studied by an equilibrium dialysis technique (9). Cellulose dialysis tubing—27/32 in. (A. H. Thomas Co., Philadelphia, PA)—was suspended in deionized water for 0.5 hr prior to use and then blotted dry. The tubing was filled with 3 ml albumin solution and suspended in 12 ml of a solution of the drug in phosphate buffer in a 50 ml centrifuge tube, which was then agitated at 4° for 48 hr. Except for two studies in which the pH was varied, studies were carried out at pH 7.4. Measurements of binding were performed in duplicate. Binding was calculated from the difference between the drug concentration inside the dialysis bag and that outside. The concentrations of DDS and MADDS were measured fluorometrically (10) in duplicate, by means of a Farrand spectrophotofluorometer. Binding was analyzed by means of the

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[2] Submitted to the Graduate Division, University of California, San Francisco, in partial fulfillment of the requirements for the MS in Pharmacology. Present address: Department of Oral Surgery, Harbor General Hospital, Torrance, CA.

Scatchard equation (11).

Results. Binding of MADDS to HSA was studied with MADDS concentrations of 1 to 15 μg/ml and HSA concentrations of 0.1, 0.5, and 0.6 g/100 ml. A Scatchard plot (Fig. 1) was constructed according to the relationship:

$$\nu/A = K_a(N - \nu),$$

in which ν is the mole ratio of bound drug to albumin, A the concentration of unbound drug, N the number of binding sites per albumin molecule, and K_a the binding (association) constant. N = the value of ν at ν/A = 0; K_a may be calculated from the value of ν/A at ν = 0 and that determined for N. The least squares line describing the regression of ν/A on ν in this plot is:

$$\nu/A = (2.35\text{–}2.73\,\nu) \times 10^5.$$

The 95% confidence limits (12) for the value of ν/A at ν = 0 are (2.35 ± 0.34) × 10^5, and for the value of ν at ν/A = 0 are 0.86 ± 0.16. Thus, N may be taken to equal 1, and K_a = 2.35 × 10^5 liters mole^{-1}.

Binding of DDS to HSA was studied with DDS concentrations of 0.5 to 30 μg/ml and HSA concentration as for the study of MA DDS binding. The Scatchard plot constructed from the results of this study is shown in Fig. 2. The regression of ν/A on ν is:

$$\nu/A = (1.17\text{–}2.66\,\nu) \times 10^4.$$

The 95% confidence limits for ν/A at ν = 0 are (1.17 ± 0.29) × 10^4, and for ν at ν/A = 0 are 0.45 ± 0.08. N may be taken to equal 0.5, and K_a = 2.34 × 10^4 liters mole^{-1}. Binding of DDS by HSA was studied at pH 5.8 and 9.6 as well as at pH 7.4. No evidence of binding could be detected at pH 9.6; at pH 5.6, the binding was reduced by about half, compared to that at pH 7.4.

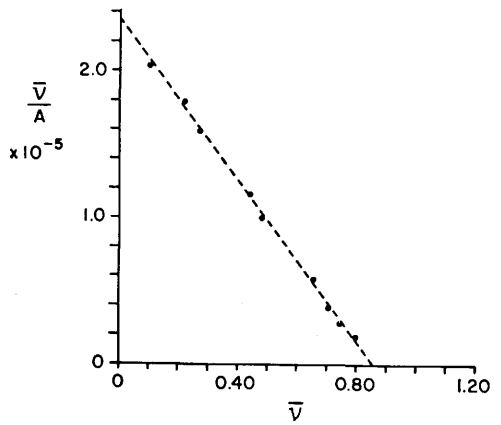

FIG. 1. Scatchard plot of the binding of MADDS by HSA. The equation of the regression of ν/A on ν is: ν/A = (2.35–2.73 ν) × 10^5; N = 0.86 ± 0.16 (mean and 95% confidence limits); K_a = (2.35 ± 0.34) × 10^5.

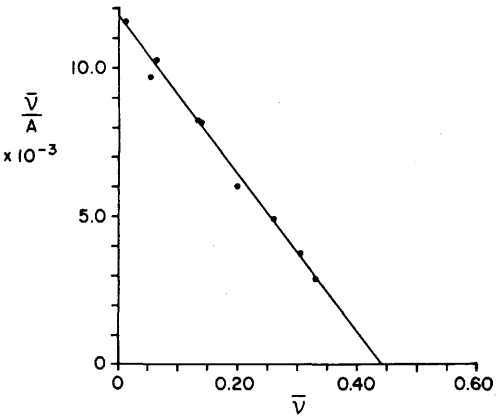

FIG. 2. Scatchard plot of the binding of DDS by HSA. The equation of the regression of ν/A on ν is: ν/A = (1.17–2.66 ν) × 10^4; N = 0.45 ± 0.08; K_a = (2.34 ± 0.58) × 10^4.

Binding of DDS to MPA was studied with DDS concentration of 0.5 to 10 μg/ml and MPA concentrations of 0.1 and 0.5 g/100 ml. The resulting Scatchard plot, shown in Fig. 3, yields the regression of ν/A on ν:

$$\nu/A = (8.36\text{–}18.77\,\nu) \times 10^3.$$

The 95% confidence limits for ν/A at ν = 0 are (8.36 ± 0.30) × 10^3, and for ν at ν/A = 0 are 0.44 ± 0.02; $N \approx$ 0.5, and K_a = 1.67 × 10^4 liters mole^{-1}.

Thus, the affinity with which MADDS binds to HSA is about 10 times that for DDS binding by HSA. And binding of DDS by MPA occurs to about the same degree as that by HSA.

Discussion. The demonstration that MA DDS was more strongly bound than DDS by human plasma (1) appears to be explained by the 10-fold greater affinity of HSA for MADDS shown here. This had been suggested by Glazko and co-workers (6), who reported a greater degree of binding of MA DDS than of DDS to HSA. There appears to be one binding site per molecule for MA DDS, whereas only ½ binding site per mole-

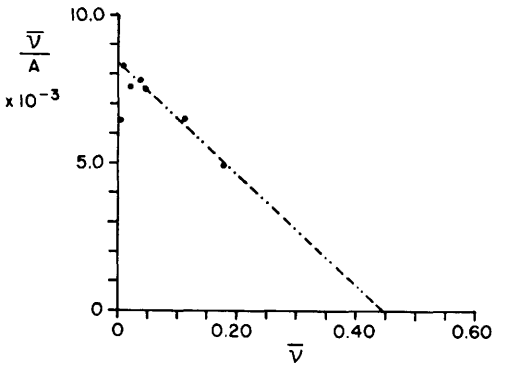

FIG. 3. Scatchard plot of the binding of DDS by MPA. The equation of the regression of ν/A on ν is: $\nu/A = (8.36\text{–}18.77\ \nu) \times 10^3$; $N = 0.44 \pm 0.02$; $K_a = (1.67 \pm 0.60) \times 10^4$.

cule could be demonstrated for DDS. That DDS has two potentially ionizable amino functions per molecule and MADDS has only one is not pertinent. The amino groups of DDS are only very weakly basic, with a pK_a of 1 (13), so that the DDS molecule is present virtually completely as its unionized species at physiologic pH. This is consistent with the demonstration here that the binding of DDS to HSA is decreased by decreasing as well as by increasing the pH of the experimental system. It has been shown, on the other hand, that the conformation of albumin changes with a change of pH in either direction (14, 15). It must be concluded, therefore, that the mechanism of binding of either DDS or MADDS by albumin is nonionic.

The mechanism of binding may well involve some interaction of the amino function with the protein molecule, however. Thus, DDS, with two free amino functions, appears to behave as a bivalent molecule. The demonstration that the albumin molecule (both HSA and MPA) possesses only ½ binding site for DDS suggests that each DDS molecule is bound to two albumin molecules.

The K_a values for DDS binding to HSA and to MPA are not significantly different; thus, the explanation for the great difference of DDS binding between dilute human and dilute mouse plasma must be sought elsewhere. It may be that there is another component of plasma which is capable of binding DDS with an affinity differing greatly between human and mouse plasma.

Summary. The characteristics of binding of DDS and MADDS by HSA and of DDS by MPA have been studied by means of an equilibrium dialysis technique and analyzed by means of the Scatchard relationship. The plot of ν/A vs ν for each of the three studies yielded a straight line, suggesting only one species of binding site. Each molecule of HSA was found to possess one binding site for MADDS. Each molecule of MPA and HSA possessed ½ binding site for DDS, suggesting that DDS behaved as a bivalent molecule, and that one molecule of DDS was bound to two albumin molecules. The binding constant of HSA binding of MADDS was 10 times greater than the constant of HSA binding of DDS. The binding constants for DDS binding by both HSA and MPA were the same.

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