

their measurements were not performed at  $\text{pH} > 11.2$ . All the facts which were given above to explain our results in the acid pH region may be used to demonstrate that the absorption maxima on Figure 2 are due to proton-transfer reactions on protein side-chain amino groups:  $-\text{NH}_3^+ + \text{OH}^- \rightleftharpoons -\text{NH}_2 + \text{H}_2\text{O}$ . From our results on BSA, over the frequency range 1–150 MHz, we found that the standard volume change associated with this reaction is between 18.5 and 46.5  $\text{cm}^3/\text{mol}$ , according to the  $\text{p}K_a$  value used in the calculations. Applegate, *et al.*,<sup>9</sup> have obtained volume changes of 26 and 36  $\text{cm}^3/\text{mol}$  for diglycine and triglycine, respectively.

These preliminary results show that ultrasonic studies of the kinetics of conformational changes in protein solutions are made difficult by the existence of an absorption due to proton-transfer reactions which becomes significant precisely in the pH regions in which occur conformational changes and dissociation of proteins in subunits.

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#### Further Remarks on the Ultrasonic Properties of Bovine Serum Albumin Solutions

*Sir:* Zana and Lang<sup>1</sup> have shown that the contribution of ultrasonic absorption in aqueous solutions of bovine serum albumin (BSA) resulting from proton transfer reactions in amino acids may be significant, particularly in the regions of the ultrasonic absorption peaks. Our recent findings<sup>2</sup> also have defined the absorption peak near pH 12. Unfortunately, there are insufficient data at this time to compare adequately the frequency dependence of the absorption peaks in BSA with those in the amino acids.<sup>3</sup>

Although there seems to be substantial agreement between some of our present conclusions and those of Zana and Lang, we wish to point out several unresolved difficulties in specifying the principal ultrasonic interaction mechanism in aqueous BSA solutions as proton transfer reactions. Comparison of the absorption peak in Figure 1 curve 1 of Zana and Lang<sup>1</sup> to those in Figures 2 to 4 of our paper,<sup>4</sup> which are at much higher BSA concentrations, shows a shift in the value of  $\text{pH}_M$

of approximately 0.8 pH unit in a direction opposite to that predicted by their equation,  $\text{pH}_M = 1/2(\text{p}K_a - \log c)$ . Furthermore, over the concentration range 0.04  $\text{g}/\text{cm}^3$  to 0.09  $\text{g}/\text{cm}^3$  we did not observe a significant shift of  $\text{pH}_M$ , within the limit of our ability of measure pH, *viz.*,  $\pm 0.05$  pH unit. That a process more complex than proton transfer is necessary to describe all of our data is indicated by the minimum in the normalized velocity of sound near pH 4.1 and displaced from  $\text{pH}_M$ , as shown in Figure 5 of our paper. This is not necessarily accounted for by chemical relaxation. The strong correlation of the velocity of sound minimum with the step increase in  $[\eta]$ , also at pH 4.1<sup>5</sup> and corresponding to the intermediate N–F' transformation<sup>6</sup> suggests that perhaps ultrasonic velocity measurements may be less susceptible to masking by additional chemical relaxation processes than the absorption coefficient, and thus ultrasonic velocity may be a more reliable indicator of conformational change in the present case. It has been shown, for example, that a dip in the velocity of sound and a change in the viscosity can occur in the region of phase transition in certain liquid crystalline systems,<sup>7</sup> and the dependence of velocity and pH may be analogous for a solution in which a configurational equilibrium is perturbed by the sound wave.<sup>8</sup> Finally, some mechanism other than proton transfer is necessary to explain the change in absorption level from neutral pH to  $\text{pH} < 1$ , *i.e.*, aside from the intervening peaking of the absorption. A similar remark pertains to the alkaline pH region.

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