tion we would expect the difference in ΔS to be 45 e.u. and in ΔH to be 18 kcal. These are in good agreement with the observed differences (Table I) when one considers that the estimates of both the number of residues involved and the changes per residue must necessarily be crude since the end effects will be large when dealing with such short segments of helix. We also have oversimplified the problem by neglecting side chain interactions. However, the denaturation region lies at lower temperatures for chymotrypsin than for chymotrypsinogen, indicating that more profound changes in the stability of the structure occur during activation of the zymogen than are revealed by the production of a new helix segment. Nevertheless, at temperatures above the denaturation region, the two proteins are very nearly identical in a_0 and b_0 values, and are thus probably in very similar conformational states. b_0 is about $-130°$ for both denatured proteins at $50°$ and in both cases there is only a small temperature dependence. a_0 for denatured chymotrypsinogen is $-538°$ at $50°$, while a_0 for denatured chymotrypsin is $-548°$ at the same temperature. a_0 also shows nearly the same temperature dependence for the two denatured proteins.

TABLE I

CHANGES IN THERMODYNAMIC FUNCTIONS DURING DENATURATION

	Chymotrypsin[1]	Chymotrypsinogen[2]	Difference
ΔS, e.u.	360	316	44
ΔH, kcal.	110	99.6	10.4

It is quite clear for both proteins that the major contributions to the entropy and enthalpy change in reversible denaturation are not associated with large changes in optical rotation. The best description of the denaturation reactions reconciling the thermodynamic and rotatory dispersion data which we have been able to find is this: The entropy change is the result of a coöperative, first-order-like melting of the tangle of side chain interactions, probably predominantly hydrophobic in nature, at the protein–solvent inteface. The melted sections are similar to liquid condensed phases in surface film studies. Water is able to penetrate the forest of side chains after denaturation, but infrequently before. Although the side chains in the melted sections gain considerable freedom, there is little unfolding of the protein in the usual sense and the conformation of the backbone is essentially unaltered in the process so that a_0 and b_0 do not change. Other studies to be reported shortly support this interpretation.

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THE EFFECT OF LITHIUM BROMIDE ON THE STRUCTURAL TRANSITION OF RIBONUCLEASE IN SOLUTION

Sir:

Interest has evidenced itself recently on the effect of aqueous lithium bromide solutions on the thermodynamic stability of the ordered structures in the fibrous and globular proteins. Contrary conclusions have been reached by different investigators.[1-5] Harrington and Schellman[1] reported a decrease in the levorotation of various proteins dissolved in such solutions, with particular attention being given to the behavior of ribonuclease. Based upon the correlations obtained between chain conformation and optical rotation for the simple homopolypeptides by Doty and co-workers,[6,7] it was deduced that the helical structures were stabilized by the action of lithium bromide. Since it is well known that the activity of water is abnormally low in such solutions,[8] the stabilization inferred could be attributed conveniently to this cause. These deductions have, however, been seriously questioned recently as a result of more extensive solution measurements, which unfortunately were restricted to a single temperature.[2]

On the other hand, investigations of the dimensional changes exhibited by a variety of fibrous proteins, when immersed in a large excess of aqueous lithium bromide solutions, have led to the conclusion that at appropriate temperatures a transformation from the ordered to disordered state occurs.[3,4,5] The transformation in these cases is accompanied by an axial contraction. A significant disparity exists, therefore, between these latter results, typical of higher polymer concentrations, and the widely accepted interpretation placed on the optical rotation data obtained in dilute solution. The suggestion has been made that the nature of the lithium bromide interaction may be strongly dependent on the protein concentration, and a mechanistic model supporting this hypothesis has been offered.[9]

Since the structural transformation of ribonuclease that occurs in pure aqueous solutions has been studied extensively,[1,10,11,12] the apparent discrepancy cited above and the associated speculation should be resolvable by studying any variations that take place in the transformation temperature caused by the addition of lithium bromide

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to the dilute solution. This would then be a direct measure of the thermodynamic stability of the ordered form.

We have investigated the optical rotation (at 546 mμ) as a function of temperature for ribonuclease (Sigma, Chromatographed Grade Type II) dissolved in aqueous solutions of increasing lithium bromide concentration. The results are summarized in Fig. 1. In pure water the variation of the specfic rotation with temperature is virtually identical with that previously reported.[1,10,11,12] The structural transformation, characterized by a relatively sharp increase in levorotation, is clearly discernible and terminates at approximately 70°. The addition of lithium bromide results in a progressive lowering of the transition temperature, indicating a decreased stability of the ordered structure present in the native form. The increase in levorotation during the transformation is still noted but the magnitude of the change in the specific rotation decreases with increasing lithium bromide concentration. For a 4.5 M lithium bromide solution the transformation temperature has been lowered to below 15°. It can be concluded that in the salt concentration range studied, lithium bromide acts as a universal transformer of the ordered structures of polypeptides and proteins. The apparent discrepancy between the results for the fibrous proteins and those for proteins in dilute solution consequently is eliminated. A continuity in the phase diagram, as the protein concentration is varied, can now be anticipated.

At any fixed temperature, as the concentration of lithium bromide increases, the optical rotation becomes less levorotatory, in accord with the observations of Harrington and Schellman,[1] this effect being more marked in the transformed state. The transformed state has been identified with a random-coil chain conformation in pure water.[10,12] The continuity of the data presented indicates that this must also characterize the transformed state obtained after the addition of lithium bromide. Thus, in this case a substantial decrease in levorotation with increasing lithium bromide concentration does not necessarily reflect the stabilization or formation of helical structures.

As has been pointed out previously,[13,14] specific solvent or medium effects can in principle change the observed optical rotation without the necessity of any concomitant structural changes occurring within the molecule. When this situation exists, an erroneous conclusion can be made when the optical rotation measurements are limited to a single temperature. This appears to be the case for the interaction of lithium bromide with proteins and polypeptides. Though the levorotation decreases, a decrease in the thermodynamic stability of the ordered structures also manifests itself. Any interpretation of changes in optical rotation based solely in terms of structural changes is, therefore, in a tenuous position in general, and erroneous in the specific case under consideration.

In addition, a survey of data in the literature shows that the addition of lithium bromide to solutions of polypeptides and proteins always

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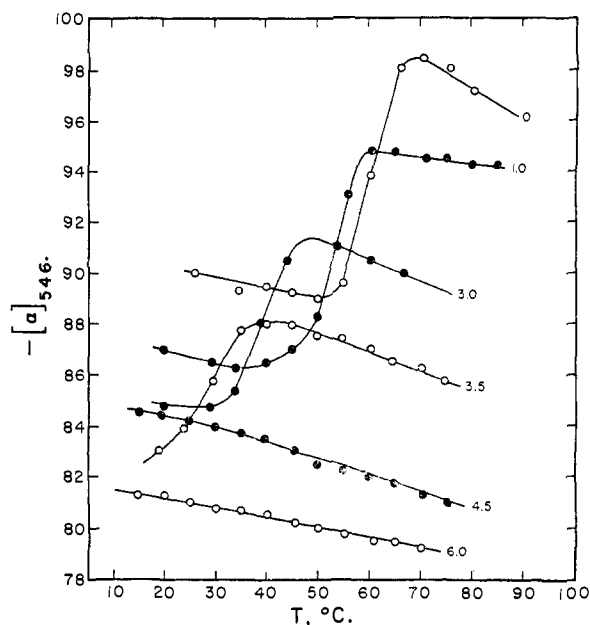(14) W. Kauzmann, *Adv. in Prot. Chem.*, **14**, 1 (1959).

Fig. 1.—Plot of specific rotation at 546 mμ ($[\alpha]_{546}$) as a function of temperature for a 1.4% ribonuclease solution dissolved in aqueous lithium bromide solution of indicated molarity.

results in a decreased levorotation.[1,2,15-19] It is particularly pronounced in the case of gelatin wherein the random coil form is maintained.[15,16] From this point of view, also, the difficulties experienced in interpreting the changes in optical rotation and accompanying inactivation of pepsin with lithium bromide can be clarified.[19]

More details of the present work, together with the complexities observed at higher lithium bromide concentration,[4,5] will be presented subsequently. Interpretation will be made in the context of the universal action of lithium bromide and similar compounds in coöperatively disrupting the ordered structures of the fibrous and globular proteins over the complete range of polymer concentration.

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THE CONFIGURATION OF $Zr(C_2O_4)_4^{-4}$ AND THE STEREOCHEMISTRY OF DISCRETE EIGHT-COORDINATION[1]

Sir:

The configurations affording a superior stereochemistry for discrete eight-coördination com-