the infrared spectrum[11] being given for comparison. In this case a well-studied functional group (guanidinium) is present; comparison with the spectrum of a fairly close analog of arginine, the methylguanidinium ion,[12,13] shows little similarity. Very few guanidinium group motions can be suggested even tentatively. This indicates that the vibrations of the guanidinium group are strongly coupled with those of the rest of the molecule.

The Raman spectrum of creatine (methylguanidine acetic acid), in the form of the hydrochloride, is given in Table VIII. This spectrum bears more resemblance to that of the methylguanidinium ion than does the spectrum of arginine. The strong line at 824 may correspond to that of the methylguanidinium ion at 915. Other lines that may be associated with this group are 534, 597, 1049, 1183, 1440 and 1460, although no definite assignments can be made.

Methionine.—The spectra of methionine, given in Table IX, with the infrared spectrum for comparison,[14] show the effect of the presence of a sulfur atom. Of the three possible C–S stretching frequencies (around 700 cm.$^{-1}$) one changes intensity appreciably on going from the hydrochloride to the sodium salt form, and this is left unassigned, as it appears extremely improbable that the C–S stretching motion would be affected by the state of ionization of the amino or carboxyl group

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The Properties of Bovine Serum Albumin in Concentrated Acetic Acid[1]

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Some of the properties of bovine serum albumin in glacial acetic acid have been determined. Most of the measurements have been done in the presence of about 0.5 weight % water since the solubility of the protein is very low under anhydrous conditions. In acetic acid, bovine serum albumin has an intrinsic viscosity of 0.134 dl. g.$^{-1}$, a sedimentation constant of 2.3 *S* and a specific optical rotation of $-31 \pm 1.5°$ compared to the values of 0.036 dl. g.$^{-1}$, 4.4 *S* and $-62°$ for the same preparation in aqueous acetate buffer. Bovine serum albumin may be recovered apparently unchanged from acetic acid solutions by lyophilization.

The fact that certain proteins are soluble in nonaqueous solvents has been only recently appreciated.[4-8] The observation that native and heat denatured bovine serum albumin (BSA) have different solubilities, not only in aqueous solutions, but also in glacial acetic acid (GAA) has prompted a more thorough investigation of BSA in the latter solvent.

Experimental

Materials.—BSA from Pentex, Inc., was deionized on an ion-exchange column, filtered through a Corning ultra-fine sintered glass filter and lyophilized. The dry protein was stored at −5°. This preparation gave a single symmetrical peak in the ultracentrifuge and had an intrinsic viscosity, $[\eta]$, of 0.036 deciliters g.$^{-1}$ in aqueous acetate buffer (0.05 *M* sodium acetate, 0.05 *M* acetic acid and 1.0 *M* sodium chloride). The specific optical rotation was −62° (λ 589 mμ) and the extinction coefficient at 278 mμ, $E^{1\%}_{1\,cm}$ 6.63.

Reagent grade glacial acetic acid (GAA) was distilled in a dry atmosphere before use.

Methods. Preparation of Solutions.—BSA dried over phosphorus pentoxide was found to be nearly insoluble in GAA but still freely soluble in water. The presence of small amounts of water in GAA or in the protein would raise the solubility greatly and accordingly the experiments were carried out on GAA solutions to which just enough water was added so that the protein would dissolve easily (about 0.5 weight % of water).

Viscosity.—Measurements were made in an Ostwald viscometer at 24°. Slight modifications to the viscometer prevented contact between the solutions and the water of the atmosphere.

Sedimentation.—Sedimentation coefficients were calculated from schlieren patterns in the Spinco Model E Ultracentrifuge and were corrected for solvent viscosity and density to water at 20° unless otherwise indicated.

Optical Rotation.—A visual polarimeter (Rudolph #80) was used at 589 mμ with 20 cm cells.

Tryptic Digestion.—The reaction mixtures contained 1% BSA, 0.01% trypsin, 0.1 *M* tris-(hydroxymethyl)-aminomethane and 0.01 *M* calcium chloride, *p*H 8.9 at 24°. At intervals, samples were withdrawn and trichloroacetic acid (TCA) was added to bring the TCA concentration to 10%. The absorption of the TCA filtrate was measured at 278 mμ.

Recovery of BSA from GAA Solution.—It was found that BSA could be effectively recovered from GAA solution by lyophilization. The dry protein thus obtained (designated BSA-AA) dissolved readily in water to give a solution (1% protein) having a *p*H of 3.8. After several successive lyophilizations from water or after dialysis against water for two hours, the *p*H of a 1% solution rose to 5.5, which is the same as that given by the native protein.

Results and Discussion

Figure 1 gives the results of the viscosity and sedimentation measurements. The value of $[\eta]$ is 0.036 dl. g.$^{-1}$ in aqueous acetate buffer and 0.134 dl. g.$^{-1}$ in GAA. The sedimentation constants ($S_{20,w}$) in the same two solvents are 4.4 and 2.3 *S*, respectively. In order to determine how important electrostatic charge effects might be in

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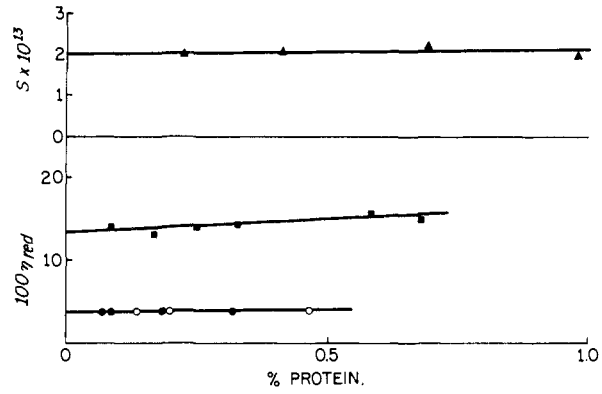

Fig. 1.—Variation of sedimentation coefficient and reduced viscosity of BSA with concentration: ●, reduced viscosity of native BSA in acetate buffer, 1.0 M NaCl, pH 4.7; ○, reduced viscosity of BSA-AA in the same buffer; ■, BSA dissolved in glacial acetic acid; ▲, sedimentation coefficients of BSA dissolved in glacial acetic acid (not corrected to $S_{20,w}$).

such a solvent as GAA, sedimentation runs were made with and without added electrolyte. Sodium formate dissolves easily in GAA (0.5% water) and a 1 M solution has a specific conductance (κ) (23° and 1000~) of 1.5×10^{-3} mho cm.$^{-1}$, which is equivalent in conductance to about 0.01 N potassium chloride in water. For GAA itself, κ is about 1×10^{-7} mho cm.$^{-1}$.

The relative viscosity at 25° for 1 M sodium formate in GAA is 2.99 and the solution density is 1.091, while that of GAA is 1.045 g. cm.$^{-3}$. The sedimentation coefficients (uncorrected) for 0.4% protein at 25° in the two solvents were 0.652 S in formate and 1.71 S in GAA, the ratio being 2.62. Assuming the partial specific volume to be constant at 0.684 cm.^{3} g.$^{-1}$ found in GAA, we would expect the ratio to be 3.36. These results suggest that there may be a primary charge effect on sedimentation in GAA, which would account for part of the difference observed between GAA solutions and aqueous solutions.

The specific rotation for the native protein was −62° in acetate buffer and −31 ± 1.5° in GAA. Yang and Doty[9] also have observed a very low levorotation for BSA under certain conditions. They give −22.5° for the optical rotation of BSA in 3:1 ethylene dichloride:dichloroacetic acid.

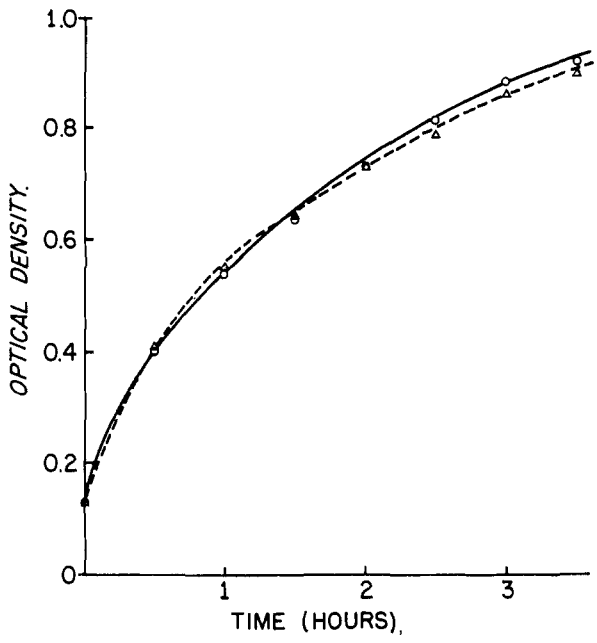

Fig. 2.—Comparison of the rates of digestion by trypsin of BSA and BSA-AA by the absorption of TCA soluble material at 278 mμ: ○, BSA; △, BSA-AA.

For the recovered protein (BSA-AA) the value of $[\eta]$ was 0.036 dl. g.$^{-1}$ in acetate buffer and the optical rotation was −61.2 ± 0.7° in the same solvent. Figure 2 shows a comparison of the rates of digestion of BSA and BSA-AA by trypsin. There is no significant difference between BSA and BSA-AA by any of these three measurements.

The results indicate that BSA and BSA-AA have probably the same structures and that all the changes occurring in GAA are reversible. The actual configuration of the molecule in GAA is not clear at present, since it is not known how much importance to attach to the changes in folding suggested by the optical rotation and to electrostatic charge effects revealed by the sedimentation data.

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