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Complex formation between bovine serum albumin and sodium deoxyribonucleate induced by heat

GREENSTEIN AND HOYER[1] observed that under special conditions, deoxyribonucleic acid (DNA) prevents the heat coagulation of serum albumin. The special conditions are: (1) The mixture of albumin and DNA must be practically salt free. (2) The pH of the mixture must be adjusted to about 5.3 prior to heating. Nearly complete protection of the albumin solutions against heat coagulation is afforded by 0.5 to 1% by weight of DNA relative to albumin. The question of the mechanism of protection was left open although in a note[2] immediately following the GREENSTEIN paper it was suggested that binding was probably not involved since two peaks were observed in the ultracentrifuge after heating.

Subsequently GEIDUSCHEK AND DOTY[3] investigated the interaction of bovine serum albumin (BSA) and DNA by light scattering at room temperature. Their procedure was to mix the DNA and BSA together and to measure the light scattering by diluting this mixture into a phosphate buffer which served as solvent. Various pH's were studied. At pH 7.47 and 6.46, no reaction was observed. At pH 5.51, DNA was found to bind 35% of its own weight of BSA. The authors felt that despite the differences between the conditions of the two experiments, the amount of binding displayed was quite inadequate to explain the protective action of DNA on BSA. It was suggested that the stronger binding that such protection appeared to require may only occur with parts of the protein structure that may be made accessible by denaturation.

This investigation represents our search for this stronger type of binding that is thought to occur.

The bovine serum albumin was a crystalline product obtained from Pentex Incorporated, Kankakee, Illinois. The deoxyribonucleic acid was prepared from salmon sperm by Dr. NORMAN SIMMONS. All solutions were heated in small glass stoppered test tubes by immersing in boiling water for ten minutes. Solutions prepared for heating experiments contained about 0.5 g BSA/dl. The albumin stock solution was prepared by the method of GREENSTEIN AND HOYER[1]. The DNA stock solution was prepared by diluting a concentrated solution in distilled water.

It was at once apparent that the products of heating are a sensitive function of the pH. If albumin alone is heated at pH 5.5, partial heat coagulation occurs as evidenced by formation of a milky opacity in the solution. However, analytical ultracentrifugation (Spinco Model E) shows that there is considerable albumin remaining in solution with the same sedimentation constant as native material. As a result of the heating, the pH rises from 5.5 to about 6.9. If one heats native albumin at pH 6.5, no heat coagulation occurs. But upon heating the albumin at pH 5.4 in 0.0015 M citrate buffer, the pH does not rise above 5.9 and all of the albumin is coagulated. Thus it appears that one of the prerequisites for heat coagulation is that the electrostatic repulsion of the albumin molecules be minimized. As the pH becomes higher, the negative charge on the albumin rises* (note: the isoelectric point of BSA is 4.5) and apparently the repulsion

* The increase in negative charge between pH 5.5 and pH 6.5 is probably accounted for by the neutralization of the imidazole groups of histidine which would be expected to titrate in this region. There are 18 histidine residues per molecule of BSA according to G. R. TRISTRAM, in H. NEURATH AND K. BAILEY, *The Proteins*, Vol. IA, Academic Press, Inc., New York, 1953, p. 215.

between albumin molecules becomes great enough above pH 6 to prevent effective contact. The repulsion caused by the charge effect should be overcome in a medium of high ionic strength, and accordingly when the albumin at pH 6.5 is heated in 0.04 M NaCl coagulation occurs.

When DNA is heated alone in aqueous solution it is severely degraded as evidenced by a large decrease in the sedimentation constant measured after the addition of salt (0.15 M). There is, however, no degradation if the ten-minute heating is carried out in the presence of salt.

With the behavior of the individual components upon heating thus clarified, an investigation of the mixtures following heating was undertaken. At a weight ratio of BSA/DNA of 15 no coagulation was observed in a salt free solution but the pH did rise from pH 5.5 to 6.9 as with the albumin alone. The heated solution exhibited two peaks of about equal area in the ultracentrifuge. A study of the concentration dependence of the slower peak indicated that it has the same sedimentation constant as native albumin. The other peak moved much too fast for DNA (native or denatured) and this fact coupled with the relative area under the fast peak suggested it must be a complex of DNA and about half the albumin present. Thus although there appeared to be considerable binding of the DNA to the albumin, the reaction had not gone to completion. However, when the DNA and albumin were heated in the presence of 0.0015 M citrate buffer, the pH went from 5.5 to 5.8 as before but in the ultracentrifuge only a fast moving component was observed. Apparently the reaction goes to completion only when the pH is controlled as in the coagulation of albumin alone*.

On the basis of these experiments, we conclude that the DNA protects the albumin from coagulating (below pH 5) by binding it, thereby preventing self-aggregation. Above pH 6 the DNA is not needed to prevent coagulation since the albumin does not coagulate under these conditions.

The completeness of the binding at a ratio of 15 in the presence of 0.0015 M citrate indicates that at least 1800 molecules of albumin can be bound per DNA molecule. The absence of gel and the presence of a single peak in the ultracentrifuge suggest that cross-linking of DNA by the albumin has not occurred. Consequently, the physical chemical properties of the completely reacted mixture were studied in order to see if the complex could be accounted for by the combination of one DNA molecule with 1800 albumin molecules. The molecular constants are listed in Table I along with the appropriate constants for native DNA and BSA. The physical measurements on the DNA–BSA complex were made in 0.005 M NaCl. Using the molecular weight of DNA and considering the weight ratio of the reactants, we may calculate the molecular weight of the BSA–DNA complex to be expected since all of the BSA reacts if there is no degradation of the DNA. This calculated molecular weight is in close agreement with the experimentally determined molecular weight and clearly supports our interpretation of the reaction. When 8 M urea is added to the BSA–DNA complex the light scattering falls drastically. This clearly suggests that the binding is mainly due to hydrogen bonds. Presumably these have been made available by the thermal denaturation of both BSA and DNA.

TABLE I

	BSA	*DNA*	*BSA–DNA*
S^0_{20}	$4.5 \cdot 10^{-13}$ sec	$22 \cdot 10^{-13}$ sec	$130 \cdot 10^{-13}$ sec
M.W.$_{\text{light scattering}}$	69,000	$8 \cdot 10^6$	$122 \cdot 10^6$ $126 \cdot 10^6$*
$[\eta]$	0.040	70	4.85

* Calculated from M.W.$_{DNA}$ + (weight ratio BSA/DNA) × M.W.$_{DNA}$ = $126 \cdot 10^6$.

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* It should be noted that when DNA and BSA are heated in 0.04 M NaCl an extremely viscous gelatinous mass is formed. This is translucent rather than opaque as with albumin alone and is probably due to the formation of a three-dimensional network. Some gel is also obtained in the presence of 0.0015 M citrate buffer when the weight ratio of BSA/DNA is as high as 60.

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