A LIGHT SCATTERING INVESTIGATION OF THE INTERACTION OF SODIUM DESOXYRIBONUCLEATE WITH BOVINE SERUM ALBUMIN

by

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The cytologist's interest in the nucleoproteins of the cell nucleus has, in recent years, been shared by investigators using the standard techniques of chemistry, with the result that several studies have been made of nucleoprotein dissociation[1-5] as well as the interaction of isolated sodium desoxyribonucleate of calf thymus (SDN) with histone[6,7], egg albumin[6,7,8], bovine serum albumin[9,10,11], and other proteins. It has been found that under suitable conditions of pH and ionic strength, SDN interacts with these proteins to form soluble complexes. The latter appear ideally suited to investigation by macromolecular techniques. Furthermore, this type of reacting system constitutes a particularly simple case of the theory of light scattering from multicomponent systems[12,13,14] applied to large molecules, and allows considerable information to be derived from the experimental data. Consequently, the interaction of sodium thymonucleate and bovine serum albumin (BSA) has been studied by light scattering with an eye to determining the relative amounts of protein and nucleic acid reacting with each other, as well as the size and shape of the soluble nucleoprotein complex. We have thus been able to give some of the qualitative information on soluble nucleoprotein complexes of SDN and bovine serum albumin[9,10] more quantitative meaning. This communication deals with the method of calculation, and some preliminary results.

THEORY

For a three-component system of two solutes (components 2 and 4) and one solvent, the reduced intensity at zero degrees, R_{ot}, is related to the other variables of the system by[13,15]

$$\frac{1000\,K'}{N_0\,R_{ot}} = \frac{m_2\left(\psi_2 - \dfrac{m_2\beta_{24}\psi_4}{1+\beta_{44}m_4}\right)^2}{1+m_2\left(\beta_{22}-\dfrac{m_4\beta_{24}^2}{1+\beta_{44}m_4}\right)} + \frac{\psi_4^2}{a_{44}} \qquad (1)$$

where N_0 is Avogadro's number, n_0 is the refractive index of the solvent, subscripts denote the components, and

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a_i are activity coefficients
m_i are molalities
$a_{ij} = (\delta a_i/\delta m_j)_{T,p,m}$ are activity coefficients
$\beta_{ij} = (\delta \gamma_i/\delta m_j)_{T,p,m}$
ψ_i are the molal refractive increments
$K' = \dfrac{2\pi^2 n_0^2}{N_0 \lambda^4}$
and λ is the wavelength of the incident light

In the limit as the concentration of both solute components 2 and 4 approaches zero, equation 1 reduces to

$$\lim_{\substack{m_2 \to 0 \\ m_4 \to 0}} \frac{N_0 R_{ot}}{1000\, K'} = m_2(\psi_2 + a\,\psi_4)^2 + (m_4 - a m_2)\psi_4^2 \tag{2}$$

where

$$a = \left(\frac{\delta m_4}{\delta m_2}\right)_{T,p,\mu_4} \tag{3}$$

is the number of moles of component 4 that has to be added to an infinitely large system in order to maintain a constant chemical potential of component 4, (μ_4), when one mole of component 2 is added to the system. It can be equivalently regarded as the number of moles of component 4 reacting with one mole of component 2 or the number of molecules of component 4 preferentially absorbed by component 2[15]. It will be referred to as the molal binding or absorption coefficient. At finite concentrations, the variation of the activity coefficients of each component with concentration and composition introduces added terms which we shall not consider, since our main preoccupation is the determination of molecular weight in a reacting system, and with it the absorption coefficient of one component for the other. Changing to weight units of concentration (g solute per gram solvent) equation 2 becomes

$$\frac{K'(c_2 + c_4)}{R_{ot}} = \frac{c_2 + c_4}{c_2 M_2 (\Phi_2 + \delta\,\Phi_4)^2 + (c_4 - \delta\, c_2) M_4 \Phi_4^2} \tag{4}$$

where Φ_i are weight refractive index increments, $(\delta n/\delta c_i)$
M_i are molecular weights
c_i are weight concentrations
and δ is the weight absorption coefficient of component 4 on component 2

$$\delta = \frac{M_2}{M_4}\, a$$

For a distribution of binding, it is easy to show that δ is the weight average absorption coefficient[16].

In our system, we shall call the sodium thymonucleate component 2, and the bovine serum albumin component 4. For the sake of simplifying the notation of the next paragraph, we shall denote the nucleic acid-protein complex as component 6. It is *not*, in the thermodynamic sense, a separate component though. The determination of the shape and size of the nucleoprotein complex proceeds as follows:

The excess scattering, over and above solvent scattering, is entirely due to the two solute components. The scattering due to the complex of components 2 and 4 can be

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calculated *only* if we know the light-scattering properties of the pure reactants, and how much of each component remains unreacted. If we write the reaction as

$$\text{SDN} + a(\text{BSA}) \rightarrow (\text{SDN})\ (\text{BSA})_a \tag{5}$$

then calling the complex (SDN) $(BSA)_a$ component 6, we have on a weight basis that $c_6 = c_2^0\ (1 + \delta)$, $c_2 = 0$ and $c_4 = (c_4^0 - \delta\, c_2^0)$ (the superscript zero denotes original concentrations) and

$$R_{\theta 6} = R_{\theta i} - K'\ (c_4^0 - \delta\, c_2^0)\ M_4\, \Phi_4^2\, P_4\ (\theta) \tag{6}$$

$R_{\theta i}$ being the reduced intensity for scattered light making an angle θ with the incident beam, and $P_i(\theta)$ being the particle-scattering factor[17]. Equation 6 is valid only so long as the second virial coefficient, B, for component 4 is zero. The light-scattering equation for component 6 is therefore given by

$$\frac{K'\, \Phi_6^2\, c_6}{R_{\theta 6}} = \frac{K_6\, c_6}{R_{\theta 6}} = \frac{1}{M_6\, P_6\ (\theta)} + 2\, B_6\, c_6\, f_6\ (\theta) \tag{7}$$

$f_6(\theta)$ being a function of the angle θ and, unlike $P(\theta)$, depending not only on the size and shape of the solute component 6 but also on solute-solvent interactions*. Φ_6 is the refractive index increment of the component 6 and is given by

$$\Phi_6 = \frac{\Phi_2 + \delta\, \Phi_4}{1 + \delta}$$

Extrapolation of the light scattering to zero angle and zero concentration then gives

$$\lim_{\substack{c \rightarrow 0 \\ \theta \rightarrow 0}} \frac{K_6\, c_6}{R_{\theta 6}} = \frac{1}{M_6} \tag{8}$$

The determination of $1/P_6(\theta)$ as a function of angle, and particularly the "limiting slope" of $P^{-1}{}_6(\theta)$, that is $\lim_{\theta \rightarrow 0} \dfrac{\mathrm{d}\, P^{-1}{}_6\ (\theta)}{\mathrm{d} \sin^2 \theta/_2}$ allows one to draw certain conclusions about the shape and the size of the nucleoprotein complex in the usual manner[18,19].

We are thus able to determine, through the quantity δ, the composition of the nucleoprotein complex and subsequently, by making the proper corrections of the total light scattering, the shape and size of the complex. However, it is proper to point out that we have simplified somewhat in regarding our system—water, buffer, SDN, and BSA—as a three-component system. To justify our neglect of interactions of the protein and SDN with the buffer salts, we cite the following information:

1. The molecular weight of SDN as determined by light scattering does not change in NaCl solutions up to 0.2 molar[20].

* For dissymetrical scattering of light, the concentration derivative of (Kc/R_θ) is in general no longer equal to $2B$ but depends also on the angle θ, approaching $2B$ as θ approaches zero. It is therefore possible to write:

$$\frac{\mathrm{d}\ (Kc/R_\theta)}{\mathrm{d}c} = 2B\ \mathrm{f}(\theta);\ \mathrm{f}(\theta) = 1$$

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2. Bovine serum albumin does not bind enough NaCl or NaSCN to affect the determination of its molecular weight in moderately concentrated salt solutions by light scattering[21]. It therefore seems reasonable to assume that *changes* in salt binding, sufficiently great to affect the accuracy of our determination of the binding coefficient of SDN for BSA will not occur.

EXPERIMENTAL METHOD AND APPARATUS

Apparatus. A Brice-Speiser light scattering photometer[22] manufactured by the Phoenix Instrument Co. of Philadelphia was used in a slightly modified form that permitted temperature regulation to $\pm$ 1.0° C and the use of a system of narrow slits (12 × 3 mm). The calibration of the instrument giving the relation between photomultiplier output and absolute intensity, R_θ, has been given previously[22,23]. For a determination of the reduced intensity as a function of angle, a hand-blown Erlenmeyer flask mounted on a suitable base was used[24]. In all our experiments we used the unpolarised radiation of the blue 436 mμ line of a mercury arc.

Viscosity measurements were made in an Ostwald-Fenske calibrated viscometer[24] in a constant temperature bath at 25.00 $\pm$ 0.02° C. Interpolated intrinsic viscosities for an average gradient, $\bar{\beta}$, of 1000 sec^{-1} are given in Table I.

Concentration measurements and refractive increment. The concentration of nucleic acid and protein was measured spectrophotometrically, using the values of the extinction coefficient of 175 for the nucleic acid at 259 mμ[25] and 6.6 at 280 mμ for the bovine serum albumin[26]. The refractive increment was taken to be 0.160 for nucleic acid[27] and that of bovine serum albumin to be 0.1935 (average of refs. 28 and 29).

Materials. The nucleic acid was prepared in this laboratory in cooperation with with Dr BARBARA H. BUNCE, according to the method of SCHWANDER AND SIGNER[30*]. Some details of the preparation are mentioned in a previous paper from this laboratory[24]. Its molecular weight in 0.2 *M* NaCl had been determined by light scattering by Dr BUNCE. The protein was Armour crystalline bovine serum albumin.

Preparation of solutions; light-scattering method. In general, all solutions were prepared from recrystallised salts, glass redistilled water and nucleic acid or protein, or both. They were freed from dust and other foreign matter by centrifuging 8 to 24 hours in a Sorvall SS-1 angle centrifuge at top speed (maximum acceleration 20,000 g) in the cold. For the light-scattering experiments on nucleic acid-protein mixtures, SDN and BSA solutions were made up separately and sufficient protein solution added to the nucleic acid to provide a given ratio of protein to nucleic acid and a nucleic acid concentration of $4 \cdot 10^{-4}$ to $5 \cdot 10^{-4}$ g/ml. The mixtures were then clarified by centrifugation. The light-scattering measurements were made by adding successive weighed portions of the clean solution to a known quantity of buffer in the Erlenmeyer cell. The final nucleic acid concentration was determined spectrophotometrically.

The following buffer solutions were used:

pH	ionic strength	moles NaH_2PO_4	moles Na_2HPO_4
7.47	0.2	0.0207	0.0623
6.46	0.2	0.0805	0.0415
5.51	0.1	0.0873	0.0036

* It is referred to elsewhere as "sample B"[24] and "Bunce-Geiduschek"[33].

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RESULTS AND DISCUSSION

The reaction between sodium thymonucleate and bovine serum albumin was investigated under the following conditions:

Expt.	pH	ionic strength	buffer	weight ratio SDN/protein	reaction
1	7.47	0.2	phosphate	1.00	none
2	6.46	0.2	phosphate	1.00	none
3, 4	5.51	0.1	phosphate	1.00	$= 0.35 \pm 0.06$

We shall discuss the reaction at pH 5.51 and ionic strength 0.1 first. In Fig. 1, $K'c_t/R_{ot}$ from equation 4 is plotted for two separate experiments, the results of which are in excellent agreement. R_{ot} is the reduced intensity at zero degrees, and has been calculated by extrapolation of the experimentally determined light scattering at many angles between 35 and 135° in the usual manner of the Zimm-type plot[18]. The light scattering from pure sodium thymonucleate is also shown in Fig. 1. It is immediately apparent that—in accordance with our expectations for an equilibrium reaction—as m_2 and m_4 approach zero, δ also approaches zero. However at total concentrations above $16 \cdot 10^{-4}$ g/ml the binding is independent of concentration. The extrapolation from this part

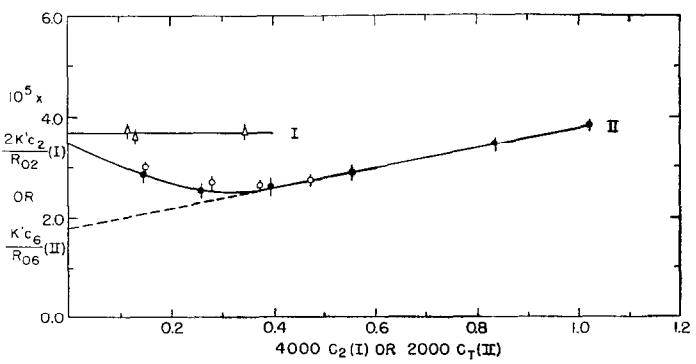

Fig. 1. Reciprocal reduced intensity plot of sodium thymonucleate (curve I) and a sodium thymonucleate-bovine serum albumin mixture (1:1 by weight) (curve II) at pH 5.51 in 0.1 ionic strength phosphate buffer. The reciprocal of reduced intensity *at zero degrees* is plotted. Concentrations are in units of g/ml.

of the curve to zero concentration therefore permits the evaluation of the absorption coefficient, δ, for those solutions whose concentration is greater than $16 \cdot 10^{-4}$ g/ml. Application of equation 4 yields $\delta = 0.35 \pm 0.06$. If B, the second virial coefficient, were zero, or B_2 and B_6 were equal, we should be able to calculate the value of δ corresponding to every concentration[31]. However, in our system, B is not zero and evidently varies continuously with δ, so that we are unable to do more than make approximate estimates of the absorption coefficient in that low concentration range in which it is changing.

In accordance with equation 6, we are now able to calculate $R_{\theta 6}$, the reduced intensity due to the nucleoprotein complex alone. Its variation with angle and concen-

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TABLE I

COMPARISON OF THE PROPERTIES OF SODIUM DESOXYRIBOSE NUCLEATE AND ITS COMPLEX WITH BOVINE SERUM ALBUMIN, IN A 1:1 MIXTURE IN 0.2 IONIC STRENGTH, pH 5.51 PHOSPHATE BUFFER AT 25° C

	SDN	*SDN – BSA complex*
Molecular weight	$2.11 \cdot 10^6$	$3.0 \cdot 10^6$
limiting dissymetry [z]	3.10	3.16
"limiting slope" of $P^{-1}(\theta)$	8.78	16.0
$(\overline{R^2})^{1/2}$ of the equivalent random coil in A.*	3260	4430
radius of gyration, R_G in A	1330	1810
intrinsic viscosity $[\eta]_{\overline{B}} = 1000$ sec.$^{-1}$	11.3	11.0
δ, absorption coefficient	—	0.35 ± 0.06

* The root-mean-square separation of the ends of a random coil having the radius of gyration determined experimentally.

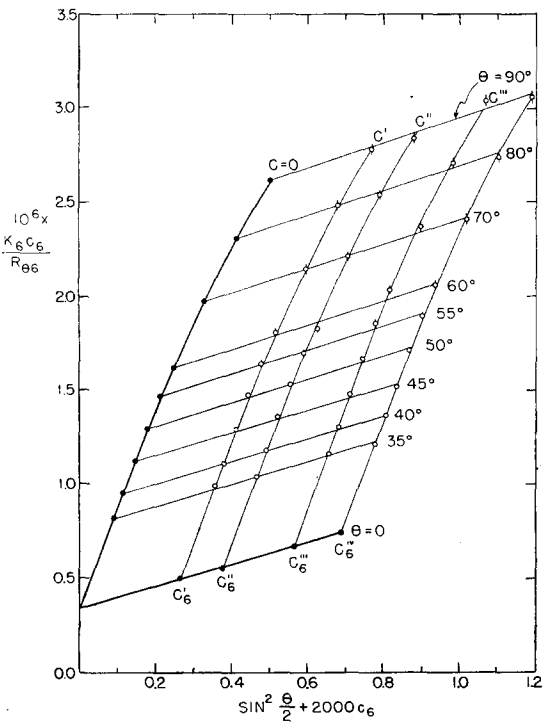

Fig. 2.* Zimm-type plot of the reciprocal reduced intensity as a function of concentration and angle for the sodium thymonucleate-bovine serum albumin complex formed in a 1:1 (weight) mixture at pH 5.51 and 0.1 ionic strength. The concentrations are

$c_6^{I} = 1.33 \cdot 10^{-4}$; $c_6^{II} = 1.88 \cdot 10^{-4}$;
$c_6^{II} = 2.83 \cdot 10^{-4}$; $c_6^{IV} = 3.45 \cdot 10^{-4}$ g/ml

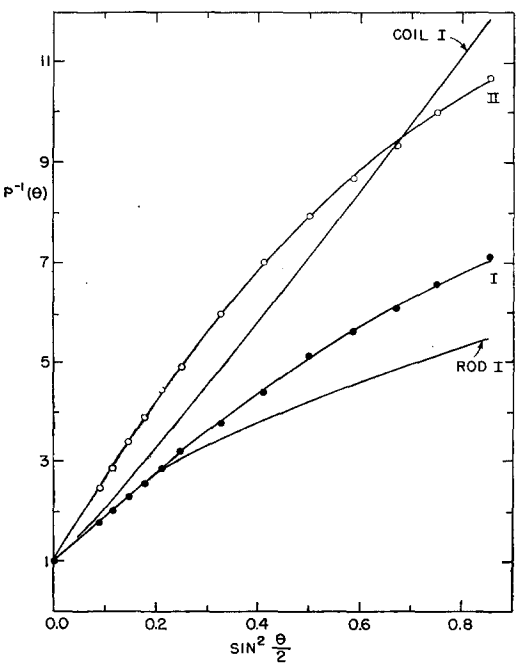

Fig. 3.* The reciprocal particle-scattering factor, $P^{-1}(\theta)$ as a function of angle for sodium thymonucleate (curve I) and its bovine serum albumin complex (curve II) at pH 5.51 and ionic strength 0.1. For comparison the reciprocal particle scattering factors for monodisperse rods of $R_G = 1330$ A (Rod I) and a monodisperse coil of $R_G = 1330$ A (Coil I) are included.

* *Note added in proof:* Recent experiments have shown that in Figs. 2 and 3, $P^{-1}(\theta)$ and $K_6 c_6 / R_6$ should probably be greater at high angles than we have shown them here. This is due to our having neglected to correct for back reflection of the transmitted beam from the exit face of the light-scattering cell, which results in a slight relative augmentation of scattering at high angles if the solution scatters dissymmetrically.

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tration is shown in the Zimm-type plot of Fig. 2. The information derivable from it is summarized in Table I. The reciprocal of the particle-scattering factor, $P^{-1}(\theta)$, is plotted in Fig. 3 together with the same quantity for pure sodium thymonucleate under the same conditions.

The radius of gyration is calculated from

$$R_G = \frac{\sqrt{3}\,\lambda'}{4\pi}\left(\frac{\mathrm{d}\,P^{-1}(\theta)}{\mathrm{d}\sin^2\frac{\theta}{2}}\right)^{1/2}_{\theta=0}$$

where $\lambda' = \lambda/n_0$ is the wavelength of light in the solution. Actually, the radius of gyration is the only quantity which can be unambiguously derived from the "limiting slope" of $P^{-1}(\theta)$ for a molecule whose extension in space does not correspond to that of a rod, random coil, or other simple models[24]. To show the extent to which sodium thymonucleate fails to conform to such models, we have drawn in Fig. 3 the reciprocal particle-scattering factor, $P^{-1}(\theta)$, for systems of monodisperse rods and random coils having the same "limiting slope" of $P^{-1}(\theta)$, that is, the same radius of gyration*. The possible interpretations of the particle scattering factor, $P(\theta)$, in terms of very polydisperse coils, crosslinked networks[24,32] and non-Gaussian chains[33] is not the main issue here. What is important to note in Fig. 3 is, that, except for a difference in the initial slope corresponding to a change in the radius of gyration, the shapes of the curves of $P^{-1}(\theta)$ for the nucleic acid and its protein complex as a function of $\sin^2\theta/2$ are almost identical. This can only mean that the shape and size of the nucleoprotein complex are almost the same as those of the original nucleic acid. The ratios of $P^{-1}(\theta)$ for the nucleoprotein complex and nucleic acid as determined between 35° and 135° are given in Table II. To emphasize the point that there is no change of form, we have compared

TABLE II

COMPARISON OF THE RECIPROCAL PARTICLE SCATTERING FACTORS OF SODIUM DESOXYRIBOSENUCLEATE $P_2^{-1}(\theta)$ AND ITS BOVINE SERUM ALBUMIN COMPLEX $P_6^{-1}(\theta)$ AT pH 5.51, IONIC STRENGTH 0.1

	$\frac{P_6^{-1}(\theta)}{P_2^{-1}(\theta)}$	$\frac{P^{-1}(\theta)_{coil}^{R_G = 1810\,A}}{P^{-1}(\theta)_{rod}^{R_G = 1880\,A}}$
35	1.39	1.49
40	1.43	—
45	1.46	1.72
50	1.51	—
55	1.53	—
60	1.53	2.15
70	1.56	2.49
80	1.57	—
90	1.56	3.01
100	1.55	—
110	1.55	3.46
120	1.53	—
135	1.51	3.88

* It must be remembered, however, that our nucleic acid is certainly polydisperse. The theoretical curves for the rod and coil are intended merely to provide some frame of reference for the experimental information.

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these values with the following hypothetical, extreme case, namely the change from a monodisperse rod with a radius of gyration of 1330 A to a monodisperse random coil with a radius of gyration of 1810 A (the values determined experimentally for the nucleic acid and its protein complex).

Such a change from an extended to a contracted form is frequently mentioned in discussions of nucleoprotein dissociation and nucleoprotein combination. It is evident that it has not occurred in the reaction between sodium thymonucleate and bovine serum albumin. The results of viscosity measurements given in Table I also point to the fact that no major changes in shape or size have taken place. They are in complete disagreement with the results of GREENSTEIN AND JENRETTE[11], who found that on addition of bovine serum albumin to SDN, both the relative viscosity and its gradient dependence decreased sharply. However, these investigators used very concentrated solutions at low ionic strength, under which conditions viscosities are even more than usually difficult to interpret. We hope to devote some effort in the near future to establishing what the situation is in native and reconstituted nucleohistones.

It is necessary to mention one reservation about our method of calculating the reduced intensity, $R_{\theta 6}$, of the nucleoprotein complex on which the above discussion is based. The calculation depends on the assumption, which has been written into equation 5, that all of the nucleic acid reacts with part of the protein. For justification of this, we depend on the observations of STENHAGEN AND TEORELL[9] and GOLDWASSER AND PUTNAM[10], that in the electrophoresis of reacting nucleic acid-bovine serum albumin mixtures, *all* of the nucleic acid moves with a changed mobility while part of the protein moves with its own characteristic mobility, and our own observation from light-scattering experiments, that the reaction is slow.

At pH 6.5 and 7.5, there is no reaction between the nucleic acid and bovine serum albumin. This is shown by the fact that a plot of $K' c_t/R_{ot}$ against concentration is linear, and lacks the concave upward portion shown in curve I of Fig. 1, and which is characteristic of a dissociating system. This is in agreement with the results of GOLDWASSER AND PUTNAM[10] who also found that at pH 5.5 and ionic strength 0.2 there was no reaction, but that as the ionic strength is lowered, interaction sets in and becomes more and more intense. On the other hand, our results, as well as those of GOLDWASSER AND PUTNAM are in disagreement with those of STENHAGEN AND TEORELL[9] who observed a change in the electrophoretic mobilities of sodium thymonucleate at pH's up to 7.9 upon the addition of bovine serum albumin. However, these authors themselves noted that their protein contained a pigmented impurity, which migrated with the nucleic acid in these nucleic acid-protein mixtures. It is quite possible, therefore, that the changed mobilities were due to the binding of the impurity rather than the bovine serum albumin itself.

As the pH is lowered, the extent of reaction increases, and about 0.1 to 0.2 pH units below the isoelectric point of the protein, precipitation occurs. We plan to extend our light-scattering studies into this region, and also to investigate the effect of changing ratios of nucleic acid to protein on the extent of reaction.

Part of the motivation in this study arose from the observation of GREENSTEIN, CARTER AND CHALKLEY[34] and GREENSTEIN AND HOYER[35] on the protective effect of SDN on albumins. These investigators found that the addition of as little as 1 part of SDN to 250 parts of bovin serum albumin in salt-free solutions was sufficient to prevent its coagulation upon being heated to 100° C for several hours. Now it would appear that

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this protection could only be conferred on the albumin if it were continuously in molecular contact with the nucleic acid, much as histones are combined in nucleoproteins. But such a state of attachment, corresponding to about one serum albumin molecule per nucleotide, represents extremely tight binding whereas our results show the interaction to be relatively weak. Even though there are differences between the conditions of the two experiments, the enormous difference between the intensity of the interaction in the two cases presents a dilemma. A possible solution may be obtained by noting the recent observations of AMBROSE AND BUTLER[36] which confirm the earlier prediction of ASTBURY[37] that the protein component of both natural and reconstituted nucleoproteins is in the β- (extended) form. This suggests that the strong binding of albumin and SDN only becomes possible when the former is denatured, at least in part. Thus in unheated solutions the interaction is the weak one that we have characterized here, and upon heating the protein attaches itself to the SDN molecule as soon as one or two regions of its α-folded polypeptide chain are converted into the β-form by the action of heat. Indeed, this concept of the protein molecule becoming firmly attached at two or three points to the SDN chain and perhaps to neighboring protein molecules may be the basis of the thermal stabilization, inasmuch as strong attachment at a few points may prevent the remainder of the molecule from undergoing the α–β transformation.

SUMMARY

The interaction of sodium desoxyribosenucleate and bovine serum albumin in phosphate buffers has been studied under various conditions of pH, by the technique of light scattering. Interpretation of the experimental data in terms of the theories of light scattering from large molecules and multicomponent systems has permitted the determination of the composition of soluble nucleoprotein complexes as well as their size and shape. There is no reaction at pH 7.47 and 6.46. At pH 5.51 and ionic strength 0.1, the nucleic acid binds 35% of its own weight of bovine serum albumin in a 1:1 mixture, that is, each molecule of nucleic acid on the average binds 11 ± 2 molecules of the protein. The nucleic acid undergoes no appreciable changes of shape upon reaction.

RÉSUMÉ

Nous avons étudié l'action mutuelle du désoxyribosenucléate de sodium et de l'albumine de sérum bovin dans des tampons phosphate sous diverses conditions de pH. La technique qui fait appel à la dispersion de la lumière a été employée. En nous basant sur la théorie de la dispersion de la lumière par les grosses molécules et les systèmes à plusieurs constituants nous avons interprété les données expérimentales, ce qui nous a permis de déterminer la composition de complexes solubles de nucléoprotéine ainsi que leur taille et leur forme. Il n'y a pas de réaction entre pH 7.47 et 6.46. A un pH de 5.51 et une force ionique de 0.1, l'acide nucléique, dans un mélange 1:1, se combine à 35% de son propre poids d'albumine de sérum bovin, c.à d. que chaque molécule d'acide nucléique se combine, en moyenne, à 11 ± 2 molécules de la protéine. L'acide nucléique ne subit pas de changements de forme considérables lors de la réaction.

ZUSAMMENFASSUNG

Es wurde die Wechselwirkung zwischen Natriumdesoxyribosenucleat und Rinderserumalbumin bei verschiedenen pH-Werten durch die Methode der Lichtstreuung untersucht. Die Auslegung der experimentellen Tatsachen in Begriffen der Theorie der Lichtstreuung an grossen Molekülen und vielfach-zusammengesetzen Systemen erlaubte die Bestimmung der Zusammensetzung löslicher Nucleoproteinkomplexe ebenso wie die ihrer Grösse und Form. Es findet keine Reaktion bei pH 7.47 und pH 6.46 statt. Bei pH 5.51 und Ionenstärke 0.1 bindet die Nucleinsäure 35% ihres eigenen Gewichtes an Rinderserumalbumin in einer Mischung 1:1, d.h. jedes Nucleinsäuremolekül bindet im Durchschnitt 11 ± 2 Moleküle des Proteins. Die Nucleinsäure erfährt keine wahrnehmbare Formänderung während der Reaktion.

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