OVALBUMIN:

A PHYSICO-CHEMICAL STUDY

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SUMMARY

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1. General Introduction

The study of the chemistry of proteins has been very greatly aided by the discovery of methods for isolating a given protein from the array of materials with which it is normally extracted from some biological source. With the significant advances in this field there arose a further problem, which is still receiving much attention, namely, the question of protein purity. At first it was believed that crystallisability was a satisfactory criterion, the existence of a protein in crystalline form being regarded as proof of homogeneity. The physical biochemists, by applying the more searching criteria of physico-chemical measurements to these crystalline proteins, however, were able to detect heterogeneity in nearly all systems studied: techniques of particular importance included electrophoresis, sedimentation, and solubility measurements. By these methods crystallisability was quickly eliminated as a criterion for purity. The next development in the study of protein heterogeneity was the discovery that the satisfaction of one criterion was insufficient proof; a protein which appears homogeneous by one method may be proved to be heterogeneous by another — indeed, a substance may be shown to be both homogeneous and heterogeneous using the same method simply by variation of the conditions used, e.g., alteration of the pH of the solution under investigation.

Unfortunately for the experimentalist, complete homogeneity of a protein is a property which cannot be demonstrated directly,
whereas deviations may be. It is therefore necessary to define a set of criteria as a standard for homogeneity of protein samples. Apart from one or two cases, where only one method of analysis was used, a protein system has yet to be proven pure, even using this operational definition of homogeneity. Thus, while great advances have been made in the study of protein homogeneity, the methods available for such study are continually being improved, and a great deal of present day research is concerned with the quest for, firstly, a set of criteria which will unequivocally prove a protein's homogeneity, and secondly, a protein which will satisfy such requirements.

2. Outline of the Field of Study

Electrophoresis of ovalbumin has revealed the presence of at least three components in samples of the protein which have been repeatedly crystallised. These three components, designated $A_1$, $A_2$ and $A_3$ in order of descending negative mobility, appear to be present in all samples of ovalbumin to the same extent, the apparent proportions, as judged from analysis of the electrophoresis patterns, being 80%, 15% and 5% respectively. Of the physico-chemical methods available for studying macromolecular systems, only electrophoresis has so far detected this heterogeneity: sedimentation studies, for example, have failed to reveal any deviation from a homogeneous system.

In a most elegant study combining enzymatic dephosphorylation experiments with electrophoretic mobility measurements, Perlmann has detected a difference in the phosphate content of the three components. After treatment of crystalline ovalbumin with the
dephosphorylating enzyme prostate phosphatase, electrophoresis
reveals the complete absence of the \( A_1 \) component and a corresponding
increase in the amount of protein with the mobility of \( A_2 \). By use
of another dephosphorylating enzyme, intestinal phosphatase, Perlmann
has also been able to convert the \( A_2 \) into a protein with the mobility
of \( A_3 \). Phosphorus analyses, done in conjunction with these
experiments, indicate that \( A_1 \), \( A_2 \) and \( A_3 \) contain two, one and zero
phosphate groups respectively. Thus she concludes that while minor
chemical differences cannot be excluded, it would appear that with
the exception of phosphate content these molecules may be identical.
If this were the case, then the individual sedimentation coefficients
would be indistinguishably close and the mixture should sediment as
one species, in agreement with observation.\(^{10,11}\) This argument
should also apply to the diffusion of the ovalbumin mixture.

Optical interference methods of particular value for studying
diffusion have been developed by Coulson, Cox, Ogston and
Philpot\(^{14}\) in England, and Kegeles and Gosting\(^{15}\) and Longsworth\(^{16}\) in
U.S.A.; as a result of the application of these methods a much
greater precision in the determination of diffusion coefficients
has been attained and thus a more critical test of the identity of
diffusion coefficients now appears possible. The procedures
developed for the determination of heterogeneity from diffusion
measurements on mixed solute systems\(^{17-19}\) have been shown to be
valid for mixtures of simple substances such as sucrose and urea,
and have been applied to a few protein systems.\(^{17,19,20}\) A study
of the diffusion behaviour of crystalline ovalbumin and also the
leading electrophoretic component, \( A_4 \), is discussed in this work. The author believes that of all the protein systems ever tested for heterogeneity, the \( A_4 \) studied in this work may well be among the purest. During the course of the discussion of these diffusion measurements it will become apparent that the interpretation of such results is complicated by two factors:

(i) the possibility of change in the configuration of the protein molecule during diffusion;

(ii) the interaction of flows between the protein and the constituent salts of the buffer solution.

Indeed, before full interpretation of diffusion results obtained with proteins can be realised, both of these effects should be investigated. In the author's opinion, the logical approach is to study the former postulate first.

One of the greatest problems associated with physical measurements on protein systems is the tendency for many proteins to denature, i.e., to change their physical structure (and hence solution properties) but not their chemical composition. This tendency for denaturation may often be correlated with the existence of reactive polar groups of the constituent amino acids of the protein. Of these, the sulphydryl groups of the cysteine residues are the most reactive: two such groups will tend to react together to form a disulphide bridge, either intramolecularly or intermolecularly. Other polar groups may also react, the above example serving only as an illustration of the type of structural rearrangement that may occur. The ease with which proteins
denature varies greatly, and in this respect, ovalbumin is one of the most unstable. With such a labile protein as ovalbumin, there is always the possibility that the material under investigation at the conclusion of a physical measurement differs from that at the commencement of the experiment. This possibility applies particularly to the case of diffusion, where a single experiment extends over several days.

A substance more suitable for physico-chemical measurements may well be obtained if these reactive groups are modified by reaction with some reagent prior to the actual physical measurement. Of course, the method of modification should be such that the modified material resembles as closely as possible the native protein. Since, as has already been mentioned, the sulphhydryl group is the most reactive, attention has been focussed mainly on this group. The modification of this group has been the subject of a large number of investigations, the results of which have been summarised in several reviews. Of the methods available for modification, the one chosen for this study is the oxidation of the sulphhydryl groups with iodine, since this reagent is one of the few reported to react specifically and stoichiometrically with all of the sulphhydryl groups of ovalbumin in the native state. The reaction involves the oxidation of sulphhydryl to disulphide according to the following equation:

\[ 2RSH + I_2 \rightarrow RSSR + 2H^+ + 2I^- \]

There are five cysteine residues per ovalbumin molecule and, indeed, five atoms of iodine per molecule are involved in the
reaction,\textsuperscript{27} this being the number required for five sulphydryl groups to be oxidised according to the above equation. However, because the sedimentation rates of the native and modified materials are essentially identical,\textsuperscript{28} Anson concludes that the reaction is entirely intramolecular, since the formation of intermolecular disulphide bridges would lead to the formation of a species with a higher sedimentation rate.

Because the oxidation requires pairs of sulphydryl groups and an odd number appear to exist in the native protein molecule, then for the above reaction to be the sole reaction occurring, at least one intermolecular disulphide bridge must be formed. In view of this anomaly, a comparative physico-chemical study of the native ovalbumin and the iodine-modified material has been undertaken in an endeavour to ascertain the exact nature of the modification.

It was felt moreover, that a comparative study of this kind would provide results likely to be of some significance in the wider field of protein structure; much attention is at present being directed to the "helix-coil" transition (i.e., the change from a peptide chain supposedly conforming in part to the Pauling and Corey \"α-helix\"\textsuperscript{29} to the random coil), and in the further development and critical testing of the theory, the availability of modified products of well defined properties will facilitate progress.

As previously mentioned, the early part of this study is concerned primarily with the diffusion of ovalbumin using the Rayleigh optical system. Accordingly, Chapter II contains a summary
of the theoretical aspects of diffusion measurement by this method; the results of the application of this theory to ovalbumin and the leading electrophoretic component, \( A_1 \), are then discussed in Chapter III.