THE SUPPOSED ROLE OF MICROBIOLOGICAL AEROSOL STABILIZERS AS SUBSTITUTES FOR BOUND WATER: A STUDY OF AN IN VITRO MODEL SYSTEM

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ABSTRACT In order to test a suggestion that inositol may take the place of water in maintaining the stability of desiccated cells, the reversible endothermic association of tobacco mosaic virus protein (TMVP) was studied turbidimetrically in presence of this substance. Its effect was to lower the temperature at which association takes place, the positive standard enthalpy and standard entropy of reaction both being increased by about 30%. The hypothesis of direct substitution of bound water by inositol at the site of macromolecular association leads to the contrary prediction that the association temperature would be raised. It is suggested that the observed effect of inositol may result from a conformation change in TMVP brought about by binding of inositol at positions adjacent to the site of reaction.

INTRODUCTION

The stability of airborne microorganisms is known to be dependent upon the type and the physiological condition of the organism, upon the chemical nature of the immediate environment, and upon the ambient physical conditions which determine the rates of the various processes by which the desiccated condition is reached (Rosebury, 1947). From the available mass of incomplete, and often conflicting, experimental data no generalization has yet emerged and perhaps none can reasonably be anticipated. The chemical additives that protect a microorganism from a dry atmosphere (Webb, 1960; Zimmerman, 1963) themselves constitute a group of substances without any other obvious common property, but within this group one does find substances such as the sugars and the polyalcohols which possess the common structural feature of a number of hydroxyl groups attached to a carbon skeleton and showing in their macroscopic behavior pronounced hydrophilic characteristics. Among the substances in this subgroup, inositol has been said to be the most effective in preserving airborne cells and viruses (Webb, 1965) and its protective effect

has been supposed to reside in the waterlike characteristics of its exposed hydroxyl groups. This hypothesis implies the binding of inositol by the cell, but demonstration of such binding by physical methods such as vapor pressure osmometry or microcalorimetry would not in itself substantiate the hypothesis. We have thought that more persuasive evidence might be obtained by studying the effect of inositol upon some macromolecular chemical reaction known to involve "bound" water. Replacement of parts of this bound water by inositol might be expected to influence both the kinetics and the equilibrium of the reaction.

The reversible endothermic association of tobacco mosaic virus protein (TMVP) is thought to be such a reaction. Evidence of various kinds gathered by Lauffer and coworkers (Lauffer, 1962) suggests strongly that the joining of each pair of protein monomer units requires the displacement of some 132 water molecules, if the process is considered to be thermodynamically equivalent to the melting of a comparable quantity of ice. If some, or all, of the bound water molecules at the locus of macromolecular association were to be replaced by inositol, one might expect the reaction to proceed less easily so that the equilibrium at any given temperature would be displaced in favor of the dissociated or monomeric form of the protein.

RESULTS

In preliminary experiments this prediction has been tested with TMVP, following in detail the turbidimetric method of Smith (1961) and Smith and Lauffer (1967), and using for calculation of the thermodynamic parameters of the reaction Flory's theory (1936) of condensation polymerization precisely in the form uesd by these same authors.

In our hands, the reaction in 0.06 M phosphate buffer at pH 6.5 appeared to be strictly reversible, and it proceeded in excellent quantitative agreement with the data of Smith and Lauffer (1967, Table I). In presence of inositol, the rise of optical density which signals the occurrence of the polymerization reaction took place at lower temperatures (Fig. 1); when the inositol concentration was 0.2 M, the displacement of the curve amounted to nearly 5°C. The corresponding changes in the standard enthalpy, free energy and entropy of reaction are shown in Table I.

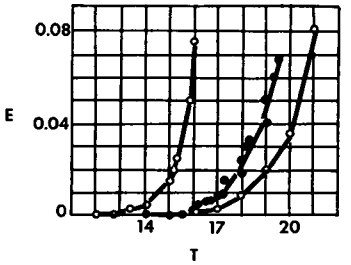

FIGURE 1 Effect of inositol on the association-dissociation of tobacco mosaic virus protein. Open circles, right, control. Closed circles, 0.10 and 0.15 M inositol. Open circles, left, 0.20 M inositol.

TABLE I

EFFECTS OF INOSITOL AND SUCROSE ON THE THERMODYNAMIC PARAMETERS OF TMVP EQUILIBRIUM AT 19°C*

Additive	Concentration of additive	$T^{\ddagger}_{0.05}$	ΔH^0	ΔF^0	ΔS^0
	M	°C	cal mole^{-1}	cal mole^{-1}	cal mole^{-1} deg^{-1}
Inositol	0.00	20.3	196,000	−4,200	+600
	0.10	18.9	260,000	−6,000	+870
	0.15	18.9	273,000	−6,000	+920
	0.20	15.8	300,000	−10,200	+960
Sucrose§	0		225,000		+860
	0.20		262,000		+936

* Reaction in 0.06 M phosphate buffer, pH 6.1. Protein concentration 0.88×10^{-3} g ml^{-1}.

‡ Temperature centigrade for an optical density increment of 0.05.

§ Data of Shalaby and Lauffer (1967).

DISCUSSION

The action of inositol is qualitatively similar to that of sucrose recently recorded by Shalaby and Lauffer (1967), but is quantitatively much more pronounced. One may reasonably connect these effects with the hydrophobic-hydrophilic character common to both molecules, but the important feature to be noted in the present context is that, far from impeding the linkage of TMVP monomers, these substances seem to facilitate it.

Shalaby and Lauffer consider that sucrose in solution can modify the behavior of TMVP by perturbing its interactions with water. It is not obvious to us that such effects of a solute could be transmitted through relatively thick layers of water at a thermodynamic activity close to unity without the appearance of anomalies in the colligative properties of the solution. We prefer to attribute the effect of sucrose to the binding of this substance to protein in the vicinity of the association centers[1].

[1] Dr. Max Lauffer reminds us of the experiments of Stevens and Lauffer (1965). It was shown that when a TMVP solution in a dialysis bag is equilibrated against a mixture of water and glycerol, water is preferentially retained in the bag in comparison to glycerol. On the average, therefore, TMVP "prefers" water to glycerol. The same is probably true for sucrose (Schachman and Lauffer, 1949) and there is no reason to suppose that inositol would behave differently. These results, however, represent the net behavior of the protein, and do not preclude the possibility of local attachment of solute at, or near, the association sites. Experimental evidence on this point appears to us to be lacking.

A reviewer remarks that "a 0.2 M solution of inositol surely changes the thermodynamic potential of the aqueous solvent and this alone might have a profound effect on a macromolecule with a very large surface area." We do not wish to question the possibility that there are solvent activity effects, but one can calculate from the published osmotic coefficients (Scatchard, Hamer, and Wood, 1938) that 0.2 M solutions of glycerol and sucrose have a water activity of about 0.996. Since solutions of different substances at this water activity may have different effects upon TMVP polymerization (Shalaby and Lauffer, 1967) we think it likely that the solute is usually dominant. It is not possible at present to select an "indifferent" solute, defined as one which will lower the solvent activity without interacting with the macromolecular component.

Conformational change in a protein molecule consequent upon binding of sucrose has been demonstrated recently in the case of β-lactoglobulin by Clement-Metral and Yon (1968). In our experiments the pronounced effect of inositol may well have been produced by a similar mechanism. Whatever the precise mechanism, however, the observed displacement of equilibrium brought about by inositol is qualitatively opposed to that anticipated from the hypothesis that bound inositol simulates bound water. While there is no direct evidence that the water binding sites in TMVP are similar to those involved in the inactivation of airborne microorganisms, the present results do suggest that the supposed hydromimetic effect of inositol in stabilizing dehydrated microorganisms requires critical review. This conclusion is subject of course to reservations concerning the validity of the turbidimetric approach, provoked partly by the difficulty in accounting for, and correcting for, the unexpectedly high turbidity of the dissociated TMVP solutions, a result noted by Smith (1961) and confirmed in our experiments. It is also unfortunate that in order to comply with limitations imposed by the Flory theory, the experimentally most accurate data have to be discarded. We hope, therefore, to be able to test our present conclusion concerning the inapplicability of Webb's hypothesis to the TMVP system by other experimental methods.

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