THE EFFECT OF FORMALDEHYDE ON THE ACIDIC AND BASIC PROPERTIES OF WOOL

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Previous papers have reported the effects of salt concentration (2) and temperature (10) on the extent of combination of wool with hydrochloric acid and sodium hydroxide. As with other proteins, the tendency of wool to combine with acids and bases is also greatly influenced by the presence of formaldehyde (3). An investigation of this influence is reported in the present paper.† Owing to the special methods which are available for studying the combination of wool with base (10), investigation of the equilibria involving wool, alkali, and formaldehyde may extend into the strongly alkaline range without complications caused by the gradual destructive effect of alkali on sulfur-containing proteins.

The pH values of alkaline solutions of proteins or amino acids are considerably decreased by the addition of formaldehyde. Over a wide range of pH, the effect is such as to make it appear that the presence of formaldehyde increases the acid dissociation constants of certain positively charged groups in the protein (principally RNH₃⁺ groups from lysine side chains in proteins, or the α-amino groups of amino acids). The dissociations of other groups (with the possible exception of guanidinium groups) appear to be very little affected. This selective action, which has been investigated in great detail (1 2, 15, 16, 21, 22), has been shown to depend on the combination of un-ionized amino groups in amino acids with the formaldehyde. Applications of this reaction to the estimation of amino acids, the measurement of protein hydrolysis, and the estimation of the primary amino groups of various proteins (5, 11–14, 18) are well established.

Procedure

Measurements were made of the amounts of hydrochloric acid and potassium hydroxide combined by wool in the presence of two concentrations of

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† This paper is not concerned with the partially irreversible combination of formaldehyde with proteins, which requires high concentrations of aldehyde and relatively long periods of time. This combination is important in the production of
formaldehyde (0.25 and 1.0 M), over the pH range 3.0 to 13.3. The measurements were made at 0°, primarily to minimize decomposition of the wool by alkali, but it is also convenient to work at low temperatures, because the dismutation of formaldehyde to methanol and formic acid at higher temperatures results in the rapid neutralization of base, and introduces large errors in the determinations of base bound by wool.

Details of the purification of the wool, determination of the acid or base combined, and correction of the results for the effects of decomposition of the wool have been described elsewhere (19, 20). Although formaldehyde is a weak acid (pK greater than 13), there was no need to estimate and correct the base bound by formaldehyde, because the base bound by wool was always determined by comparing the base present at equilibrium in aliquots of two solutions identical except for the fact that one contained wool and one did not.

Stock solutions of formaldehyde were freshly prepared by distilling paraformaldehyde into water; they were assayed by oxidation of the aldehyde with hydrogen peroxide to formic acid, which was titrated (8). The experimental solutions, containing formaldehyde and base, were made up at 0° and never exposed to higher temperatures. With alkaline solutions, 24 hours sufficed for the attainment of equilibrium; with acid solutions, 48 hours.

RESULTS AND DISCUSSION

Effect of Formaldehyde on Titration Curve of Wool

Titration curves of wool, obtained at a constant ionic strength (0.2 molal KCl) in the presence and absence of formaldehyde, are shown in Fig. 1. At the lowest pH values shown, the curves tend to come together, but, in more weakly acid solutions, formaldehyde slightly reduces the amounts of acid bound by wool. At higher pH values, the curves become widely separated. Those obtained with formaldehyde present are displaced from the control curve (no formaldehyde) in the direction of more acid pH values. The displacement reaches a maximum of about 1.9 units, when

protein plastics and artificial protein fibers (6, 9) and possibly plays a part in the protection of protein fibers from damage caused by alkali (4).

Over half of the base added to the 1.0 M formaldehyde solutions at 25° is neutralized by formic acid within 24 hours. At 0°, less than 1 per cent of the base is affected in the same time.

The ionic strength was kept constant within narrow limits by the addition of an appropriate amount of potassium chloride to each solution, calculated on the basis of preliminary experiments which permitted estimation of the equilibrium concentration of the base. In this calculation the ionization of formaldehyde was disregarded, since measurements with the glass electrode showed that it made almost negligible contributions to the ionic strength at 0° even at pH 13.
the amounts of base bound are between 0.15 and 0.20 mm per gm. of wool. The small displacement found in acid solutions depends noticeably on the formaldehyde concentration, whereas, in the alkaline range, almost a maximum effect appears to be produced by the lower of the two concentrations used. These effects on the titration curve differ from those previously reported for soluble proteins only in that they are somewhat smaller

![Figure 1](http://www.jbc.org/)

**Fig. 1.** The effect of formaldehyde on the dependence on pH of the combination of wool with hydrochloric acid and potassium hydroxide at a constant ionic strength (0.2). A separate curve has been drawn for the measurements obtained at the lower concentration of formaldehyde, only for the combination with acid. Measurements in the extremely unbuffered range near neutrality are not represented, and the curves are not defined in this region.

(consistent with the smaller lysine content of wool), and show a lower degree of dependence on the concentration of aldehyde.

Only certain of the dissociating groups in proteins are affected by formaldehyde. When the effect of the formaldehyde is very large, so that the alkaline branch of the titration curve obtained in its presence is displaced by 3 or more pH units from the control curve, a very simple analysis of the effect may be made (12). This simplicity results from the fact that practically the entire range of titration of a single set of dissociating groups (having a given dissociation constant) occurs within a range of 3 pH units. If only one such set of groups is affected by formal-
dehydrate, then, in the pH range of the displacement, the base bound by these groups in the presence of formaldehyde will be maximum throughout the entire range of the displacement, and the titration of these groups will make no contribution to the slope of the titration curve in this range of pH.

In such a simple case, and in the same range of pH, merely subtracting the ordinates of the control curve from the corresponding ordinates of the curve obtained with formaldehyde present will give the characteristic titration curve in formaldehyde of the set of dissociating groups which react with formaldehyde (12). The base bound by other groups not affected by formaldehyde will have been eliminated by the subtraction. Actually two curves will be obtained, an ascending S-shape curve just described, followed by a plateau, and then a descending S-shaped curve, which is the inverted titration curve of the same set of groups in the absence of formaldehyde. The presence of the plateau is the criterion which determines whether this simple case is actually realized. When no distinct plateau is found, the curve of the groups affected by formaldehyde has been displaced less than 3 to 4 pH units, and the maximum difference in ordinates ob-
tained is less than the total base-binding capacity of the set of groups with which we are now concerned.

An application of this reasoning to the data of Fig. 1 is shown in the upper section of Fig. 2. No distinct plateau is found. If the affected groups are identified with the ε-amino groups of lysine, the indicated lysine content of wool is greater than 2.2 per cent. The value determined by the amino nitrogen method of Rutherford, Harris, and Smith (17) is 3.3 per cent.

![Graph showing the effect of formaldehyde on the dependence on pH of the combination of wool with hydrochloric acid and potassium hydroxide, in the absence of added salt.](image)

**Fig. 3.** The effect of formaldehyde on the dependence on pH of the combination of wool with hydrochloric acid and potassium hydroxide, in the absence of added salt.

The titration constant indicated by the pH coordinates of the ascending branch of the curve of differences (Fig. 2) is roughly $10^{-9.7}$. Since the displacement caused by formaldehyde (Fig. 1) is less than 2 pH units, the negative logarithm (pK) of the titration constant of these groups in the absence of formaldehyde is less than 11.7. The value indicated by the work of Kekwick and Cannan on egg albumin (12) is about 11.4.

The fact that the descending branch of the curve is not fully symmetrical with respect to the ascending branch is discussed below.

Data obtained in the absence of salt are shown in Fig. 3. Here the

4 At pH 9.7, base bound by wool in the presence of formaldehyde is about 0.23 mM per gm. Of this amount, the ε-amino groups of lysine contribute about 0.12 mM, and histidine accounts for about 0.08 mM. The balance, 0.06 mM, presumably represents acidic groups in tyrosine side chains. Since the total tyrosine content is about 0.32
maximum separation of the curves along the pH axis is somewhat larger than in Fig. 1 (2.35 units instead of 1.90), and differences in the effects of the two concentrations of formaldehyde are much clearer than when salt is present. When the difference curves for these data are plotted as in Fig. 2 (lower section), the lack of symmetry previously noted becomes clearer, and its cause apparent. Instead of one set of groups affected by formaldehyde, two are indicated. One of these (pH values below 11) is apparently the same as that indicated in the experiment with salt, and probably corresponds to the ε-amino groups of lysine. The other occurs in a more alkaline range, suggestive of the guanidino groups of arginine. The failure of the latter to manifest themselves strongly in the data obtained with salt present appears to indicate that the combination of formaldehyde with these groups is inhibited by salt. The reason for this inhibition remains obscure. Failure to observe a pH effect of formaldehyde in the arginine groups in other proteins is probably due to the absence of data for these proteins in strongly alkaline solutions. An effect of formaldehyde on the guanidino group of arginine itself is now well known (7, 9).

The amounts of lysine and arginine indicated in the graph have significance only as minimum values. The differences attributed to arginine are somewhat more arbitrary than those attributed to lysine, because they are particularly susceptible to experimental error in the region of the steep portions of the curves in Fig. 2. However, if the analytical values for the contents of lysine, arginine, and tyrosine (20) are accepted, it is clear that the maximum base-binding capacity of wool should be considerably greater than the highest values reported in this or any previous study.

SUMMARY

1. The amounts of hydrochloric acid and potassium hydroxide combined by wool, in solutions of two concentrations of formaldehyde, have been determined and compared with earlier determinations in the absence of formaldehyde. The effect of the presence of formaldehyde has been determined both in the absence and presence of salt.

2. The effects of formaldehyde on the dissociation curve of wool have been shown to be consistent, for the most part, with existing ideas as to the combination of this substance with the amino groups of lysine. There are indications that the guanidino groups of arginine also combine with formaldehyde, at least when salt is not present.

3. Because of the strong basicity of the guanidino groups, in neither the present study nor any earlier one has the maximum base-binding capacity of wool been measured.

mM per gm., the pK of the phenolic groups in wool lies near 10.5. This is only slightly higher than the value in the free amino acid.
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