EFFECTS OF OXIDATION UPON ALCIAN BLUE STAINING OF ACID MUCOPOLYSACCHARIDES

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Since Steedman (1950) has introduced it into histochemistry, alcian blue has been advocated to reveal a staining selectivity which depends upon the presence of acidic groups in mucopolysaccharides (Mowry, 1956; Pearse, 1960). As a plausible ground of this concept the fact has been presented that the alcian blue staining of polysaccharides can be increased in intensity, when acidic groups are introduced artificially into the substances. A series of subsequent histochemical data obtained by Spicer (1960), Spicer and Meyer (1960) and by myself (Yamada, 1962 and 1963 a) have, however, not necessarily been on the side of the above concept and some types of tissue mucopolysaccharides have been shown to rather decline in alcian blue staining intensity, even after acidic groups are introduced experimentally into them. Moreover, my recent studies on the alcian blue stainability of sulfated polysaccharides of either proposed or known chemical structure have indicated that the selectivity of the basic dye is not always dependent exclusively on the presence of acidic groups in polysaccharides, but their appropriate hydroxyl groups can influence their alcian blue staining (Yamada, 1963 b).

In the present study, I have examined the alcian blue staining of several types of tissue acid mucopolysaccharides following oxidation which is known to introduce carboxyl groups into the substances, and attempted thus to obtain further evidence which helps to understand the staining mechanism of this basic dye.

MATERIALS AND METHODS

As donors of materials, use was made of 4 male and 6 female adult mice of ddN strain which are my laboratory stocks. The animals were sacrificed by decapitation and their tracheas, stomachs, small and large intestines and submaxillary glands were dissected out and sliced to small pieces of tissues. These tissues were fixed overnight in chilled ethanol-formalin as recommended by McManus and Mowry (1958), subjected to paraffin embedding and sectioned at a thickness of 6 to 8 μ. The sections were stained at room temperature by (1) 0.1% alcian blue 8GS (Schmid) acetic acid solution (pH 2.5) for 30 minutes, (2) identical procedure but after 2 hours oxidation with 5% chromic acid and

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(3) identical procedure after similar chromic acid oxidation following 5 minutes treatment with 0.5% periodic acid. In addition, each of the intact, chromic acid oxidized and periodic and chromic acids oxidized sections was stained by 0.02% azure A in 0.1 M phosphate-citrate buffer of selected pH (1.5, 3.0 and 4.5) for 30 minutes at room temperature, in order to examine its metachromatic reaction and to get information about correlation of the reaction with the alcian blue staining.

RESULTS

The several types of tissues obtained were observed in terms of tracheal cartilage matrix, gastric surface and foveolar cells, small intestinal and colonic goblets and submxillary gland alveoli, all of which are known to contain alcian blue reactive mucopolysaccharides.

Tracheal cartilage matrix and gastric surface and foveolar cells stain feebly or moderately with alcian blue, but submaxillary gland alveoli and goblets of both small intestine and colon exhibit rather intense and most intense alcian blue reactions respectively. Both types of oxidation cause a noticeable decrease in alcianophilic intensity of the cartilage matrix, and diminish conspicuously the basophilia of gastric surface and foveolar cells and both small intestinal and colonic goblets or eliminate it thoroughly, while abolishing the stainability of submaxillary gland alveoli.

When stained with the azure A solution of pH 1.5, tracheal cartilage matrix shows an intense metachromatic reaction which becomes gradually more intense as pH of the solution is raised to 3.0 and 4.5. Gastric surface and foveolar cells do not stain with the azure A solution of pH 1.5 at all, but exhibit feeble or moderate orthochromatic azurophilia at pH 3.0 and 4.5. At all the pH levels employed small intestinal goblets react doubtful or negative for the azure A stain. On the contrary, colonic goblets stain with the basic dye in a metachromatic shade which increases in intensity as pH of the dye solution is altered from 1.5 to 4.5. The azure A solution of pH 1.5 can not color submaxillary gland alveoli at all, whereas that of pH 3.0 and 4.5 stains them in an orthochromatic shade appearing more intense at the latter pH level. Either types of oxidation do not yield any significant effect on the azure A reaction at pH 1.5 of all the acidic polysaccharide containing structures, except for that of tracheal cartilage matrix which is suppressed markedly or abolished by the treatments. The metachromatic azurophilia at pH 3.0 of the cartilage matrix is decreased notably in intensity after either chromic acid or periodic and chromic acids oxidation, but the either treatments tend to increase variably the similar staining intensity at identical pH of colonic goblets. Further, the oxidations give rise to a variably positive azure A metachromatic reaction at pH 3.0 of gastric surface and foveolar cells, small intestinal goblets and submaxillary gland alveoli. Moderate and slight declines in intensity of azure A metachromasia at pH 4.5 of the cartilage matrix are observed after chromic acid and periodic and chromic acids oxidations respectively. Excepting this, the both types of oxidation increase slightly the metachromatic staining intensity at pH 4.5 of colonic goblets, and result in moderate and weak metachromatic

reactions at identical pH level of the gastric cells and submaxillary gland alveoli respectively. Although they fail to stain with azure A at pH 4.5, small intestinal goblets come to exhibit the most prominent metachromatic azurophilia at the pH level following either of the oxidations.

DISCUSSION

In the gall bladder of the guinea pig (Yamada, 1962) and toad (Yamada, 1963 a) alcian blue reactive surface mucus has been found to lose its stainability upon oxidation, while its azure A metachromasia at pH 4.5 being increased significantly in intensity following the treatment. Since metachromatic azurophilia at pH 4.5 can be assigned to the presence of carboxyl groups in substrates (Spicer, 1960), these data give support to the concept that oxidation introduces artificially carboxyl groups into the mucus, which do, however, not yield any extent of increase in alcian blue staining intensity of the substance, but rather suppress its reaction. The results of the present investigation are largely compatible with the previous data obtained from the gall bladder surface mucus; all the acidic polysaccharide containing structures examined here are found to decrease variably in intensity of their alcianophilia or to lose the stainability on oxidation, and yet their azure A reaction at pH 3.0 and 4.5 tends to increase in metachromatic staining intensity or to become metachromatic on the treatment, except for the reaction of the cartilage matrix. Thus, the above concept derived from observations upon the gall bladder surface mucus appears applicable to acidic polysaccharides involved in most of the structures examined here, and it may be concluded that experimentally oxidation introduced carboxyl groups of the polysaccharides can not necessarily result in an increase in the alcian blue stainability of the substances, but, instead, occasionally diminish the staining intensity. The suppressive effect of oxidation upon the metachromatic azurophilia at pH 3.0 and 4.5 of the cartilage matrix seems difficult to explain. In a previous study on the reactions to basic dyes of model sulfated polysaccharides the metachromatic azurophilia at pH 1.5, 3.0 and 4.5 of chondroitin sulfate C was found to be suppressed markedly in intensity by oxidation and this was interpreted as oxidative destruction of steric configurations of the substance which favor the metachromatic reaction to take place (Yamada, 1964). Therefore, the suppressive effect of oxidation upon the metachromatic reaction of the cartilage matrix may possibly be concerned with such property of chondroitin sulfates involved. From the results of my previous studies on the alcianophilia of model sulfated polysaccharides under normal, acetylated and deacetylated conditions, it has been asserted that the selectivity of alcian blue is not necessarily dependent exclusively on the presence of acidic groups in polysaccharides, but some types of hydroxyl groups in them can influence their alcian blue stainability (Yamada, 1963 b and 1964). As treatment with either chromic acid or periodic and chromic acids is thought to oxidize appropriate hydroxyl groups in polysaccharides (Casselman, 1959; Pearse, 1960) the present results indicative of the suppressive effect of oxidation upon the alcianophilia of several types of

acidic polysaccharides would be taken to be a confirmation in tissue sections of the above view derived from the experiments performed on model compounds.

SUMMARY

A histochemical study has been made of effects of oxidation upon alcian blue staining of several types of tissue acid mucopolysaccharides and the following results were obtained. The alcian blue reaction of the examined substrates is suppressed variably by either chromic acid or periodic and chromic acids oxidation. Such property of the reaction has been discussed with special reference to the metachromatic azurophilia of the intact and oxidized substrates. The present informations appear to (1) indicate that experimentally introduced carboxyl groups of some acid mucopolysaccharides can not increase the alcian blue reactivity of the substrates and (2) coincide well with my previous data that the selectivity of this copper phthalocyanine dye is not always dependent exclusively upon the presence of acidic groups in polysaccharides, but appropriate hydroxyl groups in them can influence their alcian blue stainability.

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