THE REACTION OF GELATIN WITH HYPOBROMITE

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The reaction of gelatin with alkaline hypobromite cannot be used to show the participation of the guanidino groups in gelation, contrary to a recent report, because of severe reduction of molecular weight. Hypobromite and alkali degrade gelatin more than alkali alone.

Recently Davis [1] reported that the destruction of the guanidino groups of gelatin by alkaline hypobromite prevents gelation of 2·5 % gelatin at 15°C (and also destroys its protective colloid effect for various precipitates). Also, the amount of hypobromite required to prevent gelation was increased when the amino groups were converted to guanidino groups. Davis found that treatment of gelatin with alkali alone did not prevent gelation. He assumed that the hypobromite-alkali combination does not reduce the molecular weight more than alkali alone. Grabar and Morel [2] also found that alkaline hypobromite prevents gelation. They cited the work of Goldschmidt and Wiberg [3] to show that peptides (without free α-amino groups) are not cleaved by hypobromite, although their own later work suggests that hypobromite treatment of gelatin reduces the molecular weight.[4]

We have investigated the effect of alkaline hypobromite and of alkali alone on the molecular weight of gelatin, as indicated by intrinsic viscosity. We will show that the assumption of non-degradation by hypobromite is incorrect and that the hypobromite reaction is not suitable for determination of the role of the guanidino groups in gelation of gelatin.

EXPERIMENTAL

The gelatins used were an acid extracted pigskin gelatin (Wilson Laboratories, Chicago; U—COP—CO, Special Non-Pyrogenic) and a limed calfskin (U.S. Gelatin Co., obtained from Prof. F. R. Eirich). All hypobromite reactions were carried out with solutions freshly prepared according to Davis, and containing 0·5 M potassium hypobromite, 0·5 M potassium bromide and 0·8 M potassium hydroxide. The control solutions of alkali were the same except that the hypobromite was replaced by an equimolar amount of bromide. Reactions were carried out for 130 sec at 39°C (Davis used 120 sec at 40°C), neutralized quickly with N acetic acid to final pH of 5·0-5·2 and diluted with water to 2·5 % gelatin. In hypobromite reactions 2·7 mmole of hypobromite was used per g of gelatin, the quantity required to prevent gelation, according to Davis.

Viscosities were run at 38·6 ± 0·1°C, in an Ostwald-type viscosimeter having an outflow time of 260 sec for 5 ml of buffer. Dilutions were made with a buffer of the same salt content and pH as the reaction products. Concentrations were checked by drying to constant weight at 108°C. Some browning occurred, due to traces of unreacted hypobromite; in one run this was eliminated by addition of 2 mg 95 % hydrazine to the reaction mixture. There was no significant difference due to the presence or absence of hydrazine.

RESULTS AND DISCUSSION

The results shown in table 1 make it clear that hypobromite and alkali degrade gelatin much more than alkali alone. Gelatin degraded by other means to an intrinsic viscosity of 0·1 dl./g does not gel.[5] It is, therefore, not possible to say that the non-gelation is the result of destruction of the guanidino groups.

* Contribution no. 18.

TABLE 1

type of gelatin	treatment	$[\eta]$ dl./g	gelation at 15°C	gelation at 0°C
acid pigskin	none	0·47	yes	yes
	0·8 M KOH	0·34	yes	yes
	0·8 M KOH and 0·5 M KOBr	0·095	no	no
	0·8 M KOH and 0·5 M KOBr *	0·11	no	no
limed calfskin	none	0·56	yes	yes
	0·8 M KOH	0·42	yes	yes
	0·8 M KOH and 0·5 M KOBr	0·11	no	very weak

* hydrazine used to destroy excess hypobromite.

The increase in hypobromite required to prevent gelation of guanidinated gelatin does not prove the participation of these groups in gelation, as is shown by the following considerations. We have found that of the 2·7 mmole of hypobromite per g of gelatin used, 2·5-2·6 were consumed (titration with thiosulphate of the iodine liberated from acidified potassium iodide). Since gelatin contains about 0·5 mmole of guanidino groups per g,[6] and 80 % of the guanidino groups were attacked (according to Davis), only 1·2 mmole were consumed by the guanidino groups. (This is based on the reaction of three moles of hypobromite per mole of guanidino group; we found that α-N-acetylarginine consumed 3·2.) Therefore, 1·3-1·4 mmole was consumed in other reactions including the chain breaking reaction. Conversion of all the amino groups to guanidino groups (about 0·4 mmole/g)[6] should increase the consumption of hypobromite by 1·2 mmole/g, if all the new groups react. Davis found an increase of 1·2-1·3 mmole/g, in the quantity of hypobromite required to prevent gelation. A reasonable explanation is that the deguanidination reaction and side reactions, including cleavage, are concurrent reactions of similar rates, and that to obtain sufficient cleavage to prevent gelation of the guanidinated gelatin additional hypobromite must be added to make up for that consumed by the additional guanidino groups.

The work of Goldschmidt and coworkers on the reaction of hypobromite with peptides and proteins is of interest. Goldschmidt and Wiberg [3] found that benzoylleucylglycine consumes no hypobromite in 30 min at 0°C. Goldschmidt and Steigerwald [7] found that under very mild conditions (0·3 M hypobromite and 0·003 N alkali, at 0°C) benzoylalanylglycine consumed 9·5 mmole hypobromite per mole of peptide group, relatively little reaction. (The reaction conditions of Davis and of this work are much more vigorous, and more rapid reaction would be expected.) It is not valid to rely on the very small extent of reaction of small peptides in considering extensions to macromolecular systems, in which molecular weight and all properties dependent on molecular weight are greatly affected by a relatively small number of random breaks in the chain. Thus a very high molecular weight protein, if cleaved at only one peptide residue in a hundred, will be reduced to an average molecular weight of 10,000. For our pigskin gelatin, for which a viscosity-molecular relation has been established,[5] the molecular weight is reduced to about 30,000 weight average or about 15,000 number average. This corresponds to less than 1 % peptide cleavage.

[1] Davis, *Trans. Faraday Soc.*, 1957, **53**, 1390.
[2] Grabar and Morel, *Bull. Soc. Chim. biol.*, 1950, **32**, 643.
[3] Goldschmidt and Wiberg, *Annalen*, 1927, **456**, 1.
[4] Morel and Grabar, *J. Chim. phys.*, 1951, **48**, 632.
[5] Bello, Bello and Vinograd, 133rd meeting of the *American Chemical Society* (San Francisco, April, 1958).
[6] Eastoe, *Biochem. J.*, 1955, **61**, 589.
[7] Goldschmidt and Steigerwald, *Ber.*, 1925, **58**, 1346.