GUANIDINO GROUPS

Guanidino groups in proteins are strongly basic ($pK > 12$) and remain protonated except in strongly alkaline solution. They are resistant to most chemical treatments. In the last few years, several methods have been developed for their modification. These methods are similar in that each involves reaction with a dicarbonyl compound or a derivative of a dicarbonyl. The modified groups are not ionized at neutral $pH$ values. The procedure of Toi et al. (1967) for reaction with 1,2-cyclohexanediione in 0.2 M NaOH is comparatively specific for arginine but too harsh for many proteins. The following procedure using less alkaline conditions is applicable to a greater number of proteins, although it is less specific in that some modification of amino groups also takes place (Liu et al., 1968).

The protein (10 to 20 mg) and an equal amount of 1,2-cyclohexanediione are dissolved in 5.0 ml of water and adjusted to pH 11 by addition of triethylamine and then stored in the dark at room temperature for 12 to 24 hours. To determine the effects of the high pH a control must be run without 1,2-cyclohexanediione. Extensive, but not quantitative modification of guanidine groups occurs under these conditions. The extent of reaction can be calculated from the loss of arginine as measured by amino acid analysis after acid hydrolysis. The yellow color of the protein after modification is due to the formation of an unidentified product from reaction with amino groups. This reaction usually involves some (10-40%) of the amino groups and is decreased by higher pH values. The unidentified product absorbs maximally near 440 nm.

Modification of guanidino groups can be accomplished under less alkaline conditions by reaction with a condensation trimer of diacetyl. Preparation of the reagent, and its use for the modification of bovine serum albumin, has been described by Yankeelov et al. (1968).

The reagent (trimer) is prepared by mixing 196 g of diacetyl (2,3-butanedione) with 415 g of clean, alkali-washed (0.1 N NaOH, H$_2$O), dry, powdered glass and by incubating with occasional mixing for five days at 25°C. The product is extracted from the hardened mass with diethyl ether and dried with anhydrous magnesium sulfate. Evaporation of the ether and storage overnight in the cold produces approximately 76 g (39%) of product which can be recrystallized from ether as white needles [m.p. 112.5°C-114°C; $\gamma_{max} = 286$ m$\mu$ (in 1 mM HCl)]. A crude preparation of the reagent can also be prepared by incubating a 15% solution of diacetyl for 24 to 48 hours in Tris, borate, or phosphate buffer adjusted to pH 8.8. The pH of the solution slowly decreases and must periodically be readjusted. The reagent is usable when upon 15-fold dilution its absorption at 400 m$\mu$ reaches 0.45 (Grossberg and Pressman, 1968).

In a typical experiment, 6 mg/ml of bovine plasma albumin was allowed to react with 0.4 $M$ reagent in 0.5 $M$ phosphate buffer (pH 7.0) at 25°C. After 40 hours, less than 20% of the initial arginine and about 70% of the initial lysine remained (see Figure 10-2). The decreased arginine and lysine contents can be determined by amino acid analysis after acid hydrolysis. The red color ($\gamma_{max} = 530$ m$\mu$) which forms during the reaction is presumably due to reaction with amino groups.
Phenylglyoxal is another useful reagent which reacts with guanidino groups of proteins near neutral pH (Takahashi, 1968). The reaction is reversible and the guanidino groups can be partially recovered after modification by incubation at neutral or alkaline pH values in the absence of the reagent. The procedure given below is taken from Takahashi (1968) for the modification of bovine pancreatic ribonuclease.

The reaction mixture is prepared by adding a 0.3-3% solution of phenylglyoxal hydrate dissolved in 0.2 M N-ethylmorpholine acetate buffer (pH 8.0) to an equal volume of a 1-6% solution of ribonuclease dissolved in the same buffer. The solution is incubated at 25°. At suitable intervals, samples can be removed for assay of enzymatic activity. With 0.5% ribonuclease and 1.5% phenylglyoxal, the decrease in enzymatic activity is complete in about 2 hours. The modified protein can be freed from the excess reagent by passing it through a column of Sephadex G-25 preequilibrated with 0.01 M ammonium acetate at 4°. It is stable below pH 4 but decomposes at neutral or alkaline pH values, regenerating the original guanidino groups (~80%).

REFERENCES