LETTER

On the Heterogeneity of Calf Thymus Histone

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Since the studies of Kossel and Lilienfeld[1], histones have been a well-known class of proteins, but their chemical and physicochemical properties are not yet clearly understood. Recent workers[2] have discovered a heterogeneity of calf thymus histone, but because of the preparation methods employed in these investigations it is possible that some changes in the protein had been produced during the purification processes.

In the present investigation histone was prepared carefully from acid extracts of calf thymus gland, and examined for its heterogeneity. An attempt was also made to fractionate the preparations.

Method 1. Fresh calf thymus glands were frozen, minced, homogenized in 0.05 M sodium citrate at *p*H 7.0 (or in dilute citric acid) and centrifuged. The sediment was washed with the solvent several times to remove cytoplasmic contaminants and then the histone was extracted from the sediment with 0.1 or 0.2 N sulfuric acid (Extract A). After clarifying "Extract A", the histone was precipitated by addition of two volumes of ethanol at low temperature (sometimes below 0°C.) and reprecipitated several times.

The preparation obtained was free from deoxypentose nucleic acid and its nitrogen content was 18.2%. Tryptophane content was estimated to be less than 0.2%. This preparation was found to be inhomogeneous both in electrophoresis and ultracentrifuge.

Fractional precipitation of the purified histone preparation by ethanol yielded mainly two fractions. Fraction I, precipitated at lower ethanol concentrations, revealed two components in the ultracentrifuge, the sedimentation constants ($s_{20,w}$) of which were 1.9 S and about 7 S respectively in an acetate buffer of *p*H 5.0 and ionic strength 0.2. The heavier component amounted to about one-third of fraction I and its sedimentation constant varied with different preparations. Fraction II, precipitated at higher ethanol concentrations, was ultracentrifugally almost homogeneous ($s_{20,w}=1.0$ S), but electrophoretically not homogeneous.

Fraction II differed from fraction I in the fact that it could not be precipitated by addition of ammonia to its solution, but like fraction I was soluble in H_2SO_4-$HgSO_4$ reagent[3].

Method 2. From the above-mentioned "Extract A" histone could also be salted out by addition of ammonium sulfate. The fraction, which was obtained at 80% saturation of ammonium sulfate, contained two components ($s_{20,w}=2.1$ and 12 S). A second fraction, which precipitated between 80% and 100% saturation of ammonium sulfate, was also inhomogeneous ($s_{20,w}=0.8$, 1.9 and 15 S).

Method 3. According to the method of Mirsky and others[3,4] histone was extracted from thymus nuclei with 0.2 N hydrochloric acid and precipitated by bringing the dialyzd extract to a *p*H value of about 10 by sodium hydroxide. Fractional precipitation, by in-

1) A. Kossel, "The Protamines and Histones," London and New York (1928).

2) L. Ahlström, *Arkiv. Kemi. Mineral. Geol.*, **24**A, No, 31 (1947); E. Stedman and E. Stedman, *Phil. Trans. Roy. Soc. London*, Ser. B. **235**, 565 (1951); J. Grégoire, J. Grégoire, and J. Reynand, *Compt. rend.*, **236**, 1922 (1953).

3) A. E. Mirsky and A. W. Pollister, *J. Gen. Physiol.*, **30**, 117 (1946).

4) M. M. Daly, A. E. Mirsky and H. Ris, *J. Gen. Physiol.*, **34**, 439 (1951).

creasing the *p*H of the solution, yielded at least three different fractions, and a final fraction soluble in alkaline solution was precipitated by adding ethanol at *p*H 10.9. None of these fractions were homogeneous in the ultracentrifuge.

From the above observations it was concluded that calf thymus histone is not a homogeneous protein, but consists of many fractions having different molecular weights and charges.

Studies on the homogeneity of the histone sample which was prepared without using acid are now in progress and will be published soon with the details of the present investigations.

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