COMPARATIVE ELECTROPHORETIC PROPERTIES OF HISTONES FROM CELLS OF THE MOSQUITO Aedes aegypti AND OF THE FRUITFLY Drosophila melanogaster

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Abstract. Electrophoretic mobility of histones from cell cultures of Drosophila melanogaster and of the mosquito Aedes aegypti was determined in polyacrylamide gels in the presence of different concentrations of urea. Great similarity in the electrophoretic behavior of H3, H2A, H2B and H4 histones from the two insect species was found. Histone H1 of Aedes under all conditions tested had a markedly higher electrophoretic mobility than H1 of Drosophila, but differed only slightly from H1 histones of mouse and of hamster.

As can be deduced from the mobility of Aedes H1 in the presence of sodium dodecyl sulphate its molecular weight is smaller than that of Drosophila H1 and is very close to the molecular weight of the main component of mouse H1 histone. Heterogeneity of the H1 histone from Drosophila is demonstrated. This heterogeneity is due to phosphorylation of a part of H1 molecules, since it disappears after the treatment of H1 preparations by alkaline phosphatase. Phosphorylated components were not found in the H1 of Aedes.

Thus two representatives of Diptera, Aedes and Drosophila possessing polytene chromosomes at the larval stage of development have H1 histones with markedly different primary structures. This fact demonstrates that the polytenization of chromosomes may occur in species with markedly different H1 histones.

I. INTRODUCTION

Studies of histones from different species have demonstrated a high degree of the evolutionary stability of the H3 and H4 histones and a somewhat lesser stability of histones H2A and H2B [1]. The structure of H1 histone is much more variable; among vertebrates not only species specificity but even tissue specificity is found for this histone. It has been observed, that H1 histones of most vertebrate species studied are heterogeneous and contain subfractions differing by amino acid composition, amino acid sequence, extent of postsynthetic modification and even molecular weight [e.g. 2-10]. Nevertheless the general type of structure of H1 histone molecules from all species studied is identical [11-15].
Histones of invertebrates, of insects in particular, have been studied to a significantly lesser extent. In the two Diptera species Drosophila melanogaster and Ceratitis capitata the presence of five main histone fractions was demonstrated [16-21]. H4 and H3 histones of Drosophila were quite similar to corresponding histones of calf thymus, while the amino acid composition and electrophoretic mobility of H2A and H2B histones differed markedly in these organisms. The greatest differences were found for the H1 histone.

It has been found that the H1 histone from Drosophila has a molecular weight exceeding that of calf thymus histone by 5–10%. No such data were available for Ceratitis capitata. Furthermore, the amino acid composition of H1 histones from both species of fruitflies was found to be almost identical but strongly different from the amino acid composition of H1 from mammals. H1 histones of both insect species contain two times more serine, aspartic and glutamic acid residues than the H1 histone from the rabbit thymus; the relative content of valine and isoleucine in insect H1 histones is also increased while the proportion of lysine, alanine and proline is decreased. The ratio of basic to acidic amino acids in H1 histones of both fly species is almost twice less than in H1 from rabbit thymus. Therefore the net positive charge of H1 histones of both flies is decreased and during electrophoresis in urea-containing polyacrylamide gels theses histones move markedly slower than H1 histone from calf thymus, which represents one of the 'slowest' mammalian histones. The difference in the relative mobility of H1 histones from Drosophila and calf thymus is considerably greater than the difference between the mobility of calf thymus H1 on one hand and H1 histones from Tetrahymena, echinoderms, birds and mammals on the other [2, 16, 22, 23]. Presence of a 'slow' H1 histone is generally regarded as a characteristic feature of Diptera and it was suggested that the unusual properties of this histone may be connected with a special characteristic of Dipteran chromosomes: their tendency to polytenization in various somatic tissues particularly at the larval stage [16, 19, 20]. According to this hypothesis a particular structure of H1 histone of the Drosophila type is necessary for the polytenization of chromosomes. If this hypothesis is correct, all other species of Diptera having polytene chromosomes would have H1 histones of the Drosophila type. In order to verify this suggestion we studied histones in another representative of Diptera, the mosquito Aedes aegypti.

At the larval stage of development this mosquito has polytene chromosomes and in this connection we were interested to learn whether its H1 histone belongs to the 'slow' class, just as H1 histones of the two species of flies mentioned.

II. MATERIALS AND METHODS

Cell culture
Histones were isolated from cell cultures of the diploid line of Drosophila melanogaster, tetraploid line of a mosquito Aedes aegypti [24], chinese hamster and L-mouse fibroblasts. Insects cells were grown as described in the literature [24]. L-cells and chinese hamster cells were grown as monolayers using 199 medium or Eagle medium to which 10% of serum was added.