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ENZYMATIC STUDIES OF RADIATION DAMAGE

Final Report
for Period January 1, 1979-September 30, 1981

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ABSTRACT

The susceptibility of the DNA in chromatin to single strand-specific nucleases was examined using nuclease P_1, mung bean nuclease, and venom phosphodiesterase. These experiments showed that chromatin contains a limited number of DNA sites which are susceptible to single-strand specific nucleases. These sites occur at intervals of 8 to 80 nucleosomes and are distributed throughout the chromatin.

An endogenous nuclease was found in chicken erythrocyte nuclei. This enzyme resembles the nuclease of mammalian nuclei in requirements for bivalent cations and in production of large chromatin fragments that gradually decrease in size, but differs in that the products do not go through the stage of discrete bands on gel electrophoresis.

Experiments in which chromatin fragments from micrococcal nuclease digestion were further digested with venom phosphodiesterase indicated that phosphodiesterase preferentially hydrolyzes all linkers, although this preference is not marked enough to produce and preserve particulate structures of chromatin.

Analysis of the ribose-containing components resulting from digestion of chromatin by micrococcal nuclease and P_1 nuclease led to the conclusion that a minimal amount of Poly (ADP-Rib) is necessary to form and preserve the structure of nucleosomes.

FINAL REPORT

This final report covers all research pertinent to contract DEAC02-76EV03225 (Enzymatic Studies of Radiation Damage) for the period January 1979 to September 1981. Information pertinent to previous research carried out on this contract can be found in documents COO-3225-1 through COO-3225-41.

The susceptibility of the DNA in chromatin to single-strand specific nucleases was examined using nuclease P_1, mung bean nuclease, and venom phosphodiesterase. A stage in the reaction exists where the size range of the solubilized products is similar for each of the three nucleases and is nearly independent of incubation time. During this stage the chromatin fragments sediment in the range of 30 to 100 S and contain duplex DNA ranging from 1 to 10 million daltons. Starting with chromatin depleted of histones H1 and H5 similar fragments are generated. In both cases these nucleoprotein fragments are reduced to nucleosomes and their multimers by micrococcal nuclease. Thus, chromatin contains a limited number of DNA sites which are susceptible to single-strand specific nucleases. These sites occur at intervals of 8 to 80 nucleosomes and are distributed throughout the chromatin.

Nucleosome monomers, dimers, or trimers were not observed at any stage of single-strand specific nuclease digestion of nuclei, H1- and H5-depleted chromatin, or micrococcal nuclease-generated oligonucleosomes. Each of the three nucleases converted mononucleosomes ($\sim$160 base pairs) to nucleosome cores ($\sim$140 base pairs) probably by exonucleolytic action that was facilitated by the prior removal of H1 and H5. The minichromosome of SV40 is highly

resistant to digestion by nuclease P_1.

Further studies on chicken erythrocyte nuclei revealed the presence of an endogenous nuclease. This enzyme resembles the nuclease of mammalian nuclei in requirements for bivalent cations and in production of large chromatin fragments that gradually decrease in size, but differs in that the products do not go through the stage of discrete bands on gel electrophoresis. Endogenous nuclease and micrococcal nuclease are also detectable in mononucleosomes prepared from chicken erythrocytes with the aid of micrococcal nuclease. Both nucleases are extractable with 0.35 M NaCl, and both are inhibited by pTp. In the absence of Ca^{2+}, the micrococcal nuclease is totally inactive, whereas the endogenous nuclease shows a low level of activity.

It was found that chromatin could be fragmented with micrococcal nuclease to yield three fractions composed almost exclusively of mononucleosomes, dinucleosomes, and oligonucleosomes (predominantly hexanucleosomes). Each fraction was then digested with venom phosphodiesterase. Degradation of mononucleosome proceeded via shortening of linkers; when the core particles reached the size of about 120 base pairs, they disintegrated. Dinucleosomes formed a transient, poorly defined, band in the position of mononucleosomes which disintegrated within 15 minutes. Oligonucleosomes formed poorly defined bands of consecutively smaller oligomers which never became sharply delineated. It was concluded that phosphodiesterase preferentially hydrolyzes all linkers, although this preference is not marked enough to produce and preserve particulate structures of chromatin.

The action of micrococcal nuclease on chicken erythrocyte nuclei was followed by solubility in 0.67 mM EDTA. Solubility vs time curves showed maxima and minima. Fractionation of the EDTA-soluble material on Bio-Gel A-5m yielded peaks containing oligonucleosomes which disappeared after 30', mononucleosomes which were still detectable at 50', and "acid-soluble" material which steadily increased. The nucleoside composition of all fractions was analyzed by HPLC; 4 deoxyribonucleosides, rA, and 2'(1''ribosyl)adenosine (r_2A) were detected. Although r_2A cochromatographed with rG on HPLC, the two were distinguishable because incubation with adenosine deaminase shifted the position at which r_2A (now r_2I) chromatographed, but did not affect rG. With time the % of adenosine derivatives increased in oligonucleosomes and reached ~1.5% at 30'. It showed an initial decrease in mononucleosomes, reached a minimum of about 0.5% at 15' and remained on this level as long as mononucleosomes lasted. In "acid-soluble" it showed a maximum at 15'. When the action of P_1 nuclease on chicken erythrocyte nuclei was monitored, the solubility in EDTA showed a plateau, the height of which was affected by the amount of enzyme. No mononucleosomes were detected, and most of the ribose-containing components appeared in the "acid-soluble" material in the first 10'. We conclude that a minimal amount of Poly(ADP-Rib) is necessary to form and preserve the structure of nucleosomes.

In addition, during the course of the final trimester of this contract chapters on the purification and properties of mung bean nuclease and phosphodiesterase were written, as was a review dealing with an historical perspective on nucleases (see Publications).

PUBLICATIONS

Fujimoto, M., Kalinski, A., Pritchard, A.E., Kowalski, D., and Laskowski, M., Sr. (1979) Accessibility of Some Regions of DNA in Chromatin (Chicken Erythrocytes) to Single-Strand Specific Nucleases, J. Biol. Chem. 254, 7405-7410.

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Laskowski, M., Sr., An Historical Perspective on Nucleases, Proceedings of the Cold Spring Harbor Meeting on Nucleases (in press, 1981).

Conference Reports

DOE/EV/03225/42 Takamatsu, H. (1981) Action of Venom Phosphodiesterase on Chromatin (Chicken Erythrocyte Nuclei) Fragmented with Micrococcal Nuclease, Fed. Proc. 40, 1567.

Conference Reports (continued)

DOE/EV/03225/43 Dziegielewski, T.K., and Laskowski, M., Sr., Nucleosomes Prepared from Chicken Erythrocyte Nuclei with the Aid of Micrococcal Nuclease Contain Poly(ADP-Rib), Cold Spring Harbor Meeting on Nucleases (in press, 1981).