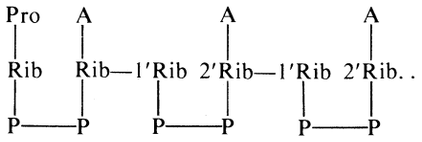
Meetings

Poly(ADP-ribose): Polynucleotide Still without an Ascribed Function

The polynucleotide, poly(adenosine diphosphoribose) [poly(ADP-ribose)], which occurs in eukaryotes, has been receiving more and more attention and interest over the last few years, although its exact functions have not been spelled out. Its biosynthesis in hen liver nuclei was first described by P. Mandel and colleagues (Strasbourg University) in 1963 when they showed that radioactivity from labeled adenosine triphosphate (ATP) in the presence of nicotinamide mononucleotide (NMN) was incorporated into an acid-insoluble product that was not digested by either ribonuclease or deoxyribonuclease. Nicotinamide adenine dinucleotide (NAD) was shown to be the precursor of poly(ADP-ribose) by O. Hayaishi (Kyoto) in 1966; and, at about the same time, groups in Tokyo (T. Sugimura) and Kyoto (Hayaishi) helped elucidate the structure and early details of poly(ADP-ribose) biochemistry.



A unique 1′,2′-glycosidic linkage between adjacent riboses that contain adenine join the ADP-ribose units of NAD. There is rather good evidence to indicate that the initial ADP-ribose unit is covalently bound to various nuclear proteins, especially histones. Polymers of as many as 20 ADP-ribose units have been shown to accumulate in preparations of thymus cell nuclei. The polymer rapidly turns over in vitro and presumably in vivo; snake venom phosphodiesterase and also a specific liver phosphodiesterase cleave the polymer to ADP-ribose units, while poly(ADP-ribose) glycohydrolase cleaves the 1′,2′-glycosidic bond. However, the polymer does not seem to be a storage form of potential NAD; NAD cannot, at least in vitro, be regenerated from poly(ADP-ribose)-protein complexes. DNA is required for the accumulation of the polymer in nuclear or chromatin preparations, presumably in part because of the inhibitory action of DNA on poly(ADP-ribose) glycohydrolase.

The biological function of this polymeric modification of nuclear proteins is not apparent, although most evidence indicates some cellular role related to DNA synthesis, repair, or structure in chromosomes. Investigators interested in poly(ADP-ribose) have met frequently to discuss the progress of their researches. A volume entitled *Poly(ADP-ribose): An International Symposium* (Fogarty International Center Proceedings No. 26, Department of Health, Education, and Welfare Publication No. 74-7477, Washington, D.C., 1973) was published outlining the state of the art as of July 1973. More recently, workers from the United States, France, West Germany, England, and Japan met (10 to 12 September 1974) in the city of Tomakomai, Hokkaido, Japan, for the third international symposium seminar on this topic. The meeting was cosponsored by the Japan Society for the Promotion of Science and the Fuiihara Foundation for Science and was organized by T. Sugimura (National Cancer Center Research Institute, Tokyo) and O. Hayaishi (Department of Medical Chemistry, Kyoto University).

The enzyme capable of polymerizing ADP-ribose groups from NAD was purified 5000-fold (the purest preparation made so far) by K. Ueda (Kyoto) and Hayaishi. The enzyme, isolated from rat liver nuclei, can use the poly(deoxyadenylate-deoxythymidylate) synthetic random polymer for its DNA requirement. As the extent of purification of the enzyme increased, shorter and shorter chains were generated on nuclear proteins, suggesting the possibility that more than one enzyme might be involved in elongation of poly(ADP-ribose). The polymerase from thymus appears to be a protein even more basic than histones (M. Gill, Harvard University).

Purification of the poly(ADP-ribose) glycohydrolase (L. O. Burzio and S. Koide, Rockefeller University) has revealed that this enzyme is inhibited by single-stranded DNA with rather strict specificity toward purine base regions. Thus it would seem that denatured purine regions in DNA might tend to cause an accumulation of the polymer in the intact cell.

Various models were postulated concerning the attachment of poly(ADP-ribose) to acceptor molecules with the existence of more than one biologically significant acceptor molecule a possibility. One model, that of H. Hilz (Hamburg), which seems consistent with the observed density of poly(ADP-ribose) proteins, would involve an acceptor molecule for the polymer containing multiple sites for ADP ribosylations. Hence the sites might be filled with polymers of very differing lengths. The number of ADP-ribose residues in rat liver has been estimated to be approximately 5.3 nanomoles per milligram of DNA.

Of the many studies dealing with the biological function of poly(ADP-ribose), none has pinpointed its roles. However, some clues are becoming evident. S. Shall (University of Sussex) showed that during asynchronous growth of mouse L cells in tissue culture the specific activity of poly(ADP-ribose) polymerase fluctuates between low activity during the peak of DNA synthesis and high specific activity as stationary maintenance of cells progresses. In confirming experiments, M. Smulson (Washington, D.C.) showed that nuclear activity is low in the HeLa cell S phase and high in the G1 phase. Examination of trout testes during spermogenesis by Shall indicates that in the developing sperm the activity of the polymerase is high when histones are the major basic nuclear protein; but, in the mature sperm cells where nuclear proteins are protamines, activity of the enzyme disappears. On his measurements of total poly(ADP-ribose) in intact mouse L cells during the cell cycle, W. R. Kidwell (National Institutes of Health) reported that two maximal accumulations of polymer were obtained, one in the mid S phase and a second accumulation during the G2 phase exploited by the use of selective G2 phase inhibitors which caused elevated levels of poly(ADP-ribose) to be accumulated in cells. Y. Kanai (Tokyo) has prepared highly specific antiserums against poly(ADP-ribose), which should allow sensitive measurements of the polymer content of cells to be made. He and his co-workers detected antibodies to ADP-ribose in the serums of patients with systemic lupus erythematosus.

The synthesis of poly(ADP-ribose) poly-

merase and its presence on the surface of polyribosomes in HeLa cells, as shown by Smulson, indicated high activity of the enzyme on ribosomes during S phase of the cell cycle. Earlier L. Burzio and S. Koide [*Biochem. Biophys. Res. Commun.* **16,** 113 (1973)] showed that generation of the polymer in vitro in intact rat liver nuclei causes significant inhibition of the template capacity of the resultant DNA nuclear protein matrix of chromatin for DNA polymerase. This work has been extended in collaboration with K. Toshihara (Mara Medical University) by the observations that rat liver nuclei contain a Ca^{2+},Mg^{2+}-dependent endonuclease capable of "activating" rat liver CNA in vitro. Furthermore, it was shown that in the presence of semipurified poly(ADP-ribose) polymerase, DNA, and NAD, a short-chain modification is transferred to the endonuclease inactivating the enzyme and its ability to generate primer sites on DNA. This modification explains the apparent inhibition of DNA template activity in rat liver. An exonuclease from rat liver, which is inhibited by free chains of poly(ADP-ribose), has also been described by M. Yamada (Tokyo) and Sugimura.

In HeLa cells, however, Smulson showed that ADP-ribosylation of nuclear proteins leads to an enhancement rather than to an inhibition in the number of primer sites for DNA polymerase. This stimulation is inversely related to the natural extent of template restriction imposed by nuclear proteins during the cell cycle and cell proliferation. For example, chromatin seems very restricted for exogenous DNA polymerase early in the G1 phase, and ADP-ribosylation of nuclear proteins releases this restriction, at least in vitro.

Two other analogous ADP-ribosylation reactions occur naturally. Diphtheria toxin catalyzes a transfer of a single ADP-ribose unit from NAD to elongation factor 2, leading to inhibition of eukaryote protein synthesis. C. Edson, K. Ueda, and O. Hayaishi (Kyoto) and E. Maxwell, E. Robinson, and O. Henriksen (NIH) have been interested in the exact residue modified in EF-2. Maxwell's group has shown that ADP-ribose is linked to an unknown, weakly basic amino moiety which does not correspond to any known amino acid and which is part of unmodified EF-2 also.

Bacteriophage T4 infection of *Escherichia coli* leads to ADP-ribosylation of the α subunits of host RNA polymerase, presumably resulting in subtle changes in enzyme specificity. W. Zillig (West Germany) and C. Goff (England) both described their studies on the identification of ADP-ribosylated residues of the α subunit as well as in vitro assays for ultimate purification of the enzyme that effects this modification.

There is still much to be learned about the relation of these nonoxidative roles of NAD in cellular proliferation and regulation. Abstracts of this meeting on this topic will be published in the *Journal of Biochemistry (Tokyo)*.

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Organotransition-Metal Chemistry

Initial progress in the field of organotransition-metal chemistry has followed the lines of interest that were generated separately by organic and inorganic chemistry. However, it is increasingly clear that organotransition-metal chemistry is not only bridging these two fields but is also finding application in other branches of science.

The stabilization and isolation of both cyclobutadiene and benzene derivatives of transition-metal organometallics were first achieved because of academic interest. However, industrial processes, such as the Ziegler-Natta olefin polymerization, the Wacker oxidation of ethylene to aldehydes and ketones, and the hydroformylation of olefins, have provided practical applications for the achievements in organotransition-metal chemistry. Still to be determined is the exact role of transition-metal organometallics in many metal biological functions and processes—such as nitrogen fixation by nitrogenase and multifunctions of vitamin B_{12}—and the functions of organomercury as a pollutant, and catalytic functions in fuel cells.

During the past decade, Japanese organic chemists have focused on research on organometallic chemistry. As a result, Japanese organotransition-metal chemists have discovered new reactions and isolated unstable organotransition-metal compounds.

Detailed mechanisms for only a few reactions of organotransition metals are known. The seminar was devoted to the following specific themes: (i) factors influencing the stability of transition metal carbon bonds and mechanisms of the form and breaking of such bonds; (ii) features of the mechanisms of reactions involving unstable organotransition-metal complexes; (iii) application to catalytic processes, such as carbonylation and related processes in which both countries have some scientific and technological interest; and (iv) application to environmental problems, notably the formation of toxic organometallic compounds, such as methylmercury.

On 1 to 3 May 1974 the first Japanese-American seminar on Prospects in Organotransition-Metal Chemistry was held at the University of Hawaii's East-West Center, in Honolulu, jointly sponsored by the National Science Foundation and the Japan Society for the Promotion of Science. The purpose of the seminar was to acquaint participants with the major trends and directions of research in Japan and the United States. Attendance was limited to 17 Japanese and 24 Americans in order to encourage informal discussion and exchange of ideas between the two nationalities.

The first session, which dealt with novel organometallic compounds, was opened by the Japanese and American organizers of the conference, M. Tsutsui and Y. Ishii. Two participants from the University of California, Los Angeles, dealt with compounds of unusual geometry: M. F. Hawthorne discussed his work in polyhedral expansion and contraction of metallocarboranes, and H. D. Kaesz described unsaturated ruthenium hydridocarbonyl cluster complexes and their possible application to catalysis. Presentations of work on compounds with novel ligands were represented by K. Itoh (Nagoya University) on the bridging, bidentate behavior of benzoyl isocyanates and by R. B. King (University of Georgia) concerning complexes of polycyano-olefins, specifically comparing the dicyanovinylidene ligand with carbon monoxide. The actinide metals paper was presented by T. Marks (Northwestern University) on tris(cyclopentadienyl)alkyl complexes.

The largest single topic of interest was homogeneous catalysis. Although the original controversy over the concerted as compared to the stepwise mechanism of various metal-catalyzed rearrangements has been largely resolved, four papers on this subject were presented. First and foremost was R. Pettit's (University of Texas, Austin) very thorough presentation on the concerted nature of the rearrangement of polycyclic hydrocarbons catalyzed by silver ions. F. Mango (Shell Oil) and R. Grubbs (Michigan State University) each discussed metallocycles as intermediates in these reactions; Mango questioned whether any reaction proceeding in this way should really be called "concerted."