COMMUNICATIONS TO THE EDITOR

INCORPORATION OF THYMIDINE TRIPHOSPHATE INTO DEOXYRIBONUCLEIC ACID BY A PURIFIED MAMMALIAN ENZYME

Sir:

Previous work from this laboratory demonstrated the incorporation of thymidine into DNA[1] catalyzed by the high-speed supernatant fraction from regenerating rat liver homogenates.[2] The incorporation of thymidine was stimulated by a mixture of DAMP, DGMP, and DCMP. Enzymes catalyzing formation of higher phosphates of deoxynucleotides have been observed by several investigators.[3] To elucidate the deoxynucleotide effect on thymidine incorporation, supernatant fraction was examined for ability to phosphorylate DAMP, DGMP and DCMP. When fortified with 5 mM. adenosine triphosphate, 5mM. Mg^{++}, and 6 mM. 3-phosphoglycerate regenerating rat liver supernatant fraction phosphorylated 5 mM. DAMP, DGMP, or DCMP at rates of approximately 1 μmole/hr./mg. protein, forming DATP, DGTP, or DCTP (respectively) as principal product. H^3-Thymidine also was phosphorylated to H^3-TTP by regenerating liver supernatant fraction, but at a much lower rate (approximately 0.03 μmole/hr./mg. protein) and this rate was lower by a factor of at least 10 in normal supernatant fraction.[4]

Deoxynucleoside triphosphates, synthesized by crude supernatant fraction as outlined above, isolated by ion-exchange,[5] and purified where necessary by chromatography on cellulose,[3c] were then incubated with a polymerizing enzyme purified about ten fold from regenerating rat liver.[6] The experiment presented in Table I demonstrates a requirement for Mg^{++}, DNA,[7] and the presence of all four deoxynucleoside triphosphates for maximal activity of purified enzyme in incorporating H^3-TTP into DNA.

Omission of a single deoxynucleoside triphosphate reduced incorporation of H^3-TTP by 66–70%. An earlier experiment with slightly less active enzyme and a different H^3-TTP preparation gave the same general result with a less definite requirement for Mg^{++}. These findings are offered as explanation for the deoxynucleotide stimulation reported in crude preparations.[2] Requirement for DNA, Mg^{++}, and presence of all four deoxynucleoside triphosphates, together with the fact that this enzyme is inhibited by pyrophosphate,[8] suggest that the reaction mechanism described for the *E. coli* enzyme[9] will also hold for rat liver enzyme.

TABLE I

REQUIREMENTS FOR H^3-THYMIDINE TRIPHOSPHATE INCORPORATION INTO DNA

Complete system contained in 0.25 ml.: DNA, 250 μg; enzyme, 380 μg. protein; H^3-TTP, 3.3 mμmoles (22,300 c.p.m.); DGTP, 8 mμmoles; DATP, 10 mμmoles; DCTP, 9 mμmoles; Mg^{++}, 2 μmoles; and TRIS:HCl, *p*H 8.0, 10 μmoles. After 60 minutes incubation at 37° 1.0 ml. cold 10% TCA was added. TCA insoluble material was washed two times with 0.5 ml. 5% TCA, hydrolyzed 20 minutes at 80° in 0.2 ml. 0.2 *M* NaOH, DNA and protein reprecipitated with dilute HCl and 5% TCA, and precipitate washed with 95% EtOH. Insoluble material dissolved in 0.5 ml. 90% formic acid and 0.1 ml. plated. (This rapid method of DNA isolation gives quantitative recovery.)

Reaction mixture	Total radioactivity[a] in DNA, c.p.m.
Complete	1,100
Omit DGTP	330
Omit DATP	400
Omit DCTP	400
Omit DGTP, DATP, and DCTP	175
Omit Mg^{++}	25
Omit DNA[b]	35
Complete, 60 minutes at 0°	45

[a] Radioactivity assayed in windowless flow counters. Since the same amount of material was plated from each reaction mixture no correction has been made for self-absorption. [b] 250 g. DNA added after incubation.

(1) These abbreviations are used: DNA, deoxyribonucleic acid; DAMP, DGMP, DCMP, and TMP for the mono- and DATP, DGTP, DCTP, and TTP for the triphosphates of deoxyadenosine, deoxyguanosine, deoxycytosine, and thymidine; TCA, trichloroacetic acid; and TRIS, tris-(hydroxymethyl)-aminomethane.

(2) F. J. Bollum and V. R. Potter, Abstracts, 132nd meeting, American Chemical Society, 19-C (1957).

(3) (a) H. Z. Sable, P. B. Wilber, A. E. Cohen and M. R. Kane, *Biochim. Biophys. Acta*, **13**, 156 (1954); (b) L. I. Hecht, V. R. Potter and E. Herbert, *ibid.*, **15**, 134 (1954); (c) H. Klenow and E. Lichtler, *ibid.*, **23**, 6 (1957); (d) E. S. Canellakis and R. Mantsavinos, *ibid.*, in press; (e) I. Leberman, A. Kornberg and E. S. Simms, *J. Biol. Chem.*, **215**, 429 (1955); (f) A. Kornberg, "The Chemical Basis of Heredity," ed. W. D. McElroy and B. Glass, Johns Hopkins Press, Baltimore, Md., 1957, p. 579; (g) S. Ochoa and L. Heppel, *ibid.*, p. 615.

(4) F. J. Bollum, P. A. Morse and V. R. Potter, unpublished.

(5) R. B. Hurlbert, H. Schmitz, A. F. Brumm and V. R. Potter, *J. Biol. Chem.*, **209**, 23 (1954).

(6) F. J. Bollum, *Federation Proc.*, **17**, in press (1958).

(7) The requirement for DNA was also demonstrated with crude supernatant fraction, see ref. 2.

(8) Inhibition was 50% at 10 m*M* and 100% at 100 m*M* pyrophosphate, tested with the crude enzyme from regenerating liver. Details to be published soon.

(9) M. Bessman, I. R. Lehman, E. S. Simms and A. Kornberg, *Federation Proc.*, **16**, 153 (1957).

(10) Postdoctoral Fellow of the National Cancer Institute, USPHS. This investigation was supported by a grant (C-646) from the National Cancer Institute, USPHS, to Prof. Van R. Potter. I wish to express sincere appreciation to Prof. Potter for interest and helpful suggestions during the course of this work. I am indebted to Miss Geraldine DeGrazia for doing the many spectrophotometric analyses required in the isolation, characterization, and purification of the deoxynucleoside triphosphates, and in checking out the validity of the rapid method for isolating DNA.

McARDLE MEMORIAL LABORATORY
UNIVERSITY OF WISCONSIN
MADISON 6, WISCONSIN

F. J. BOLLUM[10]

REACTION OF CHLORODIFLUOROMETHANE WITH LINDE MOLECULAR SIEVE 5A

Sir:

Chlorodifluoromethane reacts at room temperature with a synthetic zeolite (Linde Molecular Sieve 5A).[1] Chlorofluoromethanes have been reported stable to temperatures in excess of 400°.[2] When chlorodifluoromethane was adsorbed at 25.0° on

(1) Empirical formula $CaO \cdot Al_2O_3 \cdot 2SiO_2 \cdot nH_2O$; see also D. W. Breck *et al.*, THIS JOURNAL, **78**, 5963 (1956).

(2) A. B. Trenwith and R. H. Watson, *J. Chem. Soc.*, 2368 (1957).