A COMMENT ON "THE REPLICATION PROCESS OF SINGLE STRANDED DNA OF BACTERIOPHAGE ϕX174"

U. N. Singh

McGill - Montreal General Hospital Research Institute, Montreal

Received February 17, 1964

It was observed by Sinsheimer et al. (1962) that the replication of single stranded DNA of bacteriophage ϕx174 involved a "replicative form" with the characteristics of double stranded DNA. It was tempting to postulate that the "replicative form" might be the immediate precursor of single stranded DNA. Recently Matsubara et al. (1963) have studied the time course of incorporation of thymine-C^{14} into two types of DNA in the infected bacteria. Although, the kinetic model proposed by Matsubara et al. adequately accounted for the flow of radioactive thymine into "single DNA", there was no experimental evidence to support that the immediate precursor under consideration was indeed the "low molecular precursor" represented by the acid soluble material. The data presented by these authors (Matsubara et al.) permitted a direct evaluation of the possible role of "double DNA" as the immediate precursor of "single DNA". In contrast to their conclusion it is suggested here that the kinetic behaviour of double stranded DNA is not inconsistent with its possible role as a precursor of single stranded virus DNA.

Assuming double DNA as the immediate precursor of single DNA, Eq. (2) of Matsubara et al. can be written as:

$$\frac{dS^*}{dt} = \frac{\alpha}{D} D^*, \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where P is replaced by D. It may be pointed out that Eq. (1) is valid irrespective of whether double DNA is fully conserved or undergoes partial degredation. In the "pulse" experiment (fig. 2, Matsubara et al.) D^* increases linearly with time and is given by:

$D^* = kt$, where $k = 1.05 \times 10^3$ cpm. min^{-1}. Integrating Eq. (1) under the condition, $S^* = O$, when $t = O$, equation for S^* can be written as:

$$S^* = \frac{\alpha}{2D} kt^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$\frac{\alpha}{D}$ is estimated to be 0.88 min^{-1} from the slope of the straight line, S^* vs t^2. It is of considerable interest to note that this value of $\frac{\alpha}{D}$ closely resembles that of $\frac{\alpha}{P}$ (0.75) as estimated by Matsubara et al. This provides strong support to the possible identity of the double DNA with the "precursor" postulated by Matsubara et al. in their model.

The main objection against the concept of double DNA as the immediate precursor of single DNA raised by Matsubara et al. is based on the data reported in their "chase" experiment. It was argued that since the amount of radioactivity gained by single DNA was much in excess over the amount released from double DNA, the latter could not be the immediate precursor of the former. As shown in the following simple analysis, such a comparison is justified only when the amount of radioactivity in the immediate precursor of double DNA is '0'.

In the steady state system, low molecular precursor (L) $\longrightarrow$ double DNA (D) $\longrightarrow$ single DNA (S),

$$\frac{dD^*}{dt} = \frac{\alpha}{L} L^* - \frac{\alpha}{D} D^* \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

From Eqs. (1) and (3), $\frac{dS^*}{dt} = \frac{\alpha}{L} L^* - \frac{dD^*}{dt}$ $\ldots\ldots\ldots\ldots\ldots\ldots (4)$

It is seen from Eq. (4) that $\frac{dS^*}{dt} = -\frac{dD^*}{dt}$, only when $L^* = O$.

For all negative values of $\frac{dD^*}{dt}$ i.e. where D^* decreases with time,

$$\left|\frac{dS^*}{dt}\right| > \left|\frac{dD^*}{dt}\right|$$

If it is assumed as a rough approximation that D^* decreases linearly with time in the "chase" experiment (Fig. 3, Matsubara et al.) i.e. $D^* = D_o^* - k't$, then the equation for S^* can be written as:

$$S^* = S_o^* + \frac{\alpha}{D}(D_o^* t - 1/2\, k't^2) \quad \ldots\ldots\ldots\ldots\ldots (5)$$

Eq. (5) represents a saturation type curve for all positive values of D^* resembling the experimental curve for S^*. Calculations based on $\frac{\alpha}{D} = 0.88\ min^{-1}$ show considerable difference between the theoretical and experimental values of S^*. However, since the calculated values of S^* tend to be higher than the experimental values, this deviation does not affect the arguments presented above. On the contrary, it adds to the contention that the amount of radioactivity in double DNA is more than necessary to account for the observed increase in S^*.

In summary, the main objective of this paper is to emphasize that the kinetic data reported by Matsubara et al. do not necessarily exclude the possible precursor role of double stranded ed DNA in the synthesis of single stranded ϕx174 DNA.

The opinion expressed in this paper is my own and not that of the McGill-Montreal General Hospital Research Institute.

REFERENCES

Matsubara, K., Takai, M. and Takagi, Y., Biochem. Biophys. Res. Comm. 11, 372 (1963)

Sinsheimer, R. L., Starman, B., Nagler, C. and Guthrie, S., J. Mol. Biol., 4, 142 (1962)