THE INDUCTION OF DEOXYCYTIDYLIC ACID KINASE ACTIVITY BY A DINUCLEOTIDE FRACTION DERIVED FROM AN ENZYMATIC DNA DIGEST IN PNEUMOCOCCI[1]

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During a study concerning the effects of certain DNA degradation products on multiplication and virulence of pneumococci, it was found that such products selectively stimulated DNA synthesis over protein and RNA syntheses in virulent pneumococcal suspensions, but had no effects in avirulent suspensions (Firshein, 1961). The DNA products causing these effects consisted of a deoxyribonuclease treated DNA plus all 8 of the naturally occurring deoxynucleosides and deoxynucleotides. An examination of the role of each major moiety of the supplement revealed that the DNA digest had little effect by itself in stimulating DNA synthesis of virulent pneumococci, but became highly active in the presence of the deoxynucleosides and deoxynucleotides. In addition, the magnitude of enhancement was far out of proportion to its concentration in the supplement which was only 8 per cent. All 4 deoxynucleosides were required for optimum effects in the presence of the other parts of the supplement, whereas only 2 of the deoxynucleotides (deoxyadenylic and deoxyguanylic acids) exhibited stimulatory activity. Ion exchange chromatography of the DNA digest on ECTEOLA cellulose (Bendich et al, 1955) produced 2 major fractions, a small molecular weight fraction consisting primarily of dinucleotides, and a higher molecular weight fraction composed mainly of tri, penta,

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and octanucleotides. The small molecular weight fraction contained all of the stimulatory activity.

Several investigators have reported either on the ability of deoxynucleosides to "trigger" DNA synthesis after a critical intracellular concentration was reached (Maaloe, 1960; Hotta and Stern, 196: on an increase in deoxynucleotide kinase activity in the presence of deoxynucleosides (Hotta and Stern, 1961), or on the increase of a specific deoxynucleotide kinase (thymidylic acid kinase) in regenerati livers, but not in normal livers (Smellie, 1961). In view of these investigations and the possibility that the DNA products used in the pneumococcal studies also "triggered" DNA synthesis by increasing deoxynucleotide kinase activity, experiments were initiated to ascertai whether this increase occurred in virulent pneumococcal suspensions augmented with various DNA degradation products.

Methods and materials: Type III pneumococci were grown for 24 hr at 37 C in 9 liters of a Casitone-Tryptone (Difco)-albumin-yeast extract medium supplemented with glucose and phosphate (Marmur and Hotchkiss, 1955). Additional amounts of glucose and phosphate were added and after 2 hr incubation, the cells were centrifuged, washed once with 0.02 M phosphate buffer, pH 7.5, and suspended in tubes containing 3.0 ml of the same buffer containing glucose (1.0%), casitone (1.0%), catalase (Nutritional Biochemicals, 0.0025%), and the various DNA products to be added. The number of viable cells in each tube was approximately $2\text{-}3 \times 10^9$ per ml. Replicate tubes were prepared (at least 15 for each variable) and they were shaken vigorously in a New Brunswick gyrotory water bath shaker at 37 C for 25 min, the time period just prior to the onset of excess DNA synthesis (Firshein, 1961). After incubation, the tubes were placed in ice and the cells centrufuged after replicate tubes were pooled. They were washed once in 0.01 M K-phosphate buffer, pH 7.4, suspended in minimal amounts of the same buffer, and disrupted

in a Servall Omni Mixer by the method of Canellakis et al (1960). The debris was removed by centrifugation, and the supernatant clarified by several centrifugations at 40,000 x G for 30 min. These clear supernatants represented the source of the deoxynucleotide kinases and they were stored at - 20 C until use.

Assay of kinase activity was carried out as described by Lehman et al (1958) using C^{14} labelled deoxynucleotides as substrates. The one modification consisted of chromatographing the assay mixture after incubation, and after adding an equal volume of 70 per cent methar in a solvent consisting of isobutyric acid, 1N NH_4OH, and 0.2 M Na-versinate (50:30:0.5). The spots were detected by ultraviolet light. strips cut out, and levels of radioactivity determined by a Vanguard automatic radioactive chromatogram scanner. Specific activity was defined as the product:substrate ratio per mg protein. Protein was assayed by the method of Lowry et al (1951).

Deoxynucleosides and deoxynucleotides were purchased from the California Foundation, C^{14} deoxynucleotides were obtained from Schwarz Bioresearch, 1X recrystallized pancreatic deoxyribonuclease was purchas from Worthington Biochemicals. Highly polymerized Na-DNA was prepared by the method of Kay et al (1952), the DNA digest was prepared as described previously (Firshein, 1961), and the dinucleotide fraction wa obtained by ion-exchange column chromatography of the DNA digest on E6TEOLA cellulose (Bendich et al, 1955).

Results: Table 1 presents the data obtained after assaying the specific activity of all 4 deoxynucleotide kinases from virulent cells exposed to various moieties of the DNA-product-supplement, and from control cells. There was no difference in specific activity between controls and various supplements when the kinases of thymidylic acid, deoxyguanylic acid, or deoxyadenylic acid were examined. However, there was a significant difference between the various augmentations when deoxycytidylic acid kinase was assayed, and the striking obser-

Table 1

Deoxynucleotide kinase activity in extracts from virulent cells exposed to various DNA degradation products

Additions*			Enzyme	Specific Activity**
NS+DNT	DIN	SP. SUB.		
+	+	-	Deoxycytidylic acid Kinase	0.5
-	+	-		0.6
+	-	-		0.06
-	-	+		0.028
-	-	-		0.03
+	+	-	Thymidylic acid Kinase	0.09
-	+	-		0.08
+	-	-		0.06
-	-	+		0.08
-	-	-		0.07
+	+	-	Deoxyadenylic acid Kinase	1.4
-	+	-		1.1
+	-	-		1.4
-	-	+		1.0
-	-	-		1.2
+	+	-	Deoxyguanylic acid Kinase	1.2
-	+	-		1.5
+	-	-		1.1
-	-	+		1.1
-	-	-		1.3

Cells were exposed to the various compounds abbreviated above for 25 min and kinase activity was carried out as described in the "methods" DNS+DNT = deoxynucleosides + deoxynucleotides; DIN = dinucleotide fraction; Sp. Sub. = specific substrate of the particular kinase assayed.

Specific activity is defined as the product to substrate ratio per mg protein.

vation was that only in those extracts derived from virulent cells exposed to the dinucleotide fraction, was an enhancement of activity noted. Extracts taken from virulent cells exposed only to deoxycytidyli acid were inactive. Activities of the purine kinases were much higher than those of the pyrimidine kinases in all extracts, except where deoxycytidylic acid kinase activity was increased in those extracts which had been taken from cells exposed to the dinucleotide fraction. However, extracts taken from "dinucleotideless" cells exhibited very little deoxycytidylic acid kinase activity even in comparison to thymidylic acid kinase whose activity ranged in general from 2 to 3 times higher. Figure 1 shows the increase in total enzyme activity of deoxycytidylic acid kinase in extracts derived from cells exposed to the DNA products as a function of cell density (mg protein per ml). Again, extracts which had been taken from dinucleotide augmented cells exhibited much greater increases in total enzyme activity than supplements lacking this fraction.

Discussion: The above experiments demonstrate that fragments of DNA produced by deoxyribonuclease action can, at least in virulent pneumococci, greatly stimulate the activity of one of the 4 kinases required in the synthesis of immediate DNA precursors, the deoxynucleoside-triphosphates. However, it is not yet known why the increase occurs only in deoxycytidylic acid kinase rather than in both pyrimidine kinases, since initially, thymidylic acid kinase activity is also low in comparison to the purine kinases, and the enhancement of deoxycytidylic acid kinase activity brought about in dinucleotide-augmented cells leaves thymidylic acid kinase activity with the lowest level (10 to 20 times lower) of all the kinases in those cell suspensions which exhibit a striking increase in DNA synthesis. It would be expected that the disproportionate rate of activity existing between thymidylic acid kinase and the remaining 3 kinases would result in a limitation of the supply of one DNA precursor, thymidine-tri-phosphate. Nevertheless

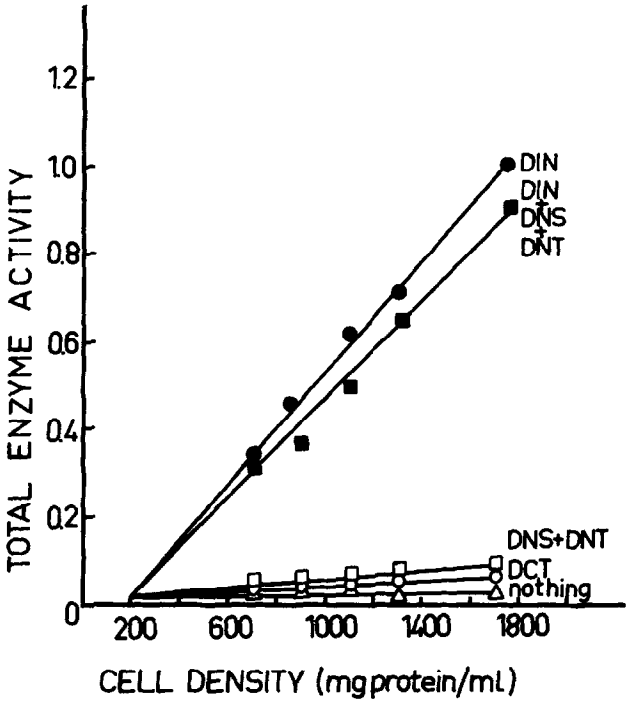

Figure 1. Total activity of deoxycytidylic acid kinase in various extracts derived from DNA-product-augmented cells as a function of cell density.
DIN = dinucleotide fraction; DNS+DNT = deoxynucleoside + deoxynucleotide mixture; DCT = deoxycytidylic acid. Extracts were taken from cells incubated for 25 min at 37 C.

the fact that excess DNA synthesis does occur under this supposedly imbalanced condition indicates that the DNA polymerase of virulent pneumococci may have a very high affinity for thymidine-tri-phosphate and possibly, a much lower one for deoxycytidine-tri-phosphate. Experiments are now in progress to ascertain whether the Michaelis Constants (Km) for the various tri-phosphates differ when the DNA polymerase is added.

The present finding that deoxycytidylic acid kinase activity can be affected, together with the previous reports of other investigators

that thymidylic acid kinase activity was alterable, suggests that pyrimidine derivatives in general may be more sensitive to control than purine compounds. This is supported by the studies of Maley and Maley (1962) who reported that thymidine-tri-phosphate could prevent the deamination of deoxycytidylic acid by a deaminase and hence control whether the deoxynucleotide would be available for conversion.

Whether the increase in deoxycytidylic acid kinase activity represents a classical case of induction is not known at the present time since 1) only the activity of the kinase may be stimulated, rather than the actual synthesis of the enzyme, and 2) the substrate, deoxycytidylic acid, did not cause the increase in activity, but a dinucleotide fraction containing among other dinucleotides, those composed of deoxycytidylic acid, was the active inducer. Work is now in progress to isolate the actual component responsible for the stimulation.

A paucity of observations exists concerning the biological role of fragments derived from DNA digests in cellular metabolism despite the presence of such fragments in every living cell during some stage of cellular growth. Butros (1959) reported on the stimulation of cleavage in Arbacia eggs by specific DNA digests derived from such eggs but the fragments were not identified. More recently, Stone and Burton (1962) found that deoxyribonucleases which were known to increase in phage infected Escherichia coli cells exhibited their greatest increase at about the same time as thymidylic acid was phosphorylated, although no implications were drawn from this observation.

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