ISOLATION AND PURIFICATION OF A SUBSTANCE INDUCING COMPETENCE AND INACTIVATING TRANSFORMING DNA IN PNEUMOCOCCUS

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Received December 16, 1967

Pakula found (1962, 1963) that transformable streptococci produce an extracellular factor, probably of enzyme nature, inducing competence in non-competent cells. A similar factor was isolated by Tomasz and Hotchkiss (1964) from the cell surface of competent cells of Pneumococcus and was also found in competent cells of Bacillus subtilis (Charpak and Dedonder, 1965; Akrigg et al., 1967) and Bacillus cereus (Felkner and Wyss, 1964). Recently Barnhart reported competence-stimulating activity in sterile filtrates from Hemophilus influenzae. The competence factor was not yet sufficiently purified to permit detailed chemical identification. A filtrate of the culture of the transformable strain of Pneumococcus contains in addition to the factor inducing competence in non-competent cells (Kohoutová and Málek, 1966) also a substance inactivating transforming DNA (Ottolenghi and Hotchkiss, 1962; Kohoutová, 1965 and 1967 a, b; Kohoutová and Málek, 1966; Kohoutová et al., 1967). As the two activities seem to be closely related with genetic transformability, we followed both of them in the filtrate of a culture of a highly transformable strain of Diplococcus pneumoniae R6bd (kindly supplied by Prof. R.D.Hotchkiss) during growth and occurrence of the competence. The substance showing the above-mentioned properties was later isolated from the filtrate, purified and analyzed in more detail.

Samples were taken at regular intervals during growth of a culture of the R6bd strain and sterilized by filtration through membrane filters. The competence of the culture was expressed as the number of streptomycin-resistant transformants per 1 ml and the competence-inducing and DNA-inactivating activities were assayed in the filtrate using the methods described at Fig. 1. Residual transforming activity is expressed as the ratio of the transforming activity of DNA treated with the filtrate to the non-treated one.

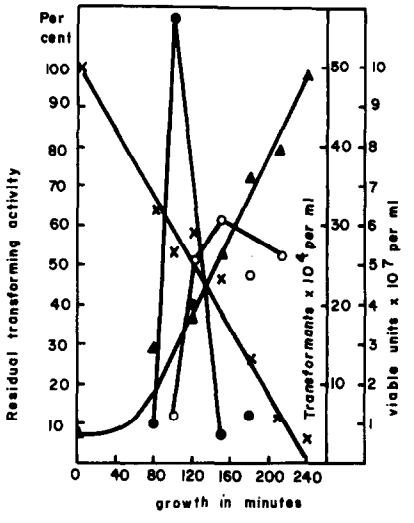

Fig.1. The substance inducing competence and inactivating transforming DNA in a filtrate of the Pneumococcus R6bd culture during growth and occurrence of competence. Assay of the competence-inducing activity: 1 ml of a 50 min old non-competent culture concentrated twice after centrifugation and resuspension into fresh transformation medium (2 - 3 x 10^7 viable cells per ml) was exposed to 0.2 ml of the filtrate for 20 min at 37°C. 1 µg of DNA was then added and after 15 min further uptake of DNA was stopped by adding DNase. Assay of the DNA - inactivating activity: 0.1 ml of the filtrates were preincubated with 0.1 ml of DNA in the region of linear response for 30 min at 37°C and then transferred to ice. Parallel samples were kept for 30 min in ice. One ml of a highly competent culture was then added and after 15 min at 37°C the DNase was added. After proper dilution of the samples on blood agar plates bacteria were grown for further 2 hr to allow phenotypic expression of the acquired streptomycin resistance character and the plates were then overlayered with agar containing 150 µg streptomycin per ml for scoring of transformants.

Δ—Δ viable units; ●——● competence of the culture; o—o competence-inducing activity; x——x DNA inactivating activity expressed as residual transforming activity.

It follows from Fig. 1 that the peak of competence occurs after 100 min of growth of the Pneumococcus culture. The maximum of the competence-inducing activity is observed 50 min later. However, even after 240 min, when the recipient cells have lost completely their competence the filtrate still exhibits a high competence-inducing activity (Kohoutová and Málek, 1966; Pakula, 1963; Barnhart, 1967). Transforming DNA is inactivated by the filtrate

collected at the peak of competence to 50% (Kohoutová, 1967), whereas the filtrate taken after 240 min decreases biological activity of the transforming DNA by 95%.

The substance inducing competence in non-competent cells was isolated from the filtrate of the Pneumococcus culture 50 min after the peak of competence and partially purified. One volume of the filtrate was precipitated with 8 volumes of precooled (-20°C) ethanol. The precipitate was dissolved in physiological saline buffered with 0.01 M K_2HPO_4 to give pH 7,6 and a portion further purified by chromatography on Sephadex G 100 or G 50. All isolation and purification procedures were carried out at 0°C.

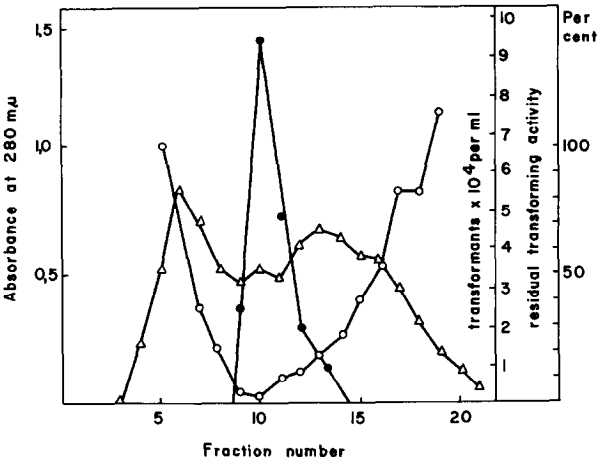

Fig. 2. The isolation of the substance inducing competence and inactivating transforming DNA on Sephadex G 100. Proteins were assayed by measuring absorption at 280 mu. The competence-inducing and DNA-inactivating activities were assayed as described at Fig. 1. Individual fractions were always used instead of the filtrate. The eluate 0.05 M Tris + 0.01 M KCl pH 7,6 was used in the control instead of the transformation medium.

Δ—Δ absorbance at 280 mμ; ●—● competence-inducing activity; o—o DNA-inactivating activity.

It can be seen in Fig. 2 that the competence-inducing substance is eluted as a sharp peak. It is of interest that fractions containing the competence-inducing substance contained in addition also the substance inactivating transforming DNA. Both activities were eluted as a single peak even during chromatography on Sephadex G 50.

The relationships between the induction of competence and time of contact of the active fraction with non-competent cells and concentration of the active fraction were also studied. It follows from Fig. 3A that optimum induction of competence occurs after about 30 min of contact. Similar results were obtained with Streptococcus (Pakula and Walczak, 1963; Dobrzański and Osowiecki, 1966).

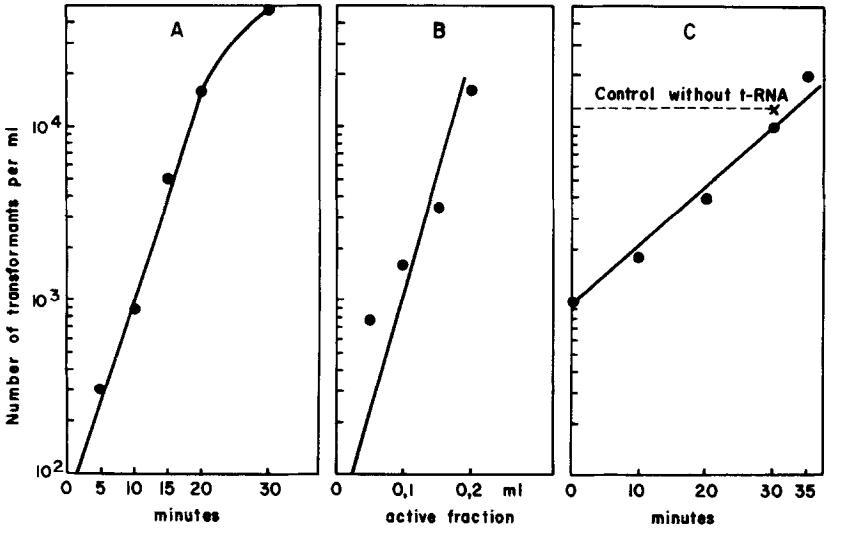

Fig. 3. Kinetics of the effect of the competence-inducing substance as a function of time (A) and concentration of the active fraction (B) on the induction of competence. Kinetics of the inhibition of induction of competence by t-RNA (50 μg) as a function of time (C).

Fig. 3B shows that there exists a linear relationship between concentration of the active fraction and induction of competence in non-competent cells after 20 min of contact. Fig. 3C shows that the most striking inhibition of the induction of competence (93%) occurs on adding t-RNA to non-competent cells at zero time, immediately before addition of the active fraction. The inhibitory effect of t-RNA decreases with the time of its addition to the complex of non-competent cells and active fraction. The addition of t-RNA to this complex after 30 min of contact results in only 18% inhibition of the induction of competence. However, in the presence of the active fraction t-RNA does not

inhibit but rather stimulates the uptake of DNA by cells that have already acquired competence.

Fig. 4A shows the inactivation of DNA in the region of linear response (2×10^{-3} µg) resulting from 30 min preincubation with a fraction containing the active competence-inducing substance. The relationship between the inactivation of DNA and time of preincubation of DNA with the fraction is linear. Fig. 4B shows the effect of various concentrations of the active

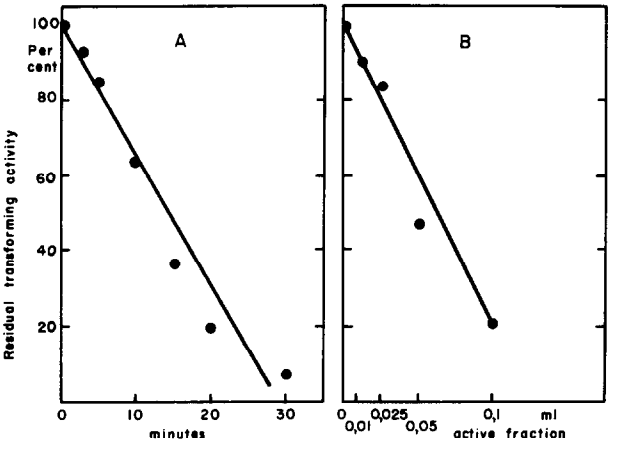

Fig. 4. Kinetics of the effect of the active fraction as a function of time (A) and concentration of the fraction (B) on inactivation of transforming DNA.

fraction on the inactivation of transforming DNA at a concentration in the region of linear response (6×10^{-4} µg). The inactivation is a linear function of the concentration of the fraction.

The inducing activity of the substance eluted from the Sephadex column is relatively thermoresistant in agreement with the data described by Pakula (1962) and Dobrzański and Osowiecki (1966) in Streptococcus and at variance with the results of Tomasz and Mosser (1966) who found that the competence factor isolated from the cell surface lost its activity completely when heated for 20 min to 100°C. The active fraction isolated here retained 70%, 45% and 30 - 40% of its initial activity when heated for 30 min at 60°C, 80°C

and 100°C, respectively. The DNA-inactivating activity was affected in a similar way.

Incubation of the fraction containing the active substance with proteolytic enzymes (trypsin, chymotrypsin and pronase at a concentration of 10 μg per assay volume) for 10 min at 37°C resulted in a complete inactivation of the substance. It was found in control experiments that these concentrations of proteases did not change the viability of the recipient cells. A similar sensitivity of the active substance isolated from competent cells of Pneumococcus to the treatment with proteolytic enzymes was described by Tomasz and Mosser (1966).

It may be concluded on the basis of our results that fractions exhibiting the competence-inducing activity always contained also the DNA-inactivating activity. Both activities are destroyed by proteolytic enzymes, are relatively thermoresistant and are inhibited by t-RNA (Kohoutová, 1967b). Further experiments are under way to establish whether a single substance or whether two separate substances with identical molecular weights are responsible for the two activities, the competence-inducing and transforming DNA inactivating activity. They should also clarify the question whether the DNA-inactivating activity takes part in the mechanism responsible for competence.

REFERENCES

Akrigg,A., Atkinson,A., Ayad,S.R. and Barker,G.R., Biochem.J., 104, 28P,(1967).
Barnhart,B.J., Biochim.Biophys.Acta, 142, 465 (1967).
Charpak,M. and Dedonder,R., Compt.Rend., 260, 5638 (1965).
Dobrzański,W.T. and Osowiecki,H., Bull.Acad.Polon.Sci.,(VI), 14, 765 (1966).
Felkner,I.C. and Wyss,O., Biochim.Biophys.Res.Commun., 16, 94 (1964).
Kohoutová,M., J.Gen.Microbiol., 38, 211 (1965).
Kohoutová,M. and Málek,I., Proc.Symp.Mutational Process. The Physiology of Gene and Mutation Expression, Academia-Prague, p.195 (1966).
Kohoutová,M., Fol.Microbiol., 12, 311 (1967a).
Kohoutová,M., Fol.Microbiol., 12, 316 (1967b).
Kohoutová,M., Braná,H. and Kopecká,H., Fol.Microbiol., 12, 385 (1967).
Ottolenghi,E. and Hotchkiss,R.D., J.Exp.Med., 116, 491 (1962).
Pakula,R., Piechowska,M., Bańkowska,E. and Walczak,W., Acta Microbiol.Polon., 11, 205 (1962).
Pakula,R. and Walczak,W., J.Gen.Microbiol., 31, 125 (1963).
Pakula,R., J.Bacteriol., 90, 1320 (1965).
Tomasz,A and Hotchkiss,R.D., Proc. Natl.Acad.Sci., 51, 480 (1964).
Tomasz,A and Mosser,J.L., Proc.Natl.Acad.Sci., 55, 58 (1966).