AN N-ACETYLATED PEPTIDE CHAIN IN TROPOMYOSIN.

R. E. Alving, E. Moczar and K. Laki

National Institute of Arthritis and Metabolic Diseases
National Institutes of Health, Public Health Service
U. S. Department of Health, Education, and Welfare
Bethesda, Maryland 20014

Received April 15, 1966

It has been reported from this laboratory (Saad and Kominz, 1961) that by the application of Sanger's DNFB method, glutamic acid could be identified as the N-terminal residue of rabbit's tropomyosin B.

Our experiments, using carboxypeptidase A suggested that tropomyosin B contained isoleucine and serine as C-terminal residues (Kominz et al., 1957). This indicated that tropomyosin consisted of two peptide chains. Recently, (Woods 1965) reported that tropomyosin B in 8 molar urea solution containing β-mercapto ethanol (to reduce S-S bonds) exhibited a molecular weight about half of that of the native molecule. This finding also suggested that tropomyosin contained two chains. But if tropomyosin consists of two chains, one of the chains must contain an N-terminal residue non-reactive to dinitrofluorobenzene.

In this communication, we report our findings which show that rabbit tropomyosin B has an N-acetylated peptide chain. After pronase digestion of tropomyosin, a tripeptide was isolated which contained acetic acid, aspartic acid, alanine and methionine in equal quantities. This finding gives further

support to the idea that tropomyosin contains two peptide chains; one starting with glutamic acid, the other one with an acetylated residue.

For these experiments, tropomyosin of high purity was prepared according to Bailey (1948) from rabbit skeletal muscle and stored in the lyophilized state. The digestion by pronase was carried out the following way: the digestion mixture, adjusted to pH 8.0 and kept at 25°C for 48 hours, contained 1 mg tropomyosin per ml and pronase in a 60 to 1 ratio (by weight). After digestion, the mixture was passed through Dowex 50 column and the effluent, containing the acetylated peptides was rechromatographed on Dowex 1 (X-8) column in the formate cycle, using a formic acid gradient. Two ml samples were collected. The main peak came out when the formic acid concentration reached 1.3 N. Acid hydrolysis of the peak yielded roughly equal amounts (Table I) of acetic acid, aspartic acid, alanine and methionine (in the form of 90% methionine sulfoxide and 10% methionine). Acetic acid was determined by gas chromatography using a sebacic acid column (Jackson, 1964). The amino acids were determined by a Spinco automatic amino acid analyzer. Hydrazinolysis of the acetylated peptide yielded

Table I.

Components of the acetylated tripeptide from tropomyosin.

Residues	μ moles per μ mole of tropomyosin digested.
Aspartic acid	0.66
Alanine	0.61
Methionine	0.55
Acetic acid	0.71

alanine, showing that this amino acid is the C-terminal residue of this tripeptide. The actual sequence of the other two amino acids remains to be established.

With the finding reported here, all three muscle proteins, actin (Alving and Laki, 1966, Gaetjens and Barany, 1966), myosin (Offer, 1965) and tropomyosin now belong to the growing class of proteins that have an acetylated N-terminus.

REFERENCES

Alving, R. E. and Laki, K., Fed. Proc., 25, 223 (1966).
Bailey, K., Biochem. J., 43, 271 (1948).
Gaetjens, E. and Barany, M., Fed. Proc. 25, 223 (1966).
Jackson, R. B., J. Chromatography, 16, 306 (1964).
Kominz, D. R., Saad, F., Gladner, J. A. and Laki, K., Arch. Biochem. Biophys., 70, 16 (1957).
Offer, G. W., Biochim. Biophys. Acta, 111, 191 (1965).
Saad, F. M. and Kominz, D. R., Arch. Biochem. Biophys., 92 541 (1961).
Woods, E. F., Nature, 207, 82 (1965).

INTERSPECIES TRANSFORMATION BETWEEN BACILLUS SUBTILIS AND BACILLUS LICHENIFORMIS

Ivan D. Goldberg, Darrel D. Gwinn and Curtis B. Thorne

U. S. Army Biological Center, Fort Detrick, Frederick, Maryland

Received April 15, 1966

Dubnau et al. (1965) reported transformation between B. subtilis and B. licheniformis. Using DNA isolated from a streptomycin-resistant strain of B. licheniformis, they were able to transform a sensitive strain of B. subtilis to streptomycin resistance at a low frequency. We have confirmed this work with a system that gives a much higher percent transformation and we have also been able to perform the reciprocal transformation. A comparison of the reciprocal crosses indicates at least a 100-fold difference in efficiency of transformation, depending on the direction of the cross. With B. licheniformis as the recipient and B. subtilis as the source of heterologous DNA, the number of interspecific transformants was 10^{-4} times the number of intraspecific transformants obtained with homologous DNA. When B. licheniformis DNA was used to transform B. subtilis the number of interspecific transformants was only 10^{-1} to 10^{-2} times the number of intraspecific transformants.

MATERIALS AND METHODS

B. subtilis W168 was isolated as a spontaneous revertant of B. subtilis 168 ind$^-$. B. licheniformis 9945A M18 arg$^-$ and M17 ad$^-$ were obtained from C. G. Leonard. B. subtilis SB-1 his$^-$, ind$^-$ was

obtained from E. W. Nester. Mutants resistant to 1 mg/ml streptomycin sulfate were isolated following exposure of spores to U.V. DNA was extracted according to the procedure of Marmur (1961). Competent cells of B. licheniformis 9945A M18 arg$^-$ and M17 ad$^-$ were prepared by the procedure of Leonard and Mattheis (1965). B. subtilis 168 ind$^-$ and SB-1 ind$^-$, his$^-$ were transformed by essentially the procedures described by Anagnostopoulos and Spizizen (1961). Transformants for auxotrophic markers were scored on minimal 10 agar (Thorne and Stull (1966)). Transformants for the streptomycin marker were scored in the following way. After DNA treatment the samples were filtered onto membrane filters (Millipore HA 0.45 μ). The membranes were then transferred to plates containing streptomycin-free growth media + 0.75% agar. The plates were incubated at 37 C for a suitable time to allow the streptomycin marker to become expressed and the membranes were then transferred to plates containing penassay agar (Difco Antibiotic Medium #3 + 0.75% agar) + 1 mg/ml streptomycin sulfate. Transformants were scored after 48 hours' incubation at 37 C. When B. licheniformis was used as recipient the expression was done on penassay agar. With B. subtilis, penassay agar could not be used. If B. subtilis streptomycin transformants were allowed to express for more than 2 hours on this medium prior to transfer to penassay agar + streptomycin, the colonies never attained normal size on the streptomycin-containing plates. Thus it was difficult to differentiate between true transformants and colonies that had started to grow on the expression plates but were killed after exposure to the antibiotic. This problem was overcome by using minimal 10 agar supplemented with 40 micrograms/ml of the required amino acid for expression of B. subtilis transformed for the streptomycin marker. With B. subtilis SB-1, the minimal 10 was supplemented with both tryptophan and histidine at a concentration

of 40 micrograms/ml. We feel that our difficulty with penassay agar was due to the extremely fast growth of B. subtilis on this medium, resulting in a heavy background of non-transformed cells that inhibited the growth of the transformants.

RESULTS

In order to obtain a meaningful measurement of the numbers of transformants for the streptomycin marker, it was first necessary to determine the time required for the expression of this marker. Under the conditions used, both B. licheniformis and B. subtilis became fully expressed after 6 hr of growth in the absence of streptomycin (fig. 1). All subsequent transformation experiments were done under conditions that gave maximum expression. Table 1 indicates the results of interspecific transformation between B. subtilis and B. licheniformis and intraspecific transformation using homologous DNA. As was shown by Dubnau et al. (1965) the interspecific transformation is significantly lower than the intraspecific transformation. This is a feature common to all the interspecific transformation systems reported to date. However, the difference in the number of transformants in the interspecific B. subtilis ⟶ B. licheniformis cross and the number obtained in the intraspecific B. licheniformis ⟶ B. licheniformis cross was 10 thousand-fold. On the other hand, in the reciprocal cross, the number of B. licheniformis ⟶ B. subtilis transformants was only 10^{-1} to 10^{-2} times the number obtained in the B. subtilis ⟶ B. subtilis cross. It should be noted that saturating levels of DNA were used in all cases. None of the auxotrophic markers tested could be transferred between the 2 species. This is in agreement with Dubnau et al. (1965), who found that only certain markers, among them streptomycin resistance, could be transferred between various species of Bacillus.

DISCUSSION

We have demonstrated interspecific transformation in both directions between 2 species with a difference in GC content calculated from T_m (Gwinn, Goldberg and Thorne, unpublished results) of 3 to 4%. Chen and Ravin (1965) compared the efficiencies of transformation obtained in reciprocal crosses between pneumo-

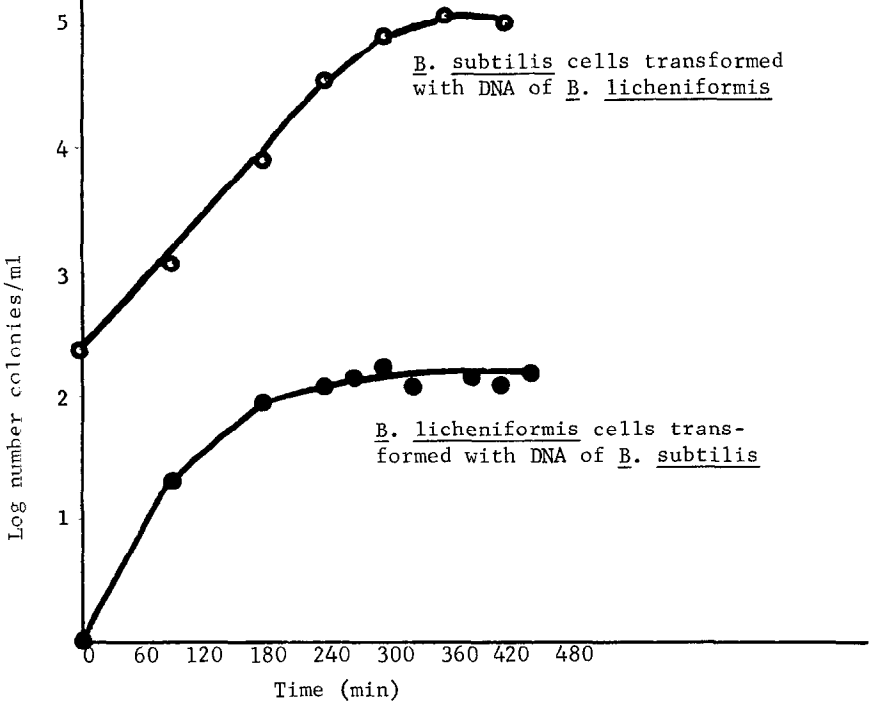
TABLE 1. Interspecific transformation between *B. subtilis* and *B. licheniformis*.

DNA source	Recipients: *B. licheniformis* 9945A M-18 (*arg*⁻) (1.7 x 10^{8}*)		*B. licheniformis* 9945A M-17 (*ad*⁻) (2.6 x 10^{8})	
	Transformants per ml		Transformants per ml	
	S^r	*arg*⁺	S^r	*ad*⁺
B. subtilis W-168 S^r	1.1 x 10^{2}	< 10	4.8 x 10^{1}	< 10
B. licheniformis 9945A S^r	1.2 x 10^{6}	2.0 x 10^{6}	6.5 x 10^{5}	3.2 x 10^{6}

DNA source	*B. subtilis* 168 (*ind*⁻) (2.9 x 10^{8})		*B. subtilis* SB-1 (*his*⁻, *ind*⁻) (2.0 x 10^{8})		
	Transformants per ml		Transformants per ml		
	S^r	*ind*⁺	S^r	*his*⁺	*ind*⁺
B. subtilis W-168 S^r	2.9 x 10^{6}	2.3 x 10^{6}	2.4 x 10^{6}	1.2 x 10^{6}	8.0 x 10^{5}
B. licheniformis 9945A S^r	1.7 x 10^{5}	< 10	2.3 x 10^{4}	< 10	< 10

* Numbers in parentheses are numbers of recipient cells/ml. All the other numbers in the table refer to numbers of transformants per ml. The concentration of *B. subtilis* DNA was 22 μg/ml; that of *B. licheniformis* DNA was 22.5 μg/ml. Both levels were saturating for intraspecific and interspecific transformations. Cells transformed to streptomycin resistance (S^r) were scored on penassay agar containing 1 mg/ml streptomycin sulfate. Cells transformed to prototrophy were scored on minimal 10 agar. With *B. subtilis* SB-1, transformants for the histidine marker were scored on minimal 10 + 40 μg/ml L-tryptophan and transformants for the indole marker were scored on minimal 10 + 40 μg/ml L-histidine. Untreated cells and cells treated with DNA, that had been preincubated with 100 μg/ml deoxyribonuclease (Worthington) were run as controls in all experiments.

FIGURE 1. Time required for expression of streptomycin resistance by B. subtilis and B. licheniformis interspecific transformants.



Millipore filters were incubated on streptomycin-free media for the times indicated and they were then transferred to plates of penassay agar containing 1 mg of streptomycin sulfate per ml. Complete procedure as in text.

coccus and the Challis strain of Streptococcus for the streptomycin resistance marker. They found the pneumococcus ⟶ Streptococcus transformation to be much more efficient than the reciprocal cross. They suggested that the pneumococcal DNA was more easily integrated into the genome of Streptococcus than was the streptococcal DNA into the pneumococcal genome. We have not eliminated the unlikely possibility in our system that B. subtilis can take up B.

licheniformis DNA more efficiently than B. licheniformis can take up B. subtilis DNA. It is also possible that B. licheniformis has a more efficient restriction mechanism (Arber and Dussoix, 1962; Boyer, 1964) than B. subtilis. Doi and Igarashi (1965) suggested on the basis of interspecies DNA-RNA hybridization studies that a small number of identical nucleotide sequences are conserved among the members of the genus Bacillus. Dubnau et al. (1965) reported interspecific nucleic acid hybridization as well as interspecific transformation among several species of Bacillus. These authors suggested that there is in the genus Bacillus a conserved core of stable genetic material that is relatively resistant to evolutionary change. This core would include the marker that determines streptomycin resistance. The fact that the efficiency of interspecific transformation is lower than the efficiency of intraspecific transformation would seem, at first glance, to argue against the core hypothesis. Presumably the same sequences of nucleotides are involved in both recombinations. However, the nucleotides adjacent to those determining a given marker might affect the frequency of transformation of that marker (Shaeffer, 1958), thus lowering the interspecific efficiency.

We wish to acknowledge the able technical assistance of T. Bryan and R. Gregoire.

REFEERENCES

Anagnostopoulos, C. and J. Spizizen, J. Bacteriol. 81, 741 (1961).
Arber, W. and D. Dussoix, J. Mol. Biol. 5, 18 (1962).
Boyer, H., J. Bacteriol. 88, 1652 (1964).
Chen, Kuo-Chun, and A. W. Ravin, Genetics 52, 434 (1965).
Doi, R. H. and R. T. Igarashi, J. Bacteriol. 90, 384 (1965).
Dubnau, D., I. Smith, P. Morell and J. Marmur, Proc. Natl. Acad. Sci. U.S. 54, 491 (1965).
Leonard, C. G. and M. J. Mattheis, J. Bacteriol. 90, 558 (1965).
Marmur, J., J. Mol. Biol. 3, 208 (1961).
Shaeffer, P., Symp. Soc. Exptl. Biol. 12, 60 (1958).
Thorne, C. B. and H. B. Stull, J. Bacteriol. 91, 1012 (1966).