IMPORTANCE OF MEMBRANE FLUIDITY IN THE INDUCTION OF ALKALINE PHOSPHATASE, A PERIPLASMIC ENZYME, IN ESCHERICHIA COLI

Kazuko Kimura and Katsura Izui

Department of Chemistry, Faculty of Science,
Kyoto University, Kyoto 606, Japan

Received April 6,1976

SUMMARY: An unsaturated fatty acid auxotroph was supplemented with either elaidate or oleate. After derepression of alkaline phosphatase by phosphate limitation at 38°C, the cells were shifted to incubation at various temperatures. Arrhenius plots of the rate of enzyme induction gave a steeper negative slope in the temperature range from 30°C to 35°C with elaidate-supplemented cells than with oleate-supplemented cells. At 25°C the induction was arrested in the former cells, while it was continued at a considerable rate in the latter. The arrest was released upon shift-back to 38°C, and precursors convertible to the active enzyme were not accumulated during incubation at 25°C. There was no marked difference in slope of Arrhenius plots of the rate of bulk protein synthesis between both types of cells, and the slope was almost equal to that of the rate of enzyme induction in the oleate-supplemented cells. The rate of β-galactosidase induction in the elaidate cells showed a similar temperature dependence to that of bulk protein synthesis.

In Escherichia coli, alkaline phosphatase [EC 3.1.3.1] is known to be located in the periplasmic space of the cell (1). It has been shown that monomers of the enzyme, which have no catalytic activity, are secreted into the periplasmic space, where they associate to form active dimers (2). However, it is not yet known if the monomers are synthesized at some special membrane-bound protein-synthesizing site similar to that found in higher organisms (3) or how the monomers are translocated across the cell membrane. It was therefore of interest to examine the effect of membrane fluidity (physical state of the membrane lipids) on the induction of alkaline phosphatase, an enzyme which is not a lipoprotein. It is now well established that the membrane fluidity influences not only the rate of transport of some low molecular substances (4,5), but also the effective assembly of the lactose transport system (6). Since the membrane fluidity depends mainly on the fatty acid composition of the membrane lipids as well as on the temperature, studies

were carried out with an unsaturated fatty acid auxotroph which had been grown with elaidate (trans-Δ^9-octadecenoate) or with oleate (cis-Δ^9-octadecenoate).

EXPERIMENTAL

Bacterial strain. Strain Ole-28E1 , an unsaturated fatty acid auxotroph of E. coli K12, was isolated as a spontaneous mutant from the parent strain Ole-28 (7). Strain Ole-28E1 can satisfy its unsaturated fatty acid requirment with elaidate, unlike its parent, which cannot utilize trans-unsaturated fatty acids. The fatty acid composition and the molecular species of phosphatidylethanolamine isolated from Ole-28E1 grown with elaidate were the same as those from Ole-28E (8) except for the complete absence of cis-unsaturated fatty acids. The doubling time of strain Ole-28E1 at 38°C with elaidate or oleate was 76 and 80 min, respectively, under the conditions employed. Elaidate did not support growth of Ole-28E1 at a temperature below 32°C, whereas oleate supported growth even at 20°C.

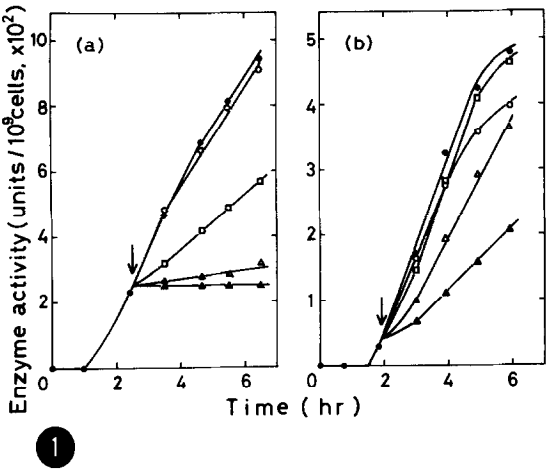
Culture media. "High-phosphate medium" consisted of medium E (9), 0.5% glycerol, 0.0075% each of the common amino acids (except no Cys, Asn, Gln, and Ser), 0.2% Triton X-100 and 0.005% elaidate or oleate. "Low-phosphate medium" consisted of 121 medium (10), 8 μM potassium phosphate and the same supplements used for high-phosphate medium.

Enzyme assays. Alkaline phosphatase and β-galactosidase were assayed by measuring the rate of hydrosis of p-nitrophenyl phosphate and of o-nitrophenyl-β-D-galactopyranoside, respectively (7). One unit of activity was defined as the activity which hydrolyzes 1 μmole of substrate per min at 30°C.

Protein-synthesis. The incorporation of amino acids into cell proteins was followed by supplementing cell cultures with [^{14}C]-L-leucine (0.33 Ci/mmole) at 0.0083 μCi/ml. At 5 minutes' intervals, 0.1-ml samples of the labeled culture were withdrawn and mixed with 1 ml of 5% trichloroacetic acid containing 0.01% unlabeled L-leucine. After the samples were heated at 90°C for 15 min, 0.2 ml of 10% trichloroacetic acid containing carrier cells (60 μg dry weight) were added. The cells were then collected by centrifugation, washed three times with 10% trichloroacetic acid, and the final pellets were dissolved in 0.5 ml of 85% formic acid. The radioactivities were determined in a liquid scintillation spectrometer using a toluene-Triton scintillator.

RESULTS AND DISCUSSION

Cells of Ole-28E1 were grown at 38°C in the high-phosphate medium supplemented with elaidate or oleate, and transferred to the low-phosphate medium at the same temperature for derepression of alkaline phosphatase (11). After allowing adequate time for the onset of derepression (about 2 hr), each culture was divided into five portions that were then incubated at various temperatures. As shown in Fig.1, the rate of appearance of enzyme activity was significantly affected by the temperature shift. The rate decreased much more as the incubation temperature was lowered with the elaidate-grown cells



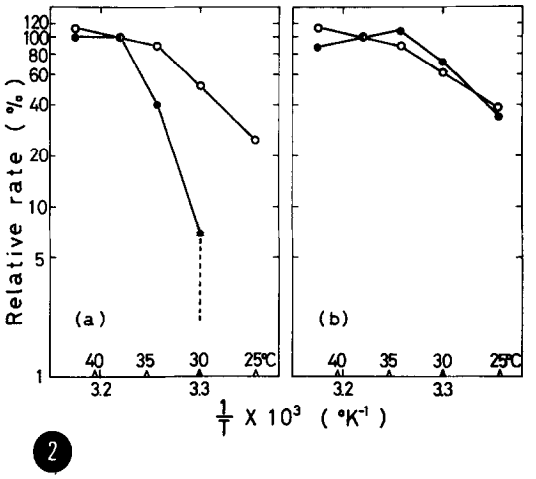


Fig. 1 Temperature dependence of alkaline phosphatase induction in strain Ole-28E1 supplemented with elaidate (a) or oleate (b). Cells were grown aerobically at 38°C to a late exponential phase (approximately 1.5×10^9 cells/ml) in 60 ml of high-phosphate medium containing elaidate or oleate. For induction of alkaline phosphatase, the cells were collected on a membrane filter and washed three times with low-phosphate medium containing elaidate or oleate. The washed cells were resuspended in the same medium to give 26 ml cultures containing about 2.3×10^9 cells/ml and were shaken at 38°C. At the times indicated by arrows, each culture was divided into 5 portions (5 ml each), and incubated with shaking at various temperatures. Samples (0.2 ml) were withdrawn at intervals and assayed for alkaline phosphatase activity (7). The cell density of each culture was followed by measuring optical density at 660 nm. It increased 2-fold for the elaidate-supplemented culture after 2 hr, and 3-fold for the oleate-supplemented culture after 3 hr at 38°C; no marked change was observed thereafter for either culture. Incubation temperature were 42°C (—o—), 38°C (—●—), 34°C (—□—), 30°C (—△—), and 25°C (—▲—).

Fig. 2 Arrhenius plot of relative rates of alkaline phosphatase induction and bulk protein synthesis in cells of Ole-28E1 supplemented with elaidate (a) or with oleate (b). The relative rates of alkaline phosphatase induction (—●—) and protein synthesis (—o—) at various temperatures are plotted against 1/T. To measure bulk protein synthesis, cells of Ole-28E1 were grown aerobically at 38°C in the same medium as used for induction of alkaline phosphatase in the experiments of Fig. 1, except for an increased concentration of potassium phosphate (1 mM) and for omission of L-leucine. At a late exponential growth phase (1.0×10^9 cells/ml), 0.1 μCi of [^{14}C]-L-leucine was added to each culture (12 ml), and the cultures were incubated at 38°C with shaking for 10 min in order to preload the cells with [^{14}C]-L-leucine (This preloading procedure was undertaken with the intention of avoiding possible limitation of the rate of [^{14}C]-L-leucine incorporation into protein fraction by the rate of its permeation into cells at lower temperatures.) Each culture was then divided into 5 portions (2 ml each) and was further shaken at various temperatures as indicated. Samples (0.1 ml) were withdrawn at 5 min intervals and processed as described in EXPERIMENTAL. The time course of incorporation of radioactivity into the protein fraction was plotted and the rate of bulk protein synthesis obtained from the slope of the curve. The rates of bulk protein synthesis at 38°C were 1680 and 1020 dpm/min /10^9cells for the elaidate and oleate cells, respectively.

than with the oleate-grown cells. It should be noted that there was no further increase of enzyme activity after shift to 25°C in the elaidate-grown cells, whereas enzyme activity continued to increase at a rate about 34% of that observed at 38°C in the oleate-grown cells.

To determine whether the characteristic responses to temperature are specific for induction of alkaline phosphatase, the temperature dependence of the rate of bulk protein synthesis was investigated with both elaidate- and oleate-grown cells. Protein synthesis was determined by [^{14}C]-L-leucine incorporation into the trichloroacetic acid-insoluble fraction. The results are shown in Fig.2, where the rate of protein synthesis at various temperatures divided by the rate at 38°C are plotted against 1/T (°K). The relative rates of alkaline phosphatase induction obtained above (Fig.1) are also plotted in this figure. There appeared to be little difference between the rates of bulk protein synthesis in the two types of cells at 38°C. In contrast to the result for alkaline phosphatase induction, protein synthesis was not arrested in the elaidate-grown cells upon temperature shift from 38°C to 25°C but continued at a rate about 25% of that observed at 38°C. Under the same conditions, the protein synthesis rate in the oleate-grown cells after the temperature shift was about 38% of that at 38°C. Activation energy, μ, were then calculated according to the Arrhenius equation for a temperature range between 30°C and 34°C. For the elaidate-grown cells, μ values were about 80 and 25 kcal for alkaline phosphatase induction and bulk protein synthesis, respectively. The respective values for the oleate-grown cells were 19 and 16 kcal. It was possible that the arrest of alkaline phosphatase induction in the elaidate-grown cells upon the temperature shift did not reflect an aberration of the secretion process but would occur for any inducible enzyme. To examine this possibility, the temperature dependence of the rate of induction of β-galactosidase, an intracellular enzyme, was investigated with the elaidate-grown cells. The enzyme synthesis was first derepressed at 38°C by the addition of 1 mM isopropyl-β-D-thiogalactoside, after which the derepressed

cells were shifted to incubation at various temperatures. The rates of β-galactosidase induction were obtained from slopes of curves plotted in the same manner as those in Fig.1. The rates relative to that at 38°C were 90, 56, 28, and 16% at 35, 30, 25, and 20°C, respectively. The data indicated that the temperature dependence of the rate of β-galactosidase induction was quite different from that of alkaline phosphatase induction but very similar to that of bulk protein synthesis. Since it has been shown that the phase transitions of membrane lipids of E. coli cells supplemented with elaidate and with oleate occur at 30°C and 13°C, respectively (12), this suggests that the failure to observe alkaline phosphatase induction in the elaidate-grown cells after the downshift from 38°C to 25°C is primarily attributable to a phase transition of the membrane lipids to a less fluid state.

Although the growth of strain Ole-28E1 in the media supplemented with elaidate essentially ceased after a shift from 38°C to 25°C, the cells did not lyse and the viability decreased only slightly during a 5-hr incubation at 25°C. When the derepressed cultures supplemented with elaidate were shifted back to 38°C after 20-min or 2-hr incubation at 25°C, alkaline phosphatase activity increased immediately as shown in Fig.3. The rate of appearance of enzyme activity after the shift-back was almost equal to that in the control culture incubated throughout at 38°C. The failure to observe a burst of enzyme activity after shift-back indicates that the monomers that are convertible to active dimers are not accumulated within the cells during incubation at 25°C. In fact, when rifampicin or chloramphenicol was added to a culture immediately before the shift-back to prevent de novo synthesis of m-RNA or protein, respectively, there was no increase in enzyme activity after shift-back to 38°C. At least two possibilities may be considered to explain events which might be taking place in the elaidate-grown cells during the incubation at 25°C: (i) The monomers of alkaline phosphatase were synthesized but their secretion was blocked, indicating that membrane fluidity is necessary for secretion but not for synthesis. The failure to observe accumulation of the monomers in the

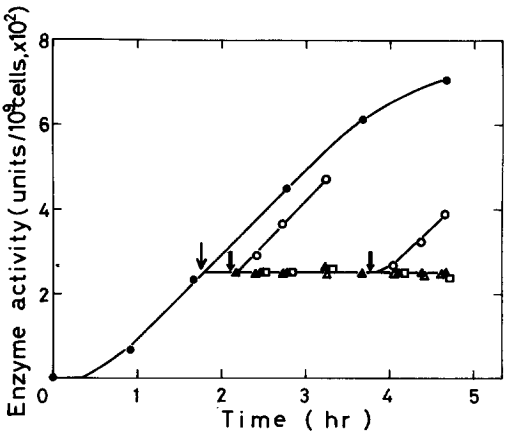


Fig. 3 Effect of shift-back of the temperature from 25°C to 38°C on induction of alkaline phosphatase in the cells supplemented with elaidate. The cells of Ole-28E1 supplemented with elaidate were induced to synthesize alkaline phosphatase at 38°C in low-phosphate medium (20 ml) (—●—). After 105 min, a portion (13 ml) of the culture was transferred to 25°C (—▲—) while the rest remained at 38°C. At 20 min and 120 min after the temperature-shift, portions of the culture were taken and shifted back to 38°C either with no addition (—○—) or after addition of rifampicin (final 100 μg/ml) (—□—) or chloramphenicol (final 200 μg/ml) (—△—). Aliquots were withdrawn at the times indicated, and alkaline phosphatase was assayed.

cells could be due to rapid degradation of the non-secreted monomers by proteases. (ii) The synthesis of the monomers was arrested, indicating that membrane fluidity is necessary also for synthesis. If it is assumed that the membrane-bound protein-synthesizing system is involved in the synthesis of monomers, the arrest of their synthesis at 25°C would be attributable to the impairment of this system at temperatures below that of the membrane lipid phase transition. This protein-synthesizing system may be tightly coupled with the secretion system as proposed in the case of higher organisms (3). Since it has been reported that the m-RNA for alkaline phosphatase was slightly but significantly enriched in the membrane fraction rather than in the cytoplasmic fraction (13), the second possibility seems more plausible. Further studies are in progress to define more precisely the function of membrane fluidity in this system.

Acknowledgment

The authors wish to express their sincere gratitude to Prof. Hirohiko Katsuki of this Department for encouragement and valuable discussions throughout this work. Thanks are also due to Profs. Takashi Yura and Makoto Kito

and to Drs. C. Hubbard and C. Edson of this University for valuable discussions and critical reading of this manuscript. This work was supported in part by research grants from the Ministry of Education of Japan, No. 778069 and No. 868025.

REFERENCES

1. Malamy, M. H. and Horecker, B. L. (1964) Biochemistry 3, 1889-1893.
2. Schlesinger, M. J. (1968) J. Bact. 96, 727-733.
3. Sabatini, D. D. and Blobel, G. (1970) J. Cell Biol. 45, 146-157.
4. Schairer, H. U. and Overath, P. (1969) J. Mol. Biol. 44, 209-214.
5. Wilson, G., Rose, S. P. and Fox, C. F. (1970) Biochem. Biophys. Res. Commun. 38, 617-623.
6. Tsukagoshi, N. and Fox, C. F. (1973) Biochemistry 12, 2816-2821.
7. Izui, K. (1971) Biochem. Biophys. Res. Commun. 45, 1506-1512.
8. Nishihara, M., Kimura, K., Izui, K., Ishinaga, M., Kato, M. and Kito, M. (1975) Biochim. Biophys. Acta 409, 212-217.
9. Vogel, H. J. and Bonner, D. M. (1956) J. Biol. Chem. 218, 97-106.
10. Torriani, A. (1968) J. Bact. 96, 1200-1207.
11. Torriani, A. (1960) Biochim. Biophys. Acta 38, 460-479.
12. Fox, C. F. (1972) in Membrane Molecular Biology (Fox, C. F. and Keith, A. D., eds), pp.345-385. Sinauer Associates Inc., Stamford, Conn.
13. Cancedda, R. and Schlesinger, M. J. (1974) J. Bact. 117, 290-301.