SYNTHESIS OF THE *E. COLI* PYRUVATE DEHYDROGENASE COMPLEX: NON-DEPENDENCE ON 3'-5'-CYCLIC AMP

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SUMMARY: A determination of the level of the pyruvate dehydrogenase complex in a 3'-5'-c-AMP deficient mutant of *E. coli* K12 has been carried out. The deficiency has no effect on specific activities for derivatives carrying either the inducible genes for two components of the complex or constitutive mutants. We conclude that synthesis of the complex is not sensitive to catabolite repression.

The *E. coli* pyruvate dehydrogenase complex, which catalyzes the formation of acetyl CoA from pyruvate and CoA, is subject to an extensive system of regulation. Enzymatic activity is sensitive to a variety of allosteric effectors (1 - 4). In addition, synthesis of at least two of the three polypeptide components of the complex is regulated by pyruvate, which induces expression of the linked *aceE* and *aceF* genes specifying the pyruvate dehydrogenase and dihydrolipoamide transacetylase components (5). We have asked whether the enzyme system is also subject to catabolite repression. Aside from its intrinsic importance to an understanding of the induction mechanism, we felt that were catabolite repression of the system demonstrable, it might then be possible to resolve the physiological role of another enzyme system, pyruvate oxidase, which produces acetate from pyruvate (6). The metabolic significance of this enzyme has hitherto been unclear (5, 7).

Measurement of an influence of catabolite repression on the synthesis of the pyruvate dehydrogenase complex is complicated by the fact that the carbon source also affects

the intracellular concentration of pyruvate, the inducer (5). To circumvent this difficulty, we have isolated a mutant deficient in adenyl cyclase activity and have examined the effect of c-AMP* on the synthesis of the complex in strains carrying either the wild-type inducible ace locus or one of several constitutive alleles (8).

RESULTS: Isolation and properties of the c-AMP deficient mutant P8-000. A c-AMP deficient mutant of E. coli K12 strain 1220 (thi leu aceF10 lac pps his) was obtained after mutagenesis with nitrosoguanidine (11) according to the method described by Perlman and Pastan (12). One Ara^- Mal^- mutant which could ferment both sugars when 0.5 mM c-AMP was added to the medium was investigated further. Fermentation properties and induction of ß-galactosidase (in a lac^+ transductant of P8-000) are identical to those of the well-characterized adenyl cyclase mutant 5336 (12) (kindly provided by Dr. R. Perlman). Transduction experiments with phage P1 grown on 5336 indicate that P8-000 carries a mutation closely linked to the site mutated in 5336; less than 10^{-8} recombinants per plaque-forming particle were obtained after selection for P8-000 transductants able to grow on succinate agar in the absence of c-AMP, although the 5336 lysate transduced P8-000 to Leu^+ at a normal frequency and P1 grown on a wild-type donor yielded Suc^+ recombinants of both 5336 and P8-000 at a frequency of 10^{-6}. We conclude that P8-000 is mutated in cya, the gene for adenyl cyclase.

Pyruvate dehydrogenase complex levels. Ace^+ derivates of P8-000 were isolated after transduction (13) with P1

*Abbreviations: c-AMP (cyclic 3'-5'-adenosine monophosphate). Genetic symbols conform to the suggestions of Demerec et al. (9) and Taylor (10). Ace^- designates a requirement for acetate.

grown on a variety of donors which are described in detail by Flatgaard et al. (8). All have the same c-AMP requirement as P8-000, but with the exception of P8-813 are Leu^+ as a result of cotransduction of leu^+ with ace^+ (14). Crude extracts (5) were prepared from cells grown aerobically to early stationary phase at 37° in minimal medium (15) containing glucose (0.4 %), thiamine (1 µg/ml), and either 0.05 % casamino acids (Difco "vitamin free") for the "minus c-AMP" samples or l-leucine, l-histidine (10 µg/ml each), and 0.5 mM c-AMP. Casamino acids were added to obviate possible secondary effects on specific activity due to generation time differences (16).

The results are summarized in Table 1. For P8-K11, which carries the inducible ace^+ locus of strain K-1-1, a slight decrease in specific activity is visible in cultures grown without c-AMP, but it is clear that the decrease is due to the presence of casamino acids rather than the absence of c-AMP; addition of c-AMP does not reverse the effect, and a depression of similar magnitude occurs in cya^+ 1220-K11 grown in the presence of casamino acids. It is likely that casamino acids diminish the intracellular concentration of pyruvate by transamination reactions, thus reducing inducer level. For constitutive derivatives P8-122, P8-616, P8-813, and P8-816, growth in the absence of c-AMP has no effect on the specific activity of the complex, and the activities correspond to those reported previously for the cya^+ constitutive parents grown on glucose (8). Two of the constitutives, P8-711 and P8-919, show a decrease in specific activity in the absence of c-AMP. The cya^+ parents are low level constitutives (8) and possibly the 711 and

TABLE 1

Specific Activity of Pyruvate Dehydrogenase Complex in Ace$^+$ P8-000 Derivatives

Strain[a]	Minus c-AMP		Plus c-AMP	
	(+CAA[b])	(-CAA)	(+CAA)	(-CAA)
P8-K11	15		14	23
1220-K11	17	23		24
P8-122	125			137
P8-616	59			59
P8-813	87		83	
P8-816	48			49
P8-711	31			42
P8-919	20			32

[a]Donors of *ace*$^+$ are indicated by the last three figures of the strain designation. K-1-1 is wild-type inducible; all other donors are constitutive.

[b]Casamino acids (0.05 %).

Specific activities were measured and are expressed according to Reed *et al.* (17).

919 *ace* alleles retain some sensitivity to inducer. If so, the relatively small decrease in activity seen here may again be due to the effect of casamino acids on inducer level.

DISCUSSION: We have found no evidence for catabolite

repression of the synthesis of the pyruvate dehydrogenase complex, in so far as lowered c-AMP levels reflect the mechanism by which the catabolic product exerts its repressive effect. Our studies on the effect of c-AMP in the ace constitutive strains were motivated by two considerations. First, although it is known that the mutations conferring constitutivity are closely linked to ace (8), their precise nature is as yet undefined, and the possibility existed that in some cases constitutivity represented an acquired insensitivity to catabolite repression. In view of the present study, this of course cannot be the case. Second, a previous report (8) suggested that growth on glucose medium depressed enzyme activity for several constitutive strains (those carrying ace^{c}-816, -813, -616, -122) compared to the levels found in succinate- or acetate-grown cells, although in all cases enzyme activity was at least twice as high as in the wild-type strain. In the P8-000 derivatives examined here, the activities are those characteristic for growth on glucose, and c-AMP produces no stimulation. Thus, the mechanism underlying this glucose effect remains as obscure as the initially mentioned role of the pyruvate oxidase.

We would finally like to point out that the present results may have an interesting bearing on Maaløe's hypothesis regarding the regulation of the synthesis of ribosomes (18). In brief, this hypothesis views ribosomal genes as structural genes whose expression is constitutive and insensitive to catabolite repression. The degree of expression of these genes is thought to depend on the number of other genes which do or do not function, in other words,

on the availability of factors such as DNA-dependent RNA polymerase. Since the synthesis of the pyruvate dehydrogenase complex is not subject to catabolite repression, synthesis of the complex in constitutive mutants should, under various growth conditions, closely parallel the expression of ribosomal genes and provide a means of testing the hypothesis.

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