Characteristics of physiological inducers of the ethanol utilization (alc) pathway in Aspergillus nidulans

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The ethanol utilization (alc) pathway in Aspergillus nidulans is one of the strongest expressed gene systems in filamentous fungi. The pathway-specific activator AlcR requires the presence of an inducing compound to activate transcription of genes under its control. We have demonstrated recently that acetaldehyde is the sole physiological inducer of ethanol catabolism. In the present study we show that compounds with catabolism related to that of ethanol, i.e. primary alcohols, primary monoamines and L-threonine, act as inducers because their breakdown results in the production of inducing aliphatic aldehydes. Such aldehydes were shown to induce the alc genes efficiently at low external concentrations. When ethanol is mixed with representatives of another class of strong direct inducers, ketones, the physiological inducer, acetaldehyde, prevails as effector. Although direct inducers essentially carry a carbonyl function, not all aldehydes and ketones act as inducers. Structural features discriminating non-inducing from inducing compounds concern: (i) the length of the aliphatic side group(s); (ii) the presence and nature of any non-aliphatic substituent. These characteristics enable us to predict whether or not a given carbonyl compound will induce the alc genes.

Key words: activation of transcription, aldehydes, catabolism, methyl ketones, toxicity.

INTRODUCTION

In fungi, a large number of inducible expression routes are responsible for the utilization of less favoured carbon sources. The ethanol pathway in the hyphal fungus *Aspergillus nidulans* is a highly inducible gene system responsible for the utilization of ethanol as sole source of carbon. This so-called alc system has been extensively studied at the genetic, biological, structural and molecular level [1–4]. These approaches have provided a profound insight into the molecular mechanisms by which *A. nidulans* responds to changes in carbon nutrient availability, by turning on or off the alc genes.

Two key elements are essential for induction of ethanol catabolism. The pathway-specific activator AlcR, encoded by the regulatory alcR gene, mediates the induction of transcription of genes under its control, but only when an inducing compound (e.g. ethanol) is present within the cell. In addition to endogenous transcriptional activation of alcR [5,6], activation of the unlinked aldA gene, as well as of a number of other alc genes, occurs [7]. These latter alc genes are tightly linked to alcR in a gene cluster on the left-hand arm of chromosome VII. The two structural genes, alcA and aldA, encode alcohol dehydrogenase I (ADHI; EC 1.1.1.1) and aldehyde dehydrogenase (ALDH; EC 1.2.1.5) respectively, the two enzymes necessary and sufficient for the oxidation of ethanol to acetate via acetaldehyde [8]. The specific structure of the promoters of alcR, alcA and aldA enables high levels of induced expression. The localization and the number of AlcR targets (featuring synergistic effects) contribute to the strength of the respective promoters [6,9,10]. Importantly, alcR autoactivation mediates the inducing signal. In addition, the specificity of the binding of the AlcR Zn–Cys binuclear cluster and the high affinity for its DNA targets [11–13] plays a prominent role in this process. In the presence of a more favoured carbon source, e.g. d-glucose, the alc system is repressed, also in the co-presence of an inducing compound. Transcriptional repression is mediated at two levels: directly, via the binding of the repressor CreA to its cognate targets located in the alc gene promoters, and indirectly, by down-regulating the antagonistic regulatory alcR gene [6,10,14–16]. Interplay between induction and repression occurs under all physiological conditions [17] and modulates the response to an inducing signal.

In addition to ethanol, the alc gene regulon is induced by other primary alcohols, primary monoamines, the amino acid L-threonine and ketones [8,18–21]. Scheme 1 displays the degradation routes of ethanol, ethylamine and L-threonine, converging on acetaldehyde as the first common catabolic intermediate. The acetate formed from acetaldehyde is further activated to acetyl-CoA to enter mainstream metabolism. Mutants in alc and ald utilize primary monoamines as sole sources of nitrogen like the wild type, indicating that the amine oxidase (EC 1.4.3.--) responsible for deamination, is expressed independently of the ethanol utilization pathway. The utilization of ethyl- and n-butylamine as growth substrates, however, requires the integrity of the alc system [22]. L-Threonine can also serve as a sole source of both carbon and nitrogen for *A. nidulans*. This amino acid is converted into acetaldehyde and glycine by threonine aldolase (EC 4.1.2.5) [23], this conversion being common in mammals and in yeasts. In baker’s yeast (*Saccharomyces cerevisiae*), this enzyme is essential for the biosynthesis of glycine when cells are grown on d-glucose [24]. The inducing capacity of L-threonine has been ascribed to its degradation product acetaldehyde, although it was impossible to show that acetaldehyde itself had inducing activity [8].

Recently, we demonstrated by several approaches that ethanol itself does not induce the alc system, thereby establishing acetaldehyde as the sole physiological inducer of ethanol catabolism by activating AlcR [10]. Here we provide evidence that ethylamine and L-threonine also promote induction because they are converted into acetaldehyde. Moreover, we show for the first time that acetaldehyde is a strong external inducer of the alc system. We have further conducted an extensive analysis of the main structural characteristics of inducing and non-inducing
Scheme 1 Related catabolism of ethanol, ethylamine and L-threonine in *A. nidulans*

A convenient representation of the chemical structure of the main catabolites is given. Enzymic conversions are indicated with arrows, labelled with the trivial name of the enzyme responsible. ADH and ALDH expression is controlled by the transcriptional activator AlcR [5]. Acetate is further metabolized to acetyl-CoA by acetyl-CoA synthase (EC 6.2.1.1), encoded by the *facA* gene [30,45,46]. Although indispensable for growth on ethanol, the catabolism of acetate is fully independent of Alc gene regulation (M. Flipphi and B. Felenbok, unpublished work). Other small primary alcohols and monoamines are converted into their corresponding carbonyl and carboxy intermediates by the same enzymes. Oxidative deamination of primary monoamines can be catalysed by copper-containing amine oxidase (EC 1.4.3.6) as well as by flavin-dependent monoamine oxidase (EC 1.4.3.4).

**MATERIALS AND METHODS**

*A. nidulans* strains, media and growth conditions

The *A. nidulans* strains used in this work were BF054 (*γA2, pabaA1*), T*gdpa*:*alcR* (*γA2 pantoB100* (argB2) arg+ (*alcR125* *gdpa*:*alcR*) and T*gdpa*:*aldA* (*pabaA1* (argB2) arg+ (*ald-A67* *gdpa*:*aldA*)) [10]. The reference given describes the relevant mutations; the other markers are in standard use [25]. Mycelia for RNA extraction were grown for 24 h at 37 °C on appropriately supplemented minimal medium [26] with 3% (w/v) lactose and 5 mM urea as sole carbon and nitrogen source respectively. Induction was achieved by addition of chemicals to a final concentration of 50 mM, unless stated otherwise. Induced biomass was harvested after a further 2.5 h of incubation (inducing conditions), while non-induced biomass was allowed to continue growing on the original growth medium during the above induction period (non-inducing conditions). Where necessary, chemicals were administered from a freshly prepared stock solution set at pH 6.8. The chemicals used were purchased from Sigma–Aldrich or Merck–Eurolabo.

**Isolation of RNA and Northern-blot analysis**

Total RNA was extracted after grinding mycelia in liquid nitrogen as described by Lockington et al. [19]. Northern analysis was carried out with glyoxal-treated total RNA [27] using Hybond N membranes (Amersham Pharmacia Biotech). Membranes were hybridized with 32P-labelled probes generated from DNA fragments from the cloned *A. nidulans* genes *alcA* (bA2) [9], *alcR* (pBSalc*RSal*) [28], *aldA* (*pAN212*) [29], *facA* (pRAS7) [30] and γ-actin (pSF5) [31]. 18S ribosomal RNA was revealed with a probe against a fragment of horseradish 18S rDNA (pRG3) [32]. Autoradiograms were exposed for various times to avoid saturation of the film. Under most inducing conditions the γ-actin gene is constitutively expressed and could be used as an internal control for the amounts of mRNA loaded. All induction experiments were repeated at least twice.

**RESULTS AND DISCUSSION**

**Induction by primary alcohols, primary monoamines and L-threonine results solely from formation of corresponding inducing aldehydes**

We have examined the inductive capabilities of compounds belonging to the three classes of convertible compounds, i.e. primary alcohols, primary monoamines and L-threonine, in order to identify the physiological inducer(s) responsible for the induction of the *alc* gene system for each of the catabolic pathways involved.

Analysis of *alc* gene transcription in response to primary alcohols showed that ethanol, n-propanol and n-butanol provoked a substantial induction, n-butanol providing the highest

### Figures

**Figure 1** Transcript analysis of the inducing potential of alcohols and monoamines

Biomass was generated on lactose/urea (non-induced growth conditions, NI) and induced with the indicated compounds to a concentration of 50 mM. Total RNA was isolated, denatured, separated and transferred to a nylon membrane as described in the Materials and methods section. Northern blots were hybridized with 32P-labelled probes for the *alcA*, *alcR* and γ-actin (*acnA*) respectively. Top panel: Northern blot showing the response of *alc* genes to primary and secondary alcohols as inducers in the wild type. Bottom panel: Northern blot showing the response to primary monoamines as inducers in the wild type. 2-butanone, butan-2-one; 2-propanol, propan-2-ol; 2-butanol, butan-2-ol.
induction (Figure 1, top panel). The responses of the *alcA* gene to ethanol and n-propanol appear, compared with that of the gratuitous inducer butan-2-one, higher than reported by Creaser et al. [20]. We have shown recently that ethanol itself is inert, and that acetaldehyde – the aldehyde formed upon its oxidation – provides the true inducing signal [10]. It is more than likely that this would be the case for all primary alcohols eliciting induction of *alc* gene transcription. Other primary alcohols, i.e. methanol and benzyl alcohol, do not provoke an induction (results not shown). Induction experiments with primary monoamines allowed us to determine the limitations for inducing potential with regard to the length of the aliphatic tail, which appeared impossible with primary alcohols because of their toxicity. But at 50 and 10 mM, we found that ethyl-, n-propyl-, n-butyl- and n-pentyl-amine induced the *alc* genes, n-butylamine eliciting the most pronounced response (Figure 1, bottom panel). In contrast, methyamine and n-hexylamine were inert. Nevertheless, methylamine penetrates *A. nidulans* with relative ease [33,34], whereas n-hexylamine enters the fungus in sufficient amounts to induce transcription of an amine oxidase-encoding gene we have cloned recently (M. Flippipi, J. Kocialkowska and B. Felenbok, unpublished work). In contrast with benzyl alcohol, benzylamine induced expression of the *alc* genes (Figure 1, bottom panel). All the monoamines tested served as sole sources of nitrogen for the fungus (not shown).

As is the case for ethanol, the formation of acetaldehyde could explain the induction observed by ethylamine, the smallest inducing primary monoamine, as well as by the amino acid l-threonine. The other products of ethylamine and l-threonine conversion, i.e. ammonium, H$_2$O$_2$ and glycine, all proved inert (results not shown). To exclude the possibility that the monoamine and the amino acid could elicit induction by themselves, we utilized two approaches to reduce the amount of acetaldehyde within the cell [10]. Figure 2 shows that the transcriptional induction provoked by 50 mM ethylamine or l-threonine is suppressed upon simultaneous addition of the aldehyde scavenger semicarbazide to 1.3 mM. At this and higher concentrations of semicarbazide, the inducing capacity of butan-2-one (50 mM) was not affected. Essentially identical results were obtained in biomass grown on l-threonine as the sole source of nitrogen (not shown), ruling out the possibility that the scavenger would interfere with uptake. In the case of the monoamine, the observed suppression exerted by semicarbazide could also be due to inhibition of copper-containing amine oxidase [35]. The results imply that both ethylamine and l-threonine are inert and that the acetaldehyde formed upon their conversion is the (common) physiological inducer. This was confirmed in a strain in which ALDH (TgpdH:aldA), is constitutively overexpressed (Figure 2, bottom panel). The aldehydes were added to wild-type biomass to the final concentrations indicated. Ethanol (50 mM) served as a reference. Top panel: acetaldehyde induction. The Northern blot was also probed for *alcA* gene transcription and for 18S ribosomal RNA (18S rRNA) as specified in the Materials and methods section. The latter species served as a control of total RNA quality and the quantity of the material loaded, but does not represent a reliable measure for the amount of mRNA in a sample. Bottom panel: 2-methylbutyraldehyde (2MB) induction. Principal manipulations were as described in the legend to Figure 1. 

**Figure 2** Suppression of *alc* gene induction on ethylamine and l-threonine

In the left panel, the induction provoked in the wild type by ethylamine or l-threonine was titrated *in vivo* with 1.3 mM semicarbazide (SC) and compared with the induced biomass without added scavenger. Mycelia to which only semicarbazide (1.3 mM) or to which butan-2-one and 8 mM semicarbazide was added provided additional controls. In the right panel, the transcriptional response to ethylamine in a transformant strain, constitutively overexpressing *alc* genes, n-butylamine eliciting the most pronounced response (Figure 1, bottom panel). All *alc* genes, n-butylamine eliciting the most pronounced response (Figure 1, bottom panel). All the monoamines tested served as sole sources of nitrogen for the fungus (not shown).

Aldehydes induce *alc* gene transcription at low external concentrations

Although we have shown that the induction provoked by ethanol, l-threonine and ethylamine can only be ascribed to acetaldehyde
formed during catabolism, we still lacked any direct evidence that acetaldehyde itself could elicit induction. Figure 3 (top panel) shows that the alc genes are indeed induced when acetaldehyde is supplied to pre-grown biomass, but only at low external concentrations. The concentration of acetaldehyde in the wild-type that elicits the highest response was around 1 mM, whereas the effect appeared stronger than that provoked by 50 mM ethanol. Even at an external concentration of 32 µM, this highly volatile compound could induce the expression of alc-A (results not shown). On the other hand, it is seen in Figure 3 (top panel) that alc transcript levels decreased rapidly with further increasing amounts of acetaldehyde.

We have also tested 2-methylbutyraldehyde, an aldehyde with a branched aliphatic tail. The induction profile as a function of the external inducer concentration was essentially similar to that of acetaldehyde (Figure 3, bottom panel). The maximal induction level elicited by this compound at about 2 mM is much higher than that of acetaldehyde, more comparable with that of butan-2-one at 50 mM (results not shown).

The experiments with aldehydes not only show that these compounds induce efficiently at low external concentrations, but also reflect their toxic nature. The level of general transcription decreases rapidly with increasing aldehyde concentration, as indicated by the decreasing γ-actin transcript level while the steady-state amount of 18 S ribosomal RNA remained unaffected (as shown in Figure 3 for acetaldehyde). Inhibition of de novo RNA synthesis, characterized by a fall in the level of actin transcript with increasing effector concentration, also occurs with other compounds such as benzyl alcohol, glycolate and acetate (M. Flipphi and B. Felenbok, unpublished work). The inhibitory effect of acetaldehyde on de novo transcription becomes apparent at those concentrations, causing malfunctions in mitosis at the level of the chromosome segregation [36]. It is generally recognized that enzyme activities depending on essential thiol groups are extremely susceptible to aldehyde inactivation. A. nidulans ALDH itself is inhibited in vitro at concentrations above 350 µM [23], which could match the intracellular concentration at which acetaldehyde starts to affect cellular functioning. Note here that formaldehyde, the smallest aldehyde, proved to be too toxic at all concentrations tested. However, the above finding that methylamine is inert suggests that it is unlikely that formaldehyde would be an inducer.

**Ketones represent a second class of direct inducers**

It has been reported that certain ketones are excellent inducers of the alc genes [20,21]. Our transcriptional analysis reconfirmed that small methyl ketones (acetone, butan-2-one and pentan-2-one) and cyclic ketones (cyclohexanone) induced a high level of transcription of the three principal genes of ethanol catabolism (alcA, alcR and aldA; Figures 4 and 5). The strong induction elicited by these substances might be explained in part by the fact that they are metabolically inert – none of them can serve as a sole carbon source for growth (results not shown). To the best of our knowledge, biotransformation of such aliphatic ketones into inducing aldehydes has never been described in A. nidulans. These ketones could therefore be considered as gratuitous inducers, constituting a second group of direct inducers. A carbonyl function appears to be the predominant characteristic of compounds acting as direct inducers of the alc genes. Interestingly, a similar situation is known in the prokaryote Escherichia coli. Expression of its lactaldehyde dehydrogenase gene is induced by small hydroxylated aldehydes, intermediates of catabolism in which the encoded enzyme plays a role, but also by the tricarboxylic-acid cycle intermediate α-oxoglutarate (‘α-ketoglutarate’), a substituted ketone [37,38].

Waring et al. [21] were the first to use mixed inducers in their study of β-tubulin expression driven from the alcA promoter. They noted that addition of a ketone could not increase the induction provoked by either ethanol or l-threonine. We conducted an analysis of alc gene transcription in response to mixtures of inducers. Figure 5 shows that, in the simultaneous presence of the strong direct inducer butan-2-one and the indirect convertible inducer ethanol (both at 50 mM), the transcript levels of the alc genes were comparable with the lower levels provoked by the alcohol alone. Similar results were obtained with cyclohexanone (Figure 5). This effect is not related to possible carbon catabolite repression arising from the catabolism of ethanol. Essentially identical results were obtained in a carbon-catabolite derepressed background (creAd30) (results not shown). It thus appears that the acetaldehyde arising from ethanol...
Structure of direct inducers of AlcR transactivation

**Figure 6** Influence of non-aliphatic substituents on the inducing capacity of small aldehydes

Top right panel: scheme depicting the chemical structures of the various double-substituted bicarbon compounds tested for their capability to promote induction of the alc genes in *A. nidulans*. Both ethanolamine and glycine are utilized as sole sources of nitrogen by the fungus, although neither compound can serve as sole carbon source. Possible interconversions are indicated with arrows, labelled with the trivial name of the enzymes responsible. Enzymes that convert glycolaldehyde into glycolate (lactaldehyde dehydrogenase; EC 1.2.1.22) and glycolate into glyoxylate (glycolate oxidase; EC 1.1.3.15) have never been described in *A. nidulans*, but are well known to occur in other organisms, e.g. in *Escherichia coli* [37,47]. Next to glycine transaminase (EC 2.6.1.4), various other transaminases can use glyoxylate as amino acceptor or glycine as amino donor. Within the induction period employed in the present study, formation of an inducing amount of glycolaldehyde from ethylene glycol occurs solely in a transformant strain that overexpresses the regulatory *alcR* gene constitutively (TgpdA:alcR) (see bottom left panel). Top right panel: double-substituted bicarbon compounds that induce the alc gene system. Mycelium was induced with ethanolamine or glyoxal at 50 mM, or with glycolaldehyde at 2 mM. Bottom left panel: ethylene glycol can induce alc gene transcription in a transformant strain constitutively overexpressing the transactivator AlcR (TgpdA:alcR). On the left the analysis in the wild type is shown and on the right that in TgpdA:alcR. Bottom right panel: glycolate and glyoxylate, bicarbon compounds that carry a carboxy substituent, do not induce the alc gene system. Transcription of the *facA* gene, encoding acetyl-CoA synthase, was additionally probed as specified in the Materials and methods section. All principal manipulations were as described in the legend to Figure 1.

Catabolism determines the level of induction of the alc genes, even though the intracellular concentration of the ketone is likely to be higher. This feature could well be a general characteristic of inducing ketones.

**Structural features of non-inducing and weakly inducing carbon compounds**

Substituted aldehydes

To further specify the induction spectrum of the alc system we have performed transcriptional analysis in mycelium supplied with a range of dicarbon compounds with various substitutions (Figure 6, top left panel).

Figure 6 (top right panel) shows the effects of hydroxy and carbonyl substituents. Glycolaldehyde (at a low concentration), ethanolamine and glyoxal provide a moderate level of induction of alc gene transcription. Ethanolamine is a substrate for amine oxidase [18], forming glycolaldehyde as it serves as sole nitrogen source for the fungus. Note that the reduced capability found for glyoxal could be due to a limited concentration of the free aldehyde in water. Unlike other aldehydes, glyoxal does not inhibit γ-actin gene transcription at 50 mM. Interestingly, ethylene glycol is inert in the wild type, but provokes induction in a strain constitutively overexpressing the transactivator AlcR (TgpdA:alcR). On the left the analysis in the wild type is shown and on the right that in TgpdA:alcR. Bottom right panel: glycolate and glyoxylate, bicarbon compounds that carry a carboxy substituent, do not induce the alc gene system. Transcription of the *facA* gene, encoding acetyl-CoA synthase, was additionally probed as specified in the Materials and methods section. All principal manipulations were as described in the legend to Figure 1.
serve as a substrate for ADHI in vivo. The results for ethylene glycol confirm the view that primary alcohols do not have intrinsic inducing capacity and only provoke induction when converted into the corresponding aldehyde.

On the other hand glycolate, glyoxylate (Figure 6, bottom right), glycine, choline, acetate and acetamide (not shown) are inert. Apparently, the aldehyde glyoxylate is unable to induce the alc genes. Nevertheless, this compound does enter the fungus, as it provokes induction of the facA gene, encoding acetyl-CoA synthase (see the legend to Scheme 1), over the basal level normally observed in lactose-grown mycelium (Figure 6, bottom right).

In conclusion, it appears that a neighbouring carboxy group neutralizes the inducing capacity of an aldehyde, whereas a hydroxy group restricts it.

Secondary alcohols and monoamines

In line with our results for ethanol and ethylamine, one would expect that a secondary alcohol or a secondary monoamine must be converted into an inducing ketone in order to induce alc gene transcription. In transcriptional analysis no more than an extremely low level of induction could be observed with either propan-2-ol or butan-2-ol (Figure 1, top panel). In a mutant lacking the complete alcA gene, we could not observe any induction on secondary alcohols (results not shown). It thus appears that secondary alcohols are inert by themselves and that ADHI is the only relevant enzyme capable of oxidizing these compounds in vivo. However, Creaser et al. [20] found that propan-2-ol and butan-2-ol were much more potent inducers of ADHI activity than primary alcohols when the fungus was cultivated for 1 day on a limiting amount of d-glucose in the presence of either alcohol as a co-substrate. Taken together, these contradictory results in fact point again towards an important role for ADHI in the onset of induction on alcohols. Creaser et al. reported in the same paper [20] that the affinity of ADHI for propan-2-ol is more than 500 times lower than its affinity for ethanol. This could explain why secondary alcohols appear to be very poor inducers under our conditions in which growing biomass is induced for only 2.5 h.

Similarly, we found that the secondary amine butyl-2-amine does not induce the alc genes (results not shown). In this case, the inducing methyl ketone butan-2-one should be formed upon oxidative deamination. Butyl-2-amine is described as an excellent gratuitous inducer of amine oxidase in A. nidulans, implying that it is taken up by the fungus, but then does not serve as a substrate for this enzyme [18].

Linear ketones

It was shown in Figure 4 that linear ketones carrying the carbonyl group in the α position are strong inducers of alc gene transcription. However, the smallest ketone carrying the carbonyl function in the β position, pentan-3-one, did not induce the alc genes (Figure 4). This compound nevertheless enters the cell, since it provokes increased transcription of one of the ketone-inducible genes clustered with alcR and alcA (J. Kociatkowska, B. Felenbok and M. Flippi, unpublished work). Apparently, any elongation of the aliphatic tail at the other end of the carbonyl group beyond a methyl group neutralizes the capacity to activate AlcR. This idea finds support in the results we obtained with dihydroxyacetone. The presence of a hydroxy-substituted methyl group at both ends of the carbonyl function apparently renders this ketone inert (results not shown). Dihydroxyacetone does enter the cell as it can serve as a sole carbon source for the fungus [39].

Activation of AlcR in relation to the structural characteristics of its inducers

It has been well established that induction of alc gene transcription requires activation of the pathway-specific transactivator AlcR. We have now established that (certain) aldehydes and ketones are direct inducers of alc gene transcription, we can speculate on the mechanism by which the inducing signal is transmitted. Several hypotheses could account for the activation of AlcR. However, the functionality of the alcA–alcR expression system in tobacco (Nicotiana tabacum) [40] would suggest that signal transmission is effected by a direct interaction of the inducer with AlcR. This could involve the formation of a Schiff base, the condensation product of carbonyl compounds (i.e. aldehydes and ketones) and primary amines. For example, Schiff-base modification of certain lysine residues in tubulin results in the inhibition of microtubule formation, a process connected with the aneugenic effect of acetaldehyde [36,41,42]. As in the case of tubulin, direct interaction of a carbonyl compound could result in conformational changes, converting AlcR into its active state. Such a mechanism occurs in the case of the regulator of t-leucine biosynthesis in S. cerevisiae, Leu3p [43], and the yeast activator necessary for the utilisation of t-proline, Put3p [44], two other Zn-Cys binuclear cluster proteins. However, other post-translational mechanisms could also occur and, at the moment, we cannot dismiss the possibility that the inducing signal is mediated indirectly, through auxiliary protein(s).

The present study revealed that there are steric and electrostatic limitations to the capacity of carbonyl compounds to activate AlcR, results that provide interesting clues about the structure of the effector site involved in this activation. (i) Linear aliphatic aldehydes are only inducing when their backbone is two to five carbon atoms long. The optimal length of the backbone appears to be four carbon atoms (butanal). Variation in the form of limited branching is allowed, as 2-methylbutyraldehyde emerged as the most powerful inducer. In addition, a single aryl substituent (benzylaldehyde) is likewise allowed. (ii) By contrast, the presence of a non-aliphatic substituent was shown to considerably restrict (hydroxy group) or even prevent (carboxy group) activation of AlcR. (iii) Linear ketones with a backbone of three to five carbon atoms are very potent activators of AlcR, provided that the carbonyl function resides in the α-position. Again, the optimal length of the backbone appears to be four carbon atoms (butan-2-one). However, cyclic aliphatic structures (cyclohexanone) also facilitate AlcR activation. With the structural features of the catalobites effecting transcriptional activation of alc genes now precisely defined, this work opens up the possibility of elucidating the molecular mechanisms underlying signal transmission to AlcR.

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