The effect of different carbon sources used for growth on the formation of DNA-protein complexes was less prominent than the effect of heme. Yet, extracts prepared from wild type cells grown on nonfermentable substrates produced a stronger band with low electrophoretic mobility than the extract prepared from cells grown on glucose. Accordingly, high glucose concentration (8 %) in the growth medium resulted in diminished formation of DNA-protein complexes as compared to extracts from cells grown in lower glucose (2 %) containing media.

We have shown previously that the expression of the *AAC2* gene was markedly reduced in *hap2* and *hap3* mutant cells [1]. These mutations, however, did not affect significantly the formation of the UAS_{AAC2} protein complexes *in vitro*.

The above results show that ABF1 functions as an important factor *trans*-acting through interaction with its binding site in UAS of the gene. In addition, they suggest that at least in the regulation of the *AAC2* gene encoding mitochondrial ADP/ATP carrier the ABF1 is acting as a carbon source- and oxygen-dependent transactivator.

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DL-Leucine Transport in a *Saccharomyces cerevisiae* Mutant Resistant to Quaternary Ammonium Salts

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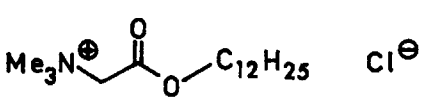
Quaternary ammonium compounds function as regulators or as antimicrobial agents (*viz.* derivatives of betaines [1]). Synthetic quaternary ammonium salts (QAS) have long been used as disinfectants, but the exact mechanism of QAS antimicrobial activity is still far for being elucidated. We tested the effect of a series of QAS differing in carbon chain length on *S. cerevisiae,* with the following conclusions:

1. The biological activity of QAS depends on alkyl carbon chain length; the most active being QAS having 12–14 carbon membered chain [2]. Chain elongation or shortening leads to a decrease in inhibitory activity.

2. Respiratory-defficient *rho*$^-$ mutants are much more sensitive to QAS than otherwise isogenic respiratory competent (*rho*$^+$) parental strains [3].

3. The minimum inhibitory concentration (MIC) of QAS for auxotrophic yeast mutants is clearly lower than for isogenic prototrophs [4] and the difference is better seen in a complete YPD medium. The primary target of QAS could thus be the transport of amino acids. In a complete medium the auxotrophs are dependent on the uptake of amino acids, whose synthesis is blocked by the mutation. In the presence of QAS, the mutants suffer from amino acid starvation whereas prototrophs carry out amino acid biosynthesis. This conclusion was supported by our earlier study [5] showing a direct effect of QAS on methionine transport in yeast.



To obtain a more direct data we isolated a series of mutants (EO) with increased resistance to one of the most active QAS: N-dodecyl-N,N,N-trimethylammonium chloride (IM). The MIC for the mutants was only a little more than double that for the parental strain Σ1278b, yet they were monogenic and resulted from a single-gene mutation as evidenced by the 2:2 segregation of QAS resistance to QAS sensitivity in tetrads of crosses of the mutants to isogenic QAS-sensitive strain of opposite mating type, and 4:0 of resistance to sensitivity segregation in mutant-to-mutant crosses [3,6]. If QAS indeed inhibit amino acid uptake, there should be some difference in the degree of the inhibition between the QAS-resistant mutants and their original QAS-sensitive strain.

The influence of the quaternary salt IM on DL-leucine transport in the EO-25 mutant under repression and derepression conditions was confronted with that obtained with its parent strain.

When Σ1278b cells grown in a complete YPD medium to the mid-exponential phase were suspended in distilled water containing 2 % glucose and supplemented with [^{14}C]DL-leucine, the amino acid uptake was inhibited by IM in a concentration-dependent manner. In the presence of 640 μmol/L IM the amino acid uptake was scarcely detectable (Fig. 1).

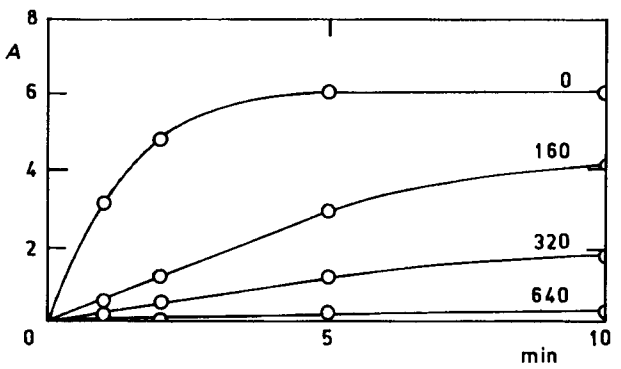


Fig. 1. DL-[^{14}C]-leucine uptake (*A*, cpm) in Σ1278b strain at different IM concentrations (μmol/L).

In IM resistant mutant cells grown in the same conditions the leucine uptake activity was nearly half of that in strain Σ1278b but was clearly less sensitive to IM inhibition (Table I). No inhibition was observed in the presence of 160 μmol/L IM while the effect of 320 μmol/L IM was about half than in the Σ1278b strain. Surprisingly, respiration-deficient Σ1278b *rho*$^-$ forms, which are otherwise much more sensitive to IM than respiration-competent *rho*$^+$ cells, did not exhibit any stronger IM-induced suppression of essential differences in leucine uptake. Likewise, no differences were observed between EO-25 *rho*$^+$ and *rho*$^-$.

The differences in leucine uptake between Σ1278b and EO-25 mutant cells were not so drastic, but one must take into account that they correspond to those in MIC; the EO-25 is only resistant to a twice higher concentration of IM than Σ1278b.

Because amino acid uptake in yeast is dependent on repression/derepression conditions, we decided to investigate the influence of IM on leucine uptake by yeast grown in repressed and derepressed conditions. Figs 2A

Table I. Effect of IM on DL-[^{14}C]-leucine uptake and assimilation by respiration-competent (*rho*$^+$) and -deficient (*rho*$^-$) sensitive wild type cells and the QAS-resistant mutant EO-25

Strain		Leucine uptake		Per cent assimilation in the presence of IM	
		Amount[a]	%	160[b]	320[b]
Σ 1278b	*rho*$^+$	615	100	67	30
	rho$^-$	543	100	82	29
EO-25	*rho*$^+$	360	100	110	61
	rho$^-$	398	100	105	52

[a]cpm/mg dry mass per min.
[b]μmol/L.

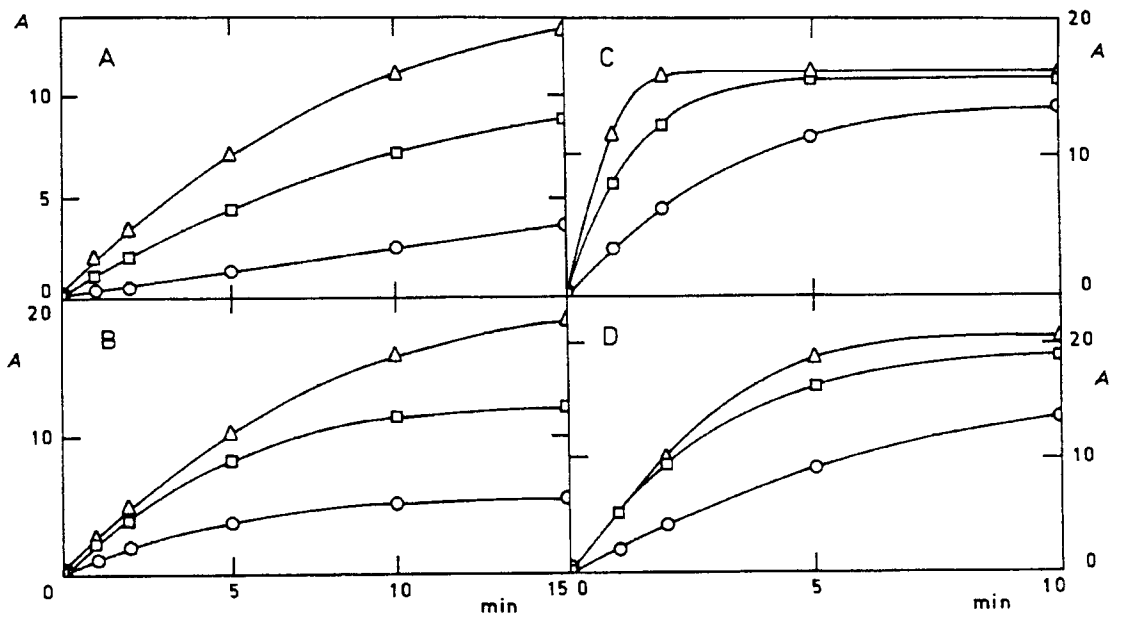


Fig. 2. Effect of IM (mmoL/L; □ – 160, O – 320; Δ – control) on the uptake of DL-[^{14}C]-leucine: (*A*) by Σ1278b yeast cells grown in a minimal medium (repressed); (*B*) by QAS-resistant mutant EO-25 grown in a minimal medium (repressed); (*C*) by Σ1278b yeast cells grown in a minimal medium and than starved for 2-h (derepressed); (*D*) by QAS-resistant mutant EO-25 grown in minimal a medium (derepressed).

and 2B show the kinetics of leucine uptake by Σ1278b and EO-25, respectively, grown in minimal Yeast Nitrogen Base medium (repressed). It can be seen that the mutant cells assimilated leucine more actively than those of Σ1278b but the relative degree of inhibition seems to be similar.

When the yeast cells were grown in the same medium and then aerated for 2 h in water supplemented with 2 % glucose, the mutant cells accumulated leucine at a lower rate than Σ1278b. On the other hand, they accumulated leucine to a higher level and the inhibitory activity of QAS was hardly detectable. In addition, 160 μmol/L IM was inactive while at 320 μmol/L the inhibition did not exceed 50 per cent (Fig. 2C and 2D).

Table II. Effect of IM on DL-[^{14}C]-leucine uptake by yeast cells grown on proline as nitrogen source

Strain	IM[a]					
	0[b]		160[b]		320[b]	
Σ1278b	3686	100	721	19	258	7
EO-25	1187	100	1066	90	808	68

[a]Inhibition – cpm/mg protein per min (first column); % (second column).
[b]μmol/L.

Howewer, when the cells were grown on Yeast Carbon Base supplemented with proline (derepressed conditions) and directly used for leucine uptake measurement (Table II), a clear-cut difference in the degree of inhibition by IM between the parent strain and the mutant was observed although the amino acid uptake by the EO-25 mutant was twice lower. The results indicate that the quaternary ammonium salt inhibited leucine transport under derepressed conditions and that this leucine transport system was resistant to quaternary ammonium salt inhibition in EO-25 mutant. A similar inhibition of L-leucine uptake by a bis-quaternary ammonium salt in derepressed yeast has been observed previously [7].

CONCLUSIONS

1. Quaternary ammonium salts seem to be potent inhibitors of amino acid uptake in *S. cerevisiae.*
2. Inhibition is the strongest in yeast with derepressed transport (growth in Yeast Carbon Base medium with proline as the sole nitrogen source).
3. The single-gene resistance to quaternary ammonium salts leads to lowering of transport inhibition.

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Catabolic Activity, Energy State and Transport of Organic Acids in *Candida utilis* at Different Osmotic Pressures

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The objective of the study was to find interrelations among catabolic activity, transport of partially oxidized intermediates and energy state of resting *Candida utilis* cells utilizing either endogenous reserves only or external ethanol (cell energization by its addition). Changes of the individual processes and/or parameters were studied under hypoosmotic, hyperosmotic and physiologically appropriate conditions.

The microorganism used was *C. utilis* strain 136 from the culture collection of the *Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology*, Prague. The medium composition and culture conditions were described previously [1]. The cells used in the experiments were obtained from a fed-batch culture. After separation, the cells were washed three times and then suspended in