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ARTICLE *in* FEBS LETTERS · JANUARY 1976

Impact Factor: 3.17 · DOI: 10.1016/0014-5793(75)80737-X · Source: PubMed

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THE USE OF A HOMOLOGOUS SERIES OF AFFINITY LABELING REAGENTS IN THE STUDY OF THE BIOTIN TRANSPORT SYSTEM IN YEAST CELLS

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Received 23 October 1975

1. Introduction

The method of affinity labeling, originally characterized for the investigation of purified enzymes [1], has found vast application in studying the structure of active sites in heterogeneous systems, e.g., antibodies [2], ribosomes [3] and other sub cellular components such as transport components on membrane vesicles [4]. Affinity labeling of active transport systems on whole cells has been attempted in the past with both varying success and practical implications [5].

A recent report of a convenient system for study [6], described the specific and irreversible inactivation of biotin transport in whole cells of the yeast, *Saccharomyces cerevisiae*, by an active-ester derivative of biotin, biotinyl-*p*-nitrophenyl ester (BNP). This system is of particular interest in that such inactivated cells could be totally reactivated upon treatment with thiols [7].

In the present investigation, we would like to report an expansion of the above approach, by using a homologous series of biotin-containing derivatives, in which the chemically reactive *p*-nitrophenyl ester group is located at increasing distances from the biologically-active ureido moiety. The affinity labels were selected in order to encompass chain-lengths corresponding to those of biotin and biocytin, both of which occur in nature. The inhibitory capacity of each derivative was examined and compared. From studies of this nature, structural information may be obtained for heterogeneous systems, in which X-ray crystallographic analysis is inconceivable.

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2. Materials and methods

2.1. *Fundamental chemical reagents*

Generous quantities of biotin, norbiotin and homobiotin were kindly supplied as gifts from the research laboratories of F. Hoffman-La Roche and Co., Basle. Unbranched ω-amino carboxylic acids were purchased from Sigma Chem. Co. (St. Louis, Missouri). Dicyclohexylcarbodiimide, dimethylformamide, *p*-nitrophenol and *N*-hydroxysuccinimide were acquired from Fluka (Buchs, Switzerland).

2.2. *Synthesis of homologous series of affinity labels*

The homologous series of biotin-containing affinity labels were synthesized by reacting biotinyl *N*-hydroxysuccinimide ester [8] and the corresponding ω-amino carboxylic acid in a mixture of dimethyl formamide and sodium bicarbonate solution. The isolated biotinyl-amino acid was further reacted with *p*-nitrophenol as described [6,7]. The synthesis of norbiotinyl- and homobiotinyl-*p*-nitrophenyl esters was accomplished in a similar fashion to that of the biotinyl derivative [6,7]. All compounds had satisfactory analyses. Experimental and analytical details will be reported in a subsequent article.

2.3. *Inactivation and reactivation of whole cells*

The strain of yeast and growth conditions used in this study are identical to those previously reported [7]. In a typical experiment, growth cultures (200 ml) were harvested, washed three times, and resuspended to 1.1×10^8 cells/ml with 50 mM potassium phosphate solution (pH 4.0). Cell samples (9 ml) were treated for 30 min at 30°C with an ethanolic solution (1 ml) containing one of the affinity label homologues (to a

final concentration of 25 μM). Ethanol (1 ml) was added to control cells. Cells treated in this manner were then washed three times with distilled water and resuspended to 10 ml. A sample (0.5 ml) was added to 50 mM potassium phosphate solution (pH 4.0) containing glucose (1% final concentration; 5.0 ml final vol). After incubation for 20 min at 30°C, 50 μl [^{14}C]biotin (5 μg/ml) was added. Aliquots (1 ml) were taken at various times, and the cells collected and washed on glass-fiber filters (Tamar, Israel). Counting of radioactive samples was performed with a Packard model 3003 Tri-Carb liquid scintillation spectrometer [9].

The remainder of the inhibited cells was centrifuged and treated with a solution (5.0 ml) of 0.2 M mercaptoethanol (pH 4.0). After 30 min at 30°C, cells were washed three times and resuspended to the original volume (9.5 ml). A sample (0.5 ml) was assayed as above for biotin uptake.

2.4. *Calculation of percent inactivation and percent reactivation*

The percent inhibition (%I) and percent reactivation (%R) were determined according to the following equations:

$$\text{equation A:} \quad \%I = \frac{C_O - C_I}{C_O} 100$$

$$\text{equation B:} \quad \%R = \frac{C_R - C_I}{C_O - C_I} 100$$

where C_O, C_I and C_R represent the amount of biotin taken up by ethanol-treated control cells, affinity labeled cells, and mercaptoethanol-treated inhibited cells, respectively.

3. Results and discussion

The homologous series of affinity labeling reagents employed in this investigation (table I) comprises nine distinct biotin-containing derivatives in which the active carbonyl-carbon is situated at varying lengths from the bicyclic ring system of the biotin moiety. Three of these compounds (I) are active-ester derivatives of biotin and its analogues: norbiotin and homobiotin. The rest (II) have unbranched ω-amino carboxylic acids attached to biotin via a peptide bond, thereby displacing the terminal carboxyl group. The shortest of the series is norbiotinyl-*p*-nitrophenyl ester, having one side-chain methylene group less than the reference compound, biotinyl-*p*-nitrophenyl ester. The longest derivative is biotinyl-ε-aminocaproyl-*p*-nitrophenyl ester which is an analogue of the native biocytin.

In order to compare the capacity of these affinity labels, the percent inhibition of normal biotin uptake as a function of the relative chain length of the affinity label is presented in fig.1. Cell samples were treated with affinity labels and assayed for biotin uptake under identical conditions. Observed differences in the inhibitory capacity of individual affinity labels are probably a function of the length of the side chain and the architecture at or near the active site, since (a) the chemical reactivity of the *p*-nitrophenyl group in homologous derivatives are probably very similar, and (b) the biological activity is attributed to the recognition of the ureido ring [10]. Inactivation of transport, therefore, is ostensibly ascribed to the relationship between the positioning of the active carbonyl-carbon and the location of a modifiable amino acid residue(s) near the active site.

It is evident from fig.1 that the shorter-length affinity labels tend to be more effective inhibitors of biotin uptake, and the inhibition decreases gradually with an increase in chain length. BNP is demonstrat-

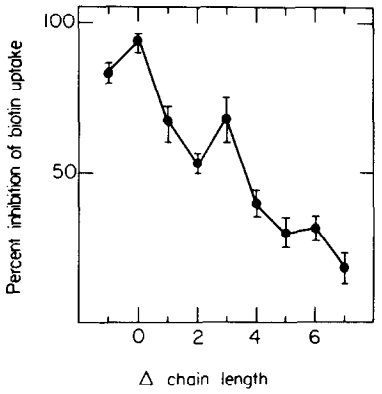
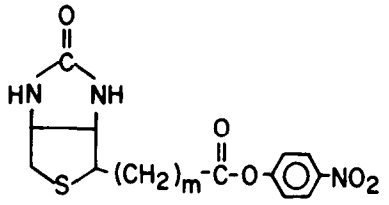


Fig.1. Percent inhibition of biotin uptake versus relative chain length of homologous affinity label. ΔChain length is defined in table 1. Percent inhibition is calculated according to equation A. The average and standard error of several experiments are shown.

Table 1

Homologous series of biotin-containing affinity labels



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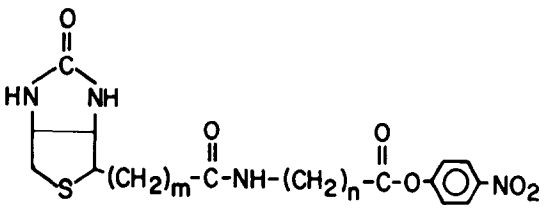

II

p-nitrophenyl ester	abbreviation	m	n	Δ chain length *
norbiotinyl	-1BNP	3	-	- 1
biotinyl	BNP	4	-	0
homobiotinyl	+1BNP	5	-	1
norbiotinyl-glycyl	-1B3NP	3	1	2
biotinyl-glycyl	B3NP	4	1	3
biotinyl-β-alanyl	B4NP	4	2	4
biotinyl-γ-aminobutyryl	B5NP	4	3	5
biotinyl-δ-aminovaleryl	B6NP	4	4	6
biotinyl-ε-aminocaproyl	B7NP	4	5	7

* Deviation of side arm from reference affinity label (BNP) in number of backbone atoms

ed to be the most effective affinity label, and reagents longer than B5NP barely affect normal uptake. However, the phenomena of covalent binding and inhibition of transport activity are not necessarily mutually inclusive. The low amounts of observed inhibition by longer derivatives could be due to competitive inhibition between free biotin and a covalently bound, long-chain inhibitor, relatively distant from the receptor site. The lack of inhibition by B7NP is somewhat surprising, since it could be expected that this compound (which is an analogue of the naturally-occurring biotin analogue, biocytin) would be as effective an inhibitor as BNP. However, as a long-chain derivative of biotin, either it does not bind covalently, or it binds, but not in the vicinity of the active site.

Since we have shown previously that the BNP-labelled cell can be reactivated with thiols [7], we wanted to see to what extent cells, inhibited with the homologous derivatives, could also be reactivated. Reactivation would implicate the modification of the same residue or a residue of a similar nature (cysteine or histidine in this case). As can be seen from table 2, it appears that (with the exception of + 1BNP-inactivated cells) cells which have undergone reasonable inhibition with a given reagent, can also be reactivated. The shortest residues, −1BNP and BNP, have the greatest potential for reactivation, and are presumably directed towards cysteine (or histidine). The increment of an additional methylene group, in the case of + 1BNP, has a marked effect

Table 2
Percentage of uptake (inhibited cells/control cells) before and after mercaptoethanol treatment

Derivative	$\frac{C_I}{C_O}$ [a]	$\frac{C_R}{C_O}$ [a]	% R [b]
– 1BNP	0.16	0.95	0.94
BNP	0.06	0.82	0.81
+ 1BNP	0.34	0.49	0.23
– 1B3NP	0.46	0.83	0.68
B3NP	0.32	0.75	0.65
B4NP	0.61	0.87	0.66
B5NP	0.71	0.74	0.09
B6NP	0.68	0.69	0.01
B7NP	0.82	0.88	0.20

[a] C_O, C_I and C_R are defined in Materials and methods.
[b] Percent reactivation is calculated according to equation B (Materials and methods).

upon the propensity for reactivation. The bulk of this reagent apparently binds to a different residue, such as lysine. The resultant peptide bond would not be labile to thiolysis. These results suggest the existence of at least two nucleophilic amino acids adjacent to the binding site of the biotin transport system.

Cells, inhibited with derivatives –1B3NP to B4NP, undergo intermediate reactivation. These reagents are apparently capable of binding both types of nucleophilic amino acid residues. It should be noted that the relationship between inhibition (and reactivation) with chain length is probably far more complicated than a simple expression of relative distances. The longer chain-lengths are probably more flexible, i.e., able to bend and form loops. This is further complicated by the presence of a peptide bond, inherent in the biotinyl-ω-amino carboxylic acid derivatives, unknown steric factors might influence the emplacement of the active carbonyl-carbon. The observed reactivation of cells, inhibited with medium chain-length derivatives, might therefore reflect the ability of these reagents to revert to a position where the reactive group can bind to residues closer to the active site.

Nevertheless, this technique can potentially provide significant structural information in heterogeneous systems. Further studies of this nature may contribute important insight regarding the mechanism involved in the transport process.

Acknowledgement

This work was supported in part by a grant from the Israel Commission for Basic Research.

References

[1] Baker, B. R. (1967) Design of Active-Site-Directed Irreversible Enzyme Inhibitors, Wiley, New York.
[2] Givol, D., Wilchek, M., Eisen, H. M. and Haimovich, J. (1972) in: Proc. Fed. Eur. Biochem. Soc. 8th Meet., Amsterdam, 1972, 26, 77–86.
[3] Sonnenberg, N., Wilchek, M. and Zamir, A. (1973) Proc. Natl. Acad. Sci. US. 70, 1423–1426.
[4] Rudnick, G., Kaback, H. R. and Weil, R. (1975) J. Biol. Chem. 250, 1371–1375.
[5] Chavin, S. I. (1971) FEBS Lett. 14, 269–282.
[6] Becker, J. M., Wilchek, M. and Katchalski, E. (1971) Proc. Natl. Acad. Sci. USA 68, 2604–2607.
[7] Viswanatha, T., Bayer, E. and Wilchek, M. (1975) Biochim. Biophys. Acta 401, 152–156.
[8] Bayer, E. and Wilchek, M. (1974) Meth. Enz. 34, 265–267.
[9] Bray, G. A. (1960) Anal. Biochem. 1, 279–285.
[10] Rogers, T. O. and Lichstein, H. C. (1969) J. Bacteriol. 100, 557–664.