Stereospecificity of the *myo*-Inositol Transport System of *Aerobacter aerogenes* and Sensitivity Towards Sulfhydryl Reagents

In a previous report from this laboratory[1], an active and inducible transport system for *myo*-inositol in *Aerobacter aerogenes* was described. The stereospecificity of the transport system has been investigated with 21 different cyclitols. Since the labelled cyclitols are not available, the inhibitory effect on *myo*- or *scyllo*-inositol uptake was tested. Cyclitols which differ from *myo*-inositol structure by no more than one modification, retain their affinity for the carrier; those which differ more extensively do not bind with a sufficient affinity. The transport system is very sensitive to sulfhydryl reagents such as *p*-hydroxymercuribenzoate (PHMB) or N-ethylmaleimide (NEM). Full protection against the latter was afforded by 0.01 *M* *myo*-inositol.

Materials and methods. *Aerobacter aerogenes* strain, growth and uptake conditions were already described[1] and the results are related to 1 mg of cells (dry weight). The sulfhydryl reagents were added after 20 min of starvation and the suspension was again shaken for 10 min. The protective effect of substrate was tested as follows: after 10 min exposure to NEM, NEM plus *myo*-inositol or *myo*-inositol alone, the cells were suspended in a carbon-free medium and starved for 30 min. The assay started with the addition of ^{3}H-*myo*-inositol. Kinetic parameters were calculated using the WILKINSON[2] hyperbolic adjustment on a 1620 IBM computer. The values were compared to those obtained by LINEWEAVER and BURK regression[3]. Depending on the concentrations of inhibitor used, the calculations of kinetic parameters were performed according to MARK and ROMANO[4] and CIRILLO[5].

Radiochemical Center (Amersham, U. K.) supplied (2-^{3}H)*scyllo*-inositol. (2-^{3}H)*myo*-inositol was purchased from NEN (Dreieichenhain, Germany).

Results and discussion. Since many bacterial transport systems have been shown to be sensitive to sulfhydryl reagents, the effects of 2 such substances, NEM and PHMB, were investigated on *myo*-inositol transport. 10^{-5} *M* PHMB produced an 80% inhibition, and complete inhibition was observed at 10^{-4} *M*. NEM was less effective: the inhibition observed was 25–30% at 10^{-4} *M* and 62–75% at 10^{-3} *M* (Figure 1). The protective effect of substrate (10 m*M*), in the presence of 1 m*M* NEM, was investigated. Controls were made either without NEM or without *myo*-inositol. The results show that the substrate, when present in saturating amounts, protects the transport system against the action of NEM (Figure 2). This suggests that essential sulfhydryl groups are located at or near the *myo*-inositol binding sites: when these sites are saturated, the inhibitor cannot react with them.

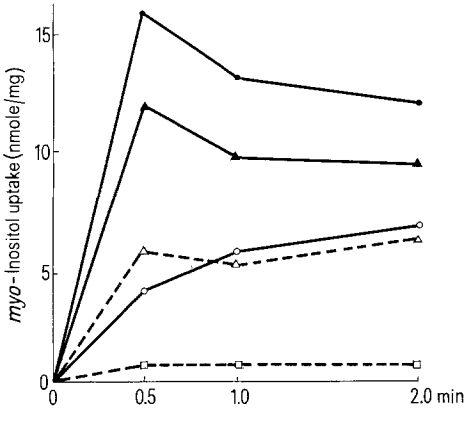

Fig. 1. Effect of sulhydryl reagents on *myo*-inositol transport. (2-^{3}H)*myo*-inositol concentration was 0.55 m*M*. ●—●, Control; ▲—▲, NEM 10^{-4} *M*; △---△, NEM 10^{-3} *M*; ■—■, PHMB 10^{-5} *M*; □---□, PHMB 10^{-4} *M*.

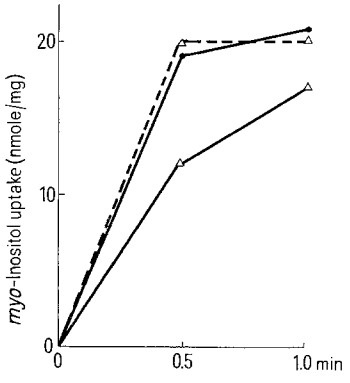

Fig. 2. Substrate protection against NEM action. Cells were treated as described in Materials and methods. △---△, NEM 10^{-3} *M* plus *myo*-inositol 10^{-2} *M*; △—△, NEM 10^{-3} *M*; ●—●, *myo*-inositol 10^{-2} *M*. Uptake assay started with addition of 0.55 m*M* (2-^{3}H) *myo*-inositol.

Since most of the cyclitols are not available as labelled compounds, the stereospecificity was studied by the inhibition of the *myo*-inositol uptake. The Table shows the steric modifications of the compound tested, in comparison with the structure of *myo*-inositol. The results presented show that in general any single modification in the *myo*-inositol molecule does not destroy the ability to inhibit, as long as the size of the new group does not exceed a certain limit. At carbon 1 and 3, inversion of hydroxyl groups diminishes the inhibitory effect less than *o*-methylation, and the lack of an hydroxyl group at the same positions hinders inhibition less than inversion. At C-2, the presence of a phosphate group completely destroys the inhibitory activity, probably because of the anionic charge introduced by the latter. Complete absence of the hydroxyl group, its inversion or its replacement by a ketone does not reduce the inhibitory effectiveness to any great extent. Substitution of the hydrogen at the same position by a methyl group makes the substance a less effective inhibitor.

There is little effect of inversion at C-4 and C-6, or when a methylene hydrogen is replaced by a methyl group.

Compounds which differ from *myo*-inositol at C-5 were tested as inhibitors of *scyllo*-inositol entry because they are also inhibitors of the dehydrogenation of *myo*-inositol. The first step of *myo*-inositol metabolism in *A. aerogenes* is a dehydrogenation at C-2 position with concommitant formation of 2-keto-*myo*-inositol[6]. Since the substrate used becomes non-radioactive upon conversion to the keto derivative, measurements of *myo*-inositol uptake

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Cyclitol	Modification	K_i (mM)
L-inositol [a]	C-1, inversion	1.8
(+) Bornesitol [c]	C-1, *o*-methyl	0.025
scyllo-Inositol [a]	C-2, inversion	0.16
Isomitilitol [b]	C-2, C-methyl	0.37
myo-Inositol-2-P [b]	C-2, *o*-phosphate	3.84
2-Keto-*myo*-inositol [b]	C-2, keto	0.22
D-inositol [a]	C-3, inversion	1.5
(−) Bornesitol [a]	C-3, *o*-methyl	0.7
(±) Viburnitol [a]	C-1(3), deoxy	0.45
5-Deoxy-inositol [b]	C-5, deoxy	1.5
neo-Inositol [c]	C-5, inversion	0.45
Sequoyitol [c]	C-5, *o*-methyl	0.02
(±) *epi*-Inositol [a]	C-4(6), inversion	0.53
Laminitol [a]	C-6, C-methyl	0.16
(−) Galaquercitol [b]	C-6, deoxy C-3, inversion	25
(+) Taloquercitol [b]	C-6, deoxy C-5, inversion	20
(±) Alloquercitol [b]	C-5, deoxy C-4(6), inversion	20
1,3/2,5-Cyclohexanetetrol [b]	C-1, C-3, deoxy	55
(±) 2,3,5/6-Cyclohexanetetrol [b]	C-1, C-4, deoxy	262
muco-Inositol [b]	simultaneous inversion C-3, C-4; C-3, C-1; C-1, C-6	51
allo-Inositol [a]	simultaneous inversion C-4, C-5; C-5, C-6; C-3, C-6; C-1, C-4; C-1, C-5; C-3, C-5	50

[a] Indicate substances (1.1 mM) tested in the presence of various concentrations of *myo*-inositol (0.27, 0.55 and 1.1 mM). [b] Indicate substances tested at a concentration 50-fold higher than *myo*-inositol (0.55 mM). [c] Indicate substances tested as uptake inhibitor of *scyllo*-inositol (0.055, 0.11 and 0.56 mM).

represents the difference between the total *myo*-inositol taken up and the radioactivity lost by dehydrogenation. We have suggested in a previous report that, at the end of the assay period, no more than 70% of the total intracellular *myo*-inositol is present as such[1]. Substances which compete with *myo*-inositol as substrate of the dehydrogenase, or which inhibit the dehydrogenase without being substrate, are thus capable of apparently stimulating uptake, since they prevent loss of label from *myo*-inositol. Accordingly those substances ((+)bornesitol, *neo*-inositol and sequoyitol) were tested as inhibitors of *scyllo*-inositol transport. The results obtained can be related to those for *myo*-inositol because of the close correspondence of the transport properties of these two cyclitols (DESHUSSES and REBER, unpublished).

Removal of any hydroxyl group, and simultaneous inversion, is not sufficient to produce a large inhibitory effect. But after 2 simultaneous inversions or removal of hydroxyl groups, the compound is completely non-inhibitory. As a general rule, it is possible to conclude that two or more modifications in the basic structure of *myo*-inositol render the molecule non-inhibitory.

Résumé. Des quantités saturantes de *myo*-inositol sont capables de protéger les groupes SH impliqués dans le transport actif du cyclitol contre les réactifs de ces groupes chez *Aerobacter aerogenes*. La spécificité du système est restreinte aux cyclitols ne présentant pas plus d'une modification par rapport à la structure du *myo*-inositol.

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Evidence for the Mediation of Indole-3-acetic Acid Effects Through its Oxidation Products

While some workers ascribe the role of detoxification of indole-3-acetic acid (IAA) to IAA-oxidase[1], others consider that it produces oxidation products to cause physiological responses characteristic of auxin[2–5]. If this view is valid, the exogenous application of oxidation products should cause effects identical to those of auxin or chemicals like sodium metabisulfite which promote the formation of initial products of IAA oxidation[3,6], should enhance a given physiological response of IAA.

Work to test this hypothesis was undertaken in this laboratory, using adventitious root formation on mung bean hypocotyl cuttings, as a bioassay. It has been reported that these cuttings do not root in water but root in 1% sucrose and more profusely in 1% sucrose + 5 mg/l IAA[7].

The seedlings were raised from uniform seeds in Petridishes lined with cotton pads in the dark in growth chambers maintained at 28 ± 2°C. 480 uniform 7-day-old seedlings were selected for experimentation. These were made into cuttings by excising the cotyledons and also

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