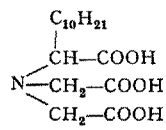
Acceleration of Metal Transport in Yeast Cells Treated with a Lipophilic Complexone

It is generally agreed that in a series of related substances the ease of penetration through a cell membrane is parallel to the lipophilic nature of the substance. The usual chelating agents, such as ethylenediaminetetraacetic acid (EDTA), do not penetrate into cells[1,2]. Assuming that lipophilic homologues of aminopolycarboxylic acids capable of penetration into cells could in some cases be highly effective as decontaminating or enzyme blocking agents, a decyl derivative of the nitrilotriacetic acid (DNTA) was prepared by reaction of α-bromolauric acid with iminodiacetic acid:



(DNTA)

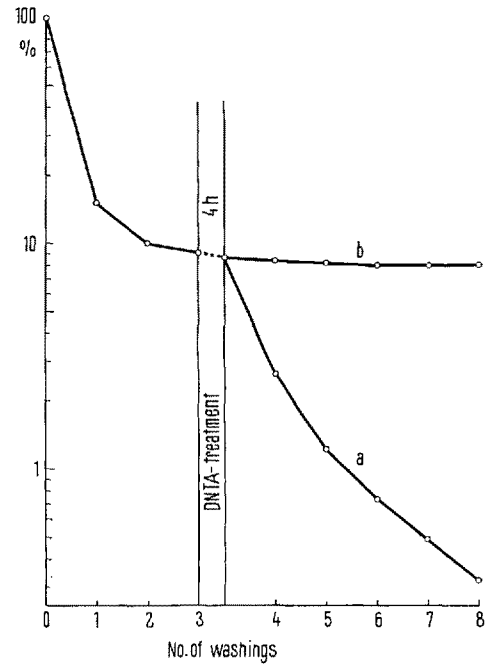
This soap-like substance is soluble in alcohols, ketones, esters, fats, and hydrocarbons. Salts are soluble in water showing surface activity. Preliminary polarographic studies with copper, zinc and mangenese indicated that the stability constants of complexes were only 1 order lower than those of the low molecular NTA, and that the acidity of carboxyl groups was not substantially altered by this substitution.

For biological experiments, baker's yeast was taken as a convenient and well-studied test organism[3,4]. Cerium was selected for the first experiments as an important fission product with high affinity to aminopolycarboxylic acids, for comparison purposes with other studies[5,6], and because its entry into enzyme systems is hardly to be expected. It was found that the adsorption of DNTA on yeast cells is very high and rapid at pH 7.4, in contrary to low molecular NTA and EDTA, the adsorption of which was not detectable. No desorption of DNTA in acetate buffer (pH 4.3) was observed. Having obtained this evidence of strong binding of DNTA with yeast cells, the following experiment was arranged in order to investigate the role of DNTA in cerium transport from yeast to EDTA solution. In all solutions, respiration was supported by aeration in presence of glucose, phosphate, and potassium.

The yeast was contamined with pure Ce^{144} in a solution of $0.08M$ $KH_2PO_4 + 0.2M$ glucose (pH 4.5, t 25 °C); mixing was accomplished by bubbling with air. No inactive cerium was added. After 90 min, the cells were separated by centrifugation and repeatedly washed with $0.05M$ EDTA in phosphate buffer (pH 6), for 15 min in intervals of 30 min (10 ml/g yeast). After 3 washings, the yeast was divided into 2 portions. Portion (A) was treated for 30 min with DNTA solution in phosphate buffer pH 7.4 ($2 \times 10^{-5} M$/g yeast), washed shortly with water and acetate-phosphate buffer (pH 4.3), and incubated for 4 h at 37 °C. Portion (B) was treated parallel in the same manner, the DNTA being substituted by the same amount of NTA. Then, washings were continued with $0.05M$ EDTA buffered to pH 4.3. The γ-activity of all supernates and of separated yeast was determined (in radioactive equilibrium) by means of a well type scintillation counter. Using these measurements, the decrease of Ce^{144}-content of yeast was calculated, expressed in % of the originally adsorbed amount, i.e. approximately 0.1 μc/g yeast (Figure).

It is evident that the greater portion of Ce is removed very rapidly, the lesser portion being bound much more strongly. Taking into account a similar study of ROTHSTEIN with Mn (II)[3,4], it can be supposed that the latter portion has passed the membrane of respiring yeast cell, becoming much less available to extracellular desorbing agents. The treatment with low-molecular NTA evidently did not influence the desorption rate (curve b). However, striking increase of desorption rate was achieved in yeast cells that had been treated with the lipophilic DNTA (curve a). The most probable interpretation of this effect is that DNTA acts as artificial 'carrier' or 'specific receptor' mediating the 'facilitated diffusion', which is 1 of the generally accepted membrane transport mechanisms.

Additional experiments indicated that: (1) desorption with diethylenetriaminopentaacetic acid is not significantly higher than that with EDTA; (2) the effectivity of 'sensibilization' with DNTA increases with time and temperature of incubation, and is lower at higher pH; (3) using palmitic acid instead of DNTA, no effect of the same order was observed; (4) although the EDTA-Ce complex formation (in competition with DNTA-Ce complex) is favourized by higher pH-value of desorbing solution, no substantial increase of decorporation rate from senzibilized cells was observed; (5) instead of EDTA, cerium or yttrium solution can be used as desorbing agent, with similar difference between DTPA-treated and non-treated cells.



Decrease of Ce^{144} content of cells before and after treatment with DNTA (curve a), and with NTA (curve b), in the course of repeated washings with EDTA (for 15 min every 30 min).

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With respect to these experiments, the transport in yeast cells is to be accepted as the rate-determining step. Having accepted the carrier mechanism as working hypothesis for further studies, it can easily be deduced that it is not necessary for the carrier to have the chelating activity desirable for an appreciable equilibrium-shift in an isolated system cell-metal-carrier. Evidently, the desorbing solution outside the cell will steadily disturb this inner equilibrium, tending to establish the equilibrium distribution of metal between the cell and the solution. The chelating activity is rather only one of more rate-determining factors. Hence, some chelating agents of medium strength with convenient selectivity and acidity can be expected to be effective too, if substituted with an alkyl chain, in altering the metal permeability of cells in a desired manner and sufficient degree.

Zusammenfassung. Es wird ein neues lipophiles Derivat der Nitrilotriessigsäure (NTA) dargestellt, eine Decylnitrilotriessigsäure (DNTA). Aus mit Ce^{144} kontaminierten Hefezellen wird durch Äthylendiamintriessigsäure (EDTA) bedeutend mehr Ce ausgewaschen, wenn die Zellen mit DNTA vorbehandelt worden sind, während Vorbehandlung mit NTA keine Steigerung bewirkt. Wahrscheinlich wirkt DNTA dank seiner Lipophilie als «Carrier» des Metalls durch die Zellmembran. Es wird auf die Möglichkeit hingewiesen, durch Alkylierung die Membrandurchlässigkeit auch anderer Chelatbildner zu verändern.

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Effect of Tween 80 on Protein-Tannic Acid Complex[1]

In previous experiments we demonstrated that Tween 80 could be used for the preparation of enzyme active extracts from the needles of adult conifers[2]. The same product has also been used by GOLDSTEIN et al.[3] to prevent tannic acid inhibition of a commercial preparation of β-glycosidase and by us of an extract from the bark of *Pinus pinea* on three purified dehydrogenases[4]. The hypothesis was advanced that tannic acid and polyphenols in general, at low concentrations, formed soluble complexes with enzymes, blocking their activities and Tween 80 reactivates these activities by separating the polyphenols from the protein.

In the present work we intended to confirm this hypothesis by studying the formation and breaking of the tannic acid-protein complex by means of Tween 80. We have measured the absorption spectra respectively of the protein, the tannic acid, and of the complex protein-tannic acid and, at last, of the same in the presence of Tween 80. Cytochrome C and metamyoglobin were chosen because the spectrum of these proteins shows a very characteristic behaviour in the visible light, whereas tannic acid and Tween 80 instead have slight and undifferentiated absorption. Cytochrome C was purchased from Boehringer, Mannheim (Germany), metamyoglobin from Kock-Light Laboratories Ltd., Colnbrook Buckinghamshire (England), tannic acid from Manetti and Roberts, Florence (Italy), Tween 80 from Fluka AG, Buchs (Switzerland).

Absorption spectra were done with a Beckman DB spectrophotometer equipped with a Sargent mod. SR recorder. The results are reported in Figures 1 and 2.

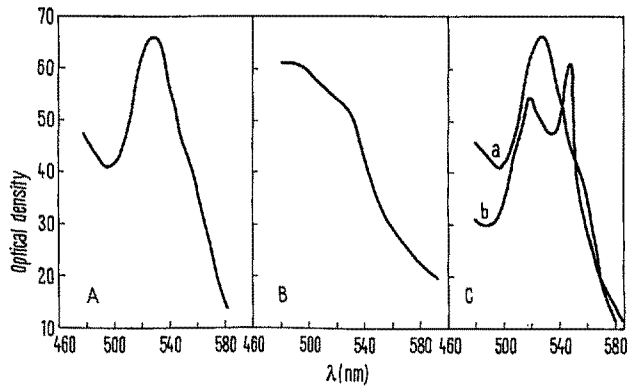

Fig. 1. (A) Spectrum of 0.1 mM oxidized cytochrome C, pH 6; final volume 2 ml. (B) Spectrum of 0.1 mM oxidized cytochrome C – 1.5 mM tannic acid complex, pH 6; final volume 2 ml. (C) (a) Spectrum of 0.1 mM cytochrome C – 1.5 mM tannic acid complex after the addition of 50 μl of Tween 80, pH 6; final volume 2 ml. (b) Spectrum of 0.1 mM cytochrome C reduced by its precipitation with 10 mM tannic acid and resuspension with 50 μl of Tween 80, pH 6; final volume 2 ml.

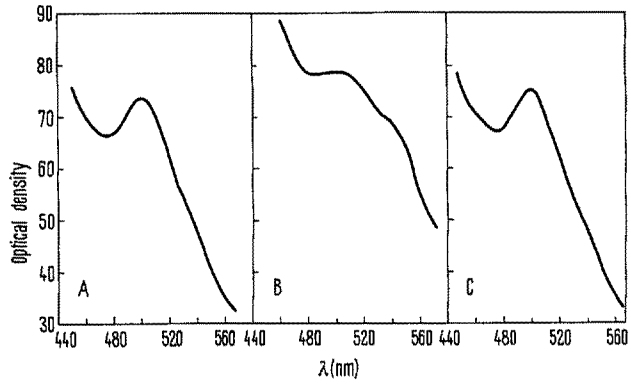

Fig. 2. (A) Spectrum of 0.1 mM metamyoglobin, pH 6; final volume 2 ml. (B) Spectrum of 0.1 mM metamyoglobin – 1.5 mM tannic acid complex, pH 6; final volume 2 ml. (C) Spectrum of 0.1 mM metamyoglobin – 1.5 mM tannic acid complex, after addition of 50 μl of Tween 80, pH 6; final volume 2 ml.

[1] This work was supported by a grant from the «Impresa Enzimologia» of the Italian Consiglio Nazionale delle Ricerche and by Grant No. FG-It-128 from the U.S. Department of Agriculture.

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