Chromatographic Forms of Baker's Yeast Cytochrome c

Chromatographic behaviour of baker's yeast cytochrome c on cation exchangers has been studied extensively. Chromatography on Amberlite IRC-50 gives three main fractions, but only the first one, regarded as the native form, has been well characterized[1-3].

This paper is concerned with the study of the various fractions, in an attempt to determine whether they are pre-existing molecular forms or conformational modifications resulting from the preparation procedure according to HAGIHARA[1].

In Table I some properties of the three fractions with yields of recovery are listed.

The first fraction arising from Amberlite IRC-50 pH 7 is identical with the preparation obtained by YAOI et al.[3] and has an E_{550}/E_{280} ratio of 0.9 after reduction with dithionite. The other fractions show a lower E_{550}/E_{280} ratio, but possess the typical absorption spectrum of cytochrome c.

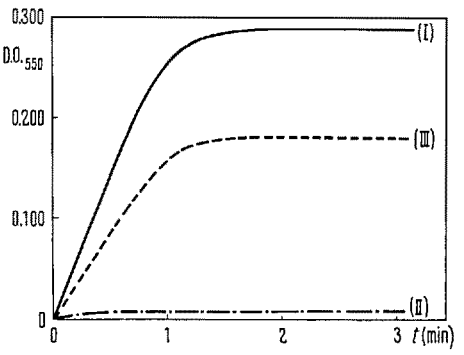
In the Figure, enzymatic reduction of all the fractions by D(−)lactic reductase[4] is plotted as increase of light absorbency at 550 mμ, measured as function of time.

The procedure for preparation of cytochrome c involves an initial treatment with ethylacetate. Since organic solvents, such as ethanol or acetone, transform cytochrome c into a mixture of fractions[5], it is quite conceivable that this step might be responsible for the presence of more than one chromatographic fraction partially or completely denatured. Indeed, fraction I (Table I), when treated again with ethylacetate (6 ml of cytochrome c $1 \cdot 10^{-4} M$ + 0.5 ml of ethylacetate at 20°C for 4 h), is transformed into three fractions indistinguishable from the ones obtained by the original preparation procedure. Since these fractions have different molecular weights[6,7], it can be assumed that the denaturation process is accompanied by a depolymerization of the native form.

In Table II, the chromatographic behaviour of the three fractions during gel-filtration is reported.

Fractions I_a and III_a, when rechromatographed by gel-filtration method, are transformed into I_b and III_b respectively; on the other hand, rechromatography of I_b, II_b and III_b fractions altogether on Amberlite IRC-50 pH 7 or on Sephadex G-50, gives only one fraction identical to fraction II (Table I).

The present data therefore suggest that preparations of cytochrome c according to HAGIHARA's method[1] contain a single native form, i.e. I, and the remaining fractions observed represent technical artifacts due to the depolymerization and denaturation procedures. In fact, the activity as electron acceptor in enzymatic reactions of fraction III, which is identical to I when measured by the usual methods[6], appears to be much lower (Figure) when tested by the more specific enzymatic assay which has been used in the experiments reported.



3 ml of reaction medium contained: 45 μm of K_2HPO_4, 5 μm of KH_2PO_4, 15 μm of sodium D-lactate pH 7.3, 0.12 μm of cytochrome c, 1 μm of sodium azide, and 0.02 ml of baker's yeast extracted particles containing 300 μg of proteins.

Table I. Characteristics of the fractions

Fractions eluted from Amberlite IRC-50	I	II	III
Recovery (mg of lyophilized protein/kg of yeast)	95.0	9.1	4.2
Electrophoretic mobility (cm per sec^{-1} V^{-1} with Whatman 3 mM, phosphate buffer pH 6.8, $\mu = 0.1$)	1.56	1 28	0.98
Mobility on Amberlite IRC-50 pH 7 (total column vol/fraction vol)	0.08	0.01	0.00
Activity as electron acceptor in enzymatic reaction (E_{550}/min in the presence of yeast respiratory particles and D(−)lactate)	0.100	0.0	0.058

Table II. Gel-filtration on 685 ml column of Sephadex G-50, 100–200 mesh. Elution with phosphate buffer pH 6.8, $\mu = 0.1$

Amberlite IRC-50 fractions	I		II		III	
Sub-fractions emerging from Sephadex G-50	a	b	a	b	a	b
Mobility on Sephadex (column vol/fraction vol)	0.38	0.48	–	0.48	0.38	0.48

Résumé. Les auteurs ont étudié les caractéristiques de trois fractions obtenues par l'extraction du citochrome c de la levure. La première est la forme naturelle, tandis qu'on peut considérer les autres comme des formes artificielles résultant du procédé d'extraction.

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