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The Stability of Dried Cytochrome c

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Cytochrome c is known to be stable even under conditions which may destroy many other enzymes. Thus it does not change at extreme pH values [1], at high temperatures in salt free solutions [2] nor upon drying. Since this last property is of great practical importance, we found it to be of interest to investigate some properties of a sample of cytochrome c, which had been kept in the dry state for 12 years. We believe this preparation to be one of the oldest of a fairly pure cytochrome c available as yet.

Patch, Morrison, Ciminera and Beyer [3] found no changes in the absorption spectrum and catalytic properties of cytochrome c which had been lyophilized and stored for four months. Drabkin [4] has found that the activity of acetone-dried cytochrome c is not completely retained when the preparation is stored at room temperature for some time. Recently Keilin and Wang [5] published an investigation of the stability of hemoglobin and certain endoerythrocytic enzymes from various animals. Their samples had been stored as solutions in ampoules and kept in darkness for 26—47 years. If the samples were taken under sterile conditions and without addition of chloroform no inactivation of the proteins occurred. These authors also give a review of some other investigations on the stability of enzymes upon storage.

Our sample of cytochrome had been prepared by Theorell in 1936 according to a method worked out by him [6], using adsorption on cellophane as an essential step. According to data from that time, the preparation was of 93 % purity, which, since pure cytochrome c at that time was believed to contain 0.34 % iron, corresponds to 0.316 % iron. It had been dialyzed against 0.2 % ammonia, evaporated to dryness in a desiccator at room temperature and then kept in a glass tube, sealed with a rubber stopper and protected against light. The tube had been standing at room temperature and humidity.

The sample was easily and almost completely soluble in redistilled water. Some insoluble particles were centrifuged down. The clear, red solution

Table 1. Analysis of old and fresh cytochrome c.

	Dry weight mg/ml	Iron content μg/ml	Iron %
Old cytochrome c	10.00	32.36	0.324
Fresh cytochrome c	4.84	18.56	0.383

showed the same absorption bands both in the reduced and oxidized states as a sample, freshly prepared according to Keilin and Hartree [7].

The slight increase in the iron content of the old cytochrome c as compared to the analysis 12 years ago indicates that at least a part of the insoluble particles were impurities.

Table 2. Optical densities at the tops of the absorption bands of old and fresh cytochrome c. To give suitable density values the old cytochrome was diluted 0.100 ml to 5.20 ml and the fresh cytochrome 0.300 ml to 8.30 ml, both with M/15 sodium phosphate buffer of pH 6.8. The values in the table are for these diluted solutions.

Wave-length in mμ	Old c	Fresh c	Density of fresh c / Density of old c
	oxidized		
278	0.613	0.322	0.53
408	1.17	1.31	1.12
528	0.117	0.129	1.10
600	0.018	0.020	1.11
690	0.008	0.009	(1.12)
	reduced		
416	1.44	1.65	1.15
520	0.168	0.195	1.16
549	0.276	0.341	1.23
650	0.003	0.006	(2.00)

The light absorption curves (Table 2) for this old cytochrome and for the fresh preparation were determined with a Beckman spectrophotometer. As neither preparation was completely pure, it is of no value to give the whole curves, and only the densities at the tops of the bands are listed. The maximal absorptions of the different bands had the same wave-lengths for the two preparations. The figures in the right column indicate that there is no considerable spectrophotometrically observable change in the old cytochrome. The low ratio at wave-length 278 mμ suggests that the impurities in the old preparation were of a protein nature.

Table 3. Catalytic activity of old and fresh cytochrome upon oxygen uptake in the oxidation of succinic acid. Reaction time 30 min. Temperature 37° C. Dry weight of kidney preparation 27.0 mg/ml. »Density» means the value calculated from tables 2 and 3 of the α-band density of added cytochrome in a final volume of 3.45 ml if it had been completely reduced. Thus for vessel 7 »density» = 0.276 × 5.2 × 0.0464/0.10 × 3.45 = 0.193.

Vessel no.	1	2	3	4	5	6	7
Well:							
0.20 ml 2 *M* KOH	→						
Main chamber:							
2.00 ml phosph. buff. *M*/15 pH 7.3	→						
0.50 ml kidney prep.	→						
ml fresh cytochrome	0	0.0162	0.0377	0.0808	0	0	0
ml old cytochrome	0	0	0	0	0.093	0.0216	0.0464
μg of Fe from added cytochrome	0.0	0.3	0.7	1.5	0.3	0.7	1.5
ml water	0.65	0.63	0.61	0.57	0.64	0.63	0.60
Side bulb:							
0.30 ml 10 % Nasuccinate pH 7.3							
0_2 uptake in μl	80.5	150.5	199	242	142	186.5	231
»Density»	0.000	0.044	0.103	0.221	0.039	0.090	0.193

For the activity determinations (Table 3) we used a modification of a method by Keilin and Hartree [8] which we have used for some other activity determinations of cytochrome c [1].

If one plots oxygen uptake *versus* micrograms of iron in the cytochrome c added to the Warburg vessels, the old cytochrome shows a slightly decreased activity. However, if one calculates the concentrations of added cytochrome c in the manometer vessels from the densities of the α-bands for the reduced cytochrome and plots the oxygen uptake against these densities, it is obvious that the old cytochrome sample has the same catalytic activity as the fresh one (Fig. 1). This again means that there must be some foreign iron in the old cytochrome.

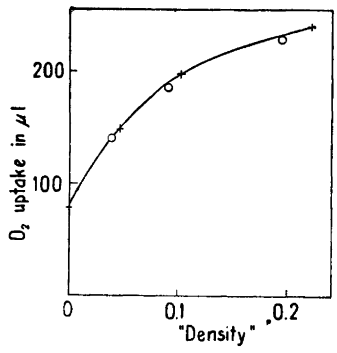

Fig. 1. Oxygen uptake plotted against concentrations of cytochrome, expressed as densities. + fresh, O old cytochrome.

SUMMARY

Cytochrome c, which had been kept in the dry state for 12 years, had the same properties as a fresh preparation with regard to solubility in water, light absorption and catalytic activity in oxidation of succinic acid.

REFERENCES

1. Paul, K.-G. *Acta Chem. Scand.* **2** (1948) 430.
2. Theorell, H. Personal communication.
3. Patch, E., Morrison, H., Ciminera, J., and Beyer, K. *Fed. Proc.* **3** (1944) 35.
4. Drabkin, D. L. *J. Biol. Chem.* **171** (1947) 409.
5. Keilin, D., and Wang, Y. L. *Biochem. J.* **41** (1947) 491.
6. Theorell, H. Unpublished experiments.
7. Keilin, D., and Hartree, E. F. *Biochem. J.* **39** (1945) 289.
8. Keilin, D., and Hartree, E. F. *Proc. Roy. Soc. London* **B 129** (1940) 277.

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