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Amino Acid Analysis of Crystalline Chicken Heart Cytochrome c

SVEN PALÉUS

Medicinska Nobelinstitutet, Biokemiska avdelningen, Karolinska Institutet, Stockholm, Sweden

Species specificity [1] has earlier been shown to occur in the amino acid sequence of a polypeptide from chicken heart cytochrome c. This preparation of cytochrome c, however, was not crystallized and therefore it was decided to make another preparation according to a recently developed method [2] and to compare its amino acid content with the already known primary structure of horse heart cytochrome c.[3,4]

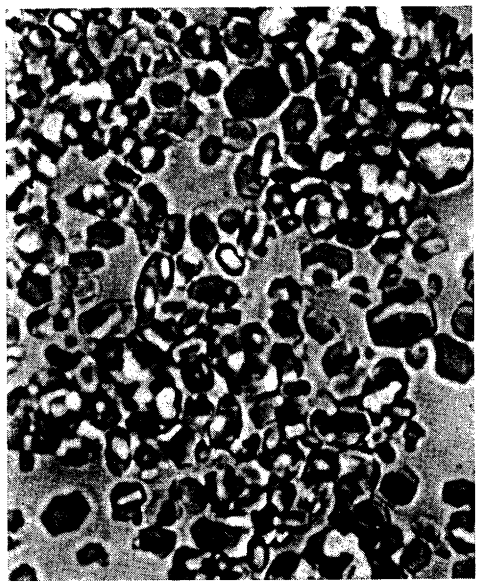

Fig. 1. Crystalline chicken cytochrome c (× 1.750.) Crystal size 3.6 × 4.4 μ. Reduced form.

Table 1.

Amino acid residue	Number per molecule Time of hydrolysis 20 h	70 h	Presumed number of amino acid residues per molecule of cytochrome c	Horse cytochrome c[3]
Aspartic acid	7.0	8.5	(9)	8
Serine	2.7	1.6	(3)	0
Threonine	5.7	5.4	(6)	10
Glutamic acid	7.8	10.0	(10)	12
Glycine	9.9	12.5	(13)	12
α-Alanine	4.1	4.9	(5)	6
Valine	2.5	3.0	(3)	3
Proline	2.1	3.2	(3)	4
Isoleucine	5.4	6.8	(7)	6
Leucine	4.8	5.7	(6)	6
Tyrosine + monochlorotyrosine	2.9		(3)	4
Lysine	14.5	17.5	(18)	19
Histidine	2.5	3.0	(3)	3
Arginine	1.6	1.8	(2)	2
Phenylalanine	3.1	3.8	(4)	4
Methionine	0.9		(1)	2
Cysteine	1.2		(2)	2
Tryptophane			(1)	1

Materials and methods. 3 kg of chicken hearts stored at −15°C were used for preparing cytochrome c. (The author expresses his thanks to AB Findus, Bjuv, for the gift of chicken hearts.) They were thawed just before use and then prepared according to Paléus.[2] The crystals (twice precipitated) are shown in Fig. 1. The yield was 94 mg.

Analytical results. The preparation described was dialyzed until it was salt free, and passed through a column of Lewatite MIH. The iron and sulfur contents were 0.45 % and 0.70 %, respectively. Amino acid analyses were performed according to Moore *et al.*[5] (Table 1). 2.393 mg and 2.053 mg were hydrolyzed with 0.5 ml 5.7 N HCl in a sealed evacuated tube for 20 and 70 h, respectively, at 110°C.

Discussion. Based on the iron analysis chicken heart cytochrome c has a minimal

molecular weight of 12 400 which is the same as that for beef and horse heart cytochrome c. According to the amino acid analysis there were more differences in the primary structure between the two cytochromes than the reported exchange of an alanine residue in the polypeptide chain in the vicinity of the prosthetic group of bovine cytochrome c for a serine residue in chicken cytochrome c.[1] A very striking difference was the three serine residues in chicken cytochrome c compared to none in horse cytochrome c. There were only six threonine residues in chicken cytochrome c compared to 10 in equine cytochrome c. Another interesting finding is the existence of only one methionine residue in chicken cytochrome c in contrast to the finding of two methionine residues in the preparation of chicken cytochrome c by Chan *et al.*[6] Together with the two cysteine residues this would, however, agree rather well with the sulfur analysis (0.70 %) which indicated the presence of three atoms of sulfur in the molecule. Small differences in the amino acid content of the two species were found for aspartic acid, glutamic acid, glycine, proline, isoleucine, and tyrosine. These differences in amino acid content imply that a different sequence exists in these cytochromes. This has been confirmed by the recently reported partial amino acid sequence analysis of chicken cytochrome c.[6]

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