The action of carboxypeptidases A and B on the separated α and β chains of normal adult human hemoglobin

The molecule of normal adult human hemoglobin is known to be composed of four polypeptide chains[1], a pair of so-called α chains and a pair of β chains. The recently reported[2] clear-cut separation of these chains by countercurrent distribution or by chromatography[3] now makes possible reliable amino acid sequence studies. As part of a general study[4] of the splitting of the separated chains by enzymes, the action of carboxypeptidases A[5] and B[6,7] is particularly interesting and timely.

The α and β chains* were isolated by countercurrent distribution[2] of normal adult human globin prepared according to TEALE[8]. Carboxypeptidase-A was the Sigma Chemical Co. preparation, a 5-times crystallized toluol–water suspension. Its concentration was assayed spectrophotometrically assuming an ε_m of $8.6 \cdot 10^{-4}$ (ref. 5). Prior to use, this enzyme was treated with DFP, by adding 10 mg Na_2CO_3 to 1.00 ml of the enzyme suspension, and then a 200-fold molar excess of DFP. Carboxypeptidase-B was prepared from acetone powders of bovine pancreas** and activated according to FOLK AND GLADNER[6,7]. The NaCl extracts of procarboxypeptidase B (2.5 g acetone powder gives 2.00 ml of NaCl extract) had an activity of 1,200 units/ml, measured by hydrolysis of hippuryl-lysine. The reactions were carried out at 25°, pH 7.65, in a 0.2 *M* Na phosphate buffer containing 0.056 *M* sodium lauryl sulfate. This detergent was used to solubilize the chains, which are otherwise insoluble at this pH. The incubation mixtures contained: α or β chain (mol. wt. 17,000), 1.4 m*M*; CBP–B, 130 units/ml of reaction mixture; CBP–A, 0.1 m*M*. Aliquots (0.4 ml) were removed at time intervals and the reaction stopped by 0.2 ml 1 *N* HCl. The amino acids present in each aliquot were determined by quantitative column chromatography[9].

Table I shows the rates of release of amino acids from the α chain. No amino acids were released by CBP–A alone. CBP–B produced a rapid release of arginine associated with a rapid release of tyrosine and a slow release of lysine. The rapid

TABLE I

AMINO ACIDS RELEASED FROM THE α CHAIN IN μMOLES/μMOLE α CHAIN

CBP-B added initially; CBP-A added after 65 min.

Time (min)	*CBP–B*		*CBP–B + CBP–A*	
	35	*65*	*105*	*255*
Arginine	0.91	0.92	0.97	0.96
Tyrosine	0.86	0.86	0.85	0.89
Lysine	0.47	0.77	0.90	1.02
Serine	0.04	0.10	0.85	1.42
Threonine	—	0.03	0.35	1.40
Leucine	—	0.02	0.41	1.20
Valine	—	—	0.29	0.93
Alanine	—	—	0.15	0.28
Phenylalanine	—	—	0.12	0.14
Histidine	—	—	0.11	0.10

Abbreviations: CBP-A, carboxypeptidase A; CBP-B, carboxypeptidase B; DFP, diisopropylfluorophosphate.

* The chains were kindly furnished by Dr. R. J. HILL.

** The powder was kindly supplied by Dr. J. E. FOLK.

release of tyrosine, even before the addition of CBP–A, was probably caused by the known contamination of CBP–B preparations by small amounts of CBP–A. After addition of CBP–A, serine was released at the most rapid rate. The data indicate the following sequence for the C-terminal residues of the α chain: –Ser–Lys–Tyr–Arg. This result is confirmed by the work of HILL AND KONIGSBERG[4] who have found a Tyr–Arg peptide on tryptic hydrolysis of the α chain.

Table II shows the rates of release of amino acids from the β chain. The only amino acids released by CBP–A were histidine, tyrosine, and a small amount of lysine,

TABLE II

AMINO ACIDS RELEASED FROM THE β CHAIN IN μMOLES/μMOLE β CHAIN

CBP-A added initially; CBP-B added after 75 min.

Time (min)	*CBP–A*	*CBP–B + CBP–A*		
	75	*110*	*140*	*330*
Histidine	1.12	1.80	1.84	2.10
Tyrosine	0.92	0.95	0.96	0.96
Lysine	0.08	0.92	1.08	1.38
Alanine	0.02	0.64	1.67	4.55
Leucine	—	0.28	0.80	1.32
Serine	—	0.10	0.26	1.92
Valine	—	0.03	0.08	1.80
Glycine	—	—	- -	0.45
Threonine	—	—	0.07	0.25
Phenylalanine	—	—	- -	0.14

the latter probably by the CBP–B which contaminates CBP–A preparations. After addition of CBP–B, a rapid release of lysine was observed, accompanied by a concomitant release of histidine and alanine, at an equal rate. These data, together with other experiments done in this laboratory which show that histidine is the first amino acid released by CBP–A, indicate the following sequence for the β chain: –(His,Ala)–Lys–Tyr–His. This sequence is concordant with the observations of previous investigators, who have shown that histidine is released by hydrazinolysis and by CBP–A from both the β chain[10] and whole hemoglobin[11–13], and who have suggested that a Tyr–His peptide might be at the C-terminal position[10,11].

The data for the other amino acids shown in Tables I and II do not allow any further conclusions to be drawn regarding the C-terminal sequence of the chains, because the rates of release of amino acids which are located several residues away from the C-terminal positions are complicated by the non-uniformity of enzymic digestion at these distant sites.

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An effect of chloramphenicol on the incorporation of tritiated thymidine into chromosomal DNA

The inhibitive action of chloramphenicol on protein synthesis has been utilized extensively and with considerable success for the elucidation of the relationships between nucleic acids and proteins in microorganisms[1–3]. The apparent absence of a direct effect of this antibiotic on DNA replication in microorganisms[3], the probability that DNA in microorganisms is less intimately associated with a protein moiety than is chromosomal DNA[4,5], and the linear pattern of DNA increase in bacteria[6,7,8] as opposed to the stepwise pattern characteristic of DNA synthesis in chromosomal systems, have led us to the notion that chromosomal protein may play a controlling role in chromosomal DNA replication in addition to the structural function recently suggested[9,10]. We have explored this possibility by studying the incorporation of tritiated thymidine into meristematic cells of onion root tips in the presence and absence of chloramphenicol; the incorporation of [^{14}C]thymidine was studied under similar experimental conditions as a control for possible tritium effects[11].

Onion roots were immersed in a solution of 300 μg/ml chloramphenicol for periods of 6 and 24 h prior to the addition of labeled thymidine, with renewal of the chloramphenicol solutions every 6 h. [^{3}H]thymidine was used at a concentration of 2.5 μC/ml and a specific activity of 360 μC/μmole. The roots were incubated with the isotope for 11 h, fixed, briefly hydrolyzed, and squashed. Kodak AR-10 stripping film was applied and the preparations were exposed for 7 days. Assay of the [^{3}H]thymidine solutions before and after incorporation indicated that ample amounts of the tracer were available for incorporation throughout the incubation period.

The semi-quantitative analysis of the [^{3}H]thymidine-labeled preparations led to the following conclusions: (a) the percentage of cells with labeled nuclei did not differ significantly from control values in the root tips treated with chloramphenicol for either 6 h or 24 h; (b) the percentage of heavily labeled nuclei (nuclei with autoradiographic grain densities above the limit for reliable direct counting) was considerably higher in the treated roots than in controls. Instrumental analysis of the heavily labeled fraction of nuclei by means of a scanning microscope designed by GULLBERG[12] resulted in the data plotted in Fig. 1. The graph shows a progressive increase in the amount of [^{3}H]thymidine incorporation in nuclei of chloramphenicol-treated roots. The effect of the drug appears even more strikingly when we consider that the heavily

Abbreviation: DNA, deoxyribonucleic acid.