CHANGES IN CARBONIC ANHYDRASE ISOENZYME CONTENT ACCOMPANYING DIFFERENTIATION IN RABBIT ERYTHROID CELLS

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1. Introduction

In most mammalian species circulating erythrocytes contain two genetically distinct forms of carbonic anhydrase (EC 4.2.1.1), isoenzymes I and II of which isoenzyme I usually comprises the bulk of the carbonic anhydrase protein [1]: rabbits may be somewhat atypical as erythrocytes contain roughly equal amounts of these two proteins [2,3]. Little is known about the relative rates of synthesis of the two isoenzymes in erythrocyte precursors although it has been suggested [4] that in humans the two proteins may be synthesised in a coordinate manner since the amounts of the two isoenzymes are significantly correlated in erythrocytes from both normal and clinically affected individuals. The advent of the velocity–sedimentation technique has allowed the separation of the various cell types through which developing erythroblasts pass during erythrocyte maturation: using this method, with bone marrow cells from rabbits, it was found that carbonic anhydrase activity increased steadily throughout the erythrocyte developmental sequence [5].

Here we have used specific radioimmunoassays [6] to measure both isoenzymes in the different cell types to determine whether or not the observed increase in catalytic activity parallels changes in the amounts of either or both isoenzymes in developing erythroblasts. We have found that in the very earliest erythroid precursor cells the amount of isoenzyme II protein exceeds that of isoenzyme I: the levels of the two isoenzymes increase, roughly in parallel, up to the polychromatic cell stage, after which there is an apparent decline in the levels of both isoenzymes that is more marked in the case of isoenzyme II. At all stages of the developmental sequence the measured catalytic activity agreed fairly closely with that expected from the measured isoenzyme content which suggests that most of the immunoreactive protein was also catalytically active.

2. Materials and methods

Rabbits were rendered anaemic and bone marrow material was prepared and fractionated as in [5]. Erythroid cells were stained and classified according to [7]. Carbonic anhydrase isoenzymes I and II were measured as protein in lysates of the various cell types using the radioimmunoassay procedure in [6]. Apoenzymes from both isoenzymes were prepared by dialysing the native enzymes [3] against phenathroline by the method in [8]. All other procedures are as given [5].

3. Results and discussion

As pointed out in the original description of the cell separation technique [7] the proportions of the various erythroid cell types observed in fractions obtained from bone marrow preparations varies with the degree of anaemia induced and is mainly a function of the individual animal. For the same reason the enzyme profiles obtained after cell separation may vary somewhat from animal to animal. However with the 6 animals used in the present study the profiles obtained were similar in all essentials. A typical sepa-

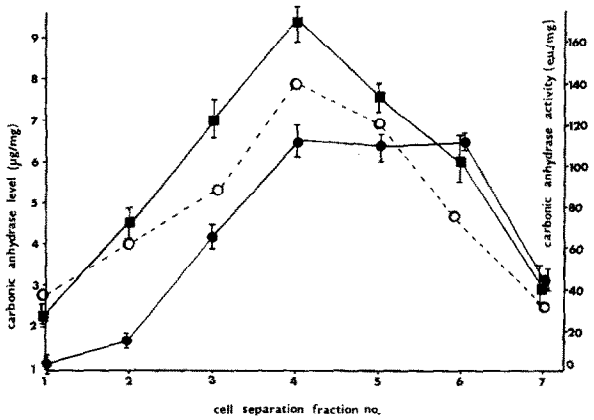

Fig.1. Carbonic anhydrase activity and isoenzyme content in lysates of rabbit erythroid cells. References to experimental methods are given in the text. Carbonic anhydrase activity (○), isoenzyme I protein (●) and isoenzyme II protein (■) per mg total soluble protein are plotted for each of the fractions listed in table 1. All estimations were done in triplicate; mean values are shown for activity measurements and mean plus range for isoenzyme content. The profile is typical of 6 such separations.

ration profile is shown in fig.1; the relative amounts of the different erythroid cell types in the corresponding fractions are given in table 1.

There appear to be several features that are common to all the separations carried out so far. In the earliest erythroid precursors the level of carbonic anhydrase II protein is consistently higher than that of carbonic anhydrase I. The amounts of both proteins increase as the developmental sequence progresses, reaching maximum in those fractions richest in polychromatic cells: the specific catalytic activity increases in parallel and reaches a maximum at the same stage. In the later stages of erythroid cell production the specific activity of the enzyme declines and this change is accompanied by an apparent fall in the amounts of both carbonic proteins but in particular of isoenzyme II. However since both catalytically inactive apoenzyme reacted exactly like the corresponding native isoenzyme in their respective immunoassays the possibility exists that the correlation between isoenzyme protein content and catalytic activity may be fortuitous and thereby mask the existence of inactive or activated forms of the enzyme; the finding that observed activity agrees fairly closely with that calculated on the basis of the measured enzyme content suggests that such forms are probably not present to any significant extent in any of the cell fractions (table 2). In particular since the calculated activity never greatly exceeds that which was observed in any of the fractions there is no direct indication for the presence of inactive apoenzymes. The simplest expla-

Table 1
Percentage composition of erythroid cell types in fractions separated from rabbit bone marrow

Cell fraction	Erythroid cell types					
	P-E	B	P	O	R	E
1	54	41	5			
2	3	80	8	6	3	
3		56	41		3	
4		24	64	10	2	
5			27	64	9	
6			4	34	62	
7					65	35

Bone marrow was separated into 6 fractions as in [5]: fraction 7 represents a lysate prepared from circulating red cells removed shortly before the animal was sacrificed.

Abbreviations: PE, pro-erythroblasts; B, basophilic; P, polychromatic; O, orthochromatic; R, reticulocytes; E, erythrocytes

Table 2
Observed and calculated carbonic anhydrase activity in lysates of cell fractions from rabbit bone marrow

Cell fraction[a]	Carbonic anhydrase activity (EU/ml lysate)	
	Obs.	Calc.[b]
1	49	39
2	133	120
3	204	217
4	367	330
5	367	290
6	169	124
7	2670	2960

[a] See fig.1

[b] Total carbonic anhydrase activity was calculated using 7050 EU/mg for isoenzyme 2: activity due to isoenzyme 1 was neglected in this calculation as this enzyme contributes < 10% of the total activity even when present in equimolar amounts [2,3]

nation therefore for the observed increase in specific catalytic activity up to the polychromatic cell stage of development is that the polypeptide chains of isoenzymes I and II are synthesised de novo in the appropriate cell types, and immediately converted into the catalytically active form of the enzyme by sequestration of Zn^{2+}.

After the orthochromatic cell stage the specific activity of carbonic anhydrase apparently declines and this is accompanied by a fall in the amount of both isoenzyme proteins. It is possible that both proteins are being degraded since that specific degradation of lactate dehydrogenase M4 has been suggested [9] to occur during erythroid differentiation. However, it seems more likely that the observed decrease, both in activity and protein content, is due to dilution of the cell contents by massive synthesis of haemoglobin which begins to accelerate rapidly at this stage of erythrocyte development. The level of isoenzyme I protein does not decline, however, to the same extent as that of isoenzyme II and this is probably because synthesis of isoenzyme I may continue in reticulocytes: incorporation of labelled amino acids into isoenzyme I was detected [10] but not into isoenzyme II in human reticulocytes and similar results with rabbit reticulocytes were obtained [3]. It would appear that the relative amounts of the two isoenzymes that are found in the mature erythrocyte of a particular species probably reflects mainly the quantity and relative stabilities of the corresponding messenger RNA molecules that are originally transcribed in the nucleated erythrocyte precursors.

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