CHANGE OF THE RELATIVE PROPORTION OF VARIOUS FORMS OF PHOSPHOENOLPYRUVATE CARBOXYKINASE IN CHICKEN LIVER POSSIBLY ASSOCIATED WITH ENHANCED GLUCONEOGENESIS

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1. Introduction

Phosphoenolpyruvate (PEP) carboxykinase (EC 4.1.132) is a key enzyme in gluconeogenesis in liver. Previously we demonstrated the occurrence of, in total, four forms of PEP carboxykinase in the liver of chick embryo and young chick, two in the mitochondria and two in the cytosol fraction, which could respectively be separated from each other by means of DEAE-Sephadex column chromatography, whereas in the liver of adult chicken there were usually only two forms of PEP carboxykinase, the one in the mitochondria and the other in the cytosol fraction. The two enzyme components that are dominant in the adult chicken are eluted from the column at relatively lower buffer concentrations (the first components) as compared to the other two PEP carboxykinase components (the second components) [1]. In the present paper we report that when the adult chicken was forced to exercise an active gluconeogenesis, the first components which have been dominant in the adult chicken liver tended to disappear and instead, the other two components (the second components), which were distinctive in the chick embryo and young chick and which appeared to disappear during adolescence, reappeared in both the mitochondria and the cytosol fraction.

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2. Materials and methods

White leghorn male chickens (body weight 900–1100 g) were used throughout the present study. For the preparation of PEP carboxykinase, livers were homogenized in 9 vol of 0.25 M sucrose containing 0.01 M Tris–HCl buffer (pH 7.2), 0.5 mM EDTA and 0.1 mM mercaptoethanol in a glass homogenizer with Teflon pestle. The mitochondria were sedimented by 20 min centrifugation between 600 *g* and 8000 *g*, and the soluble fraction was obtained by 60 min centrifugation at 105 000 *g*. The mitochondria were suspended in the same sucrose solution so that the final volume was the same to that of the initial homogenate. The mitochondrial suspension was then sonicated at 10 kcycles for 3 min, followed by centrifugation at 105 000 *g* for 60 min, and the supernatant obtained was used as the source of the mitochondrial PEP carboxykinase [1].

PEP carboxykinase was assayed by the exchange reaction between oxaloacetate and $NaH^{14}CO_3$ [2] after a slight modification as described in the previous paper [1]. The amount of [^{14}C] oxaloacetate formed was calculated on the basis of the specific radioactivity of $NaH^{14}CO_3$ (0.01 μCi/μmole) used.

The specific activity of the PEP carboxykinase preparation was expressed in terms of μmoles of ^{14}C-oxaloacetate formed per mg protein per min of the reaction.

The column chromatography of PEP carboxykinase was performed using a column of DEAE-Sephadex A-25 (1.5 × 30 cm) equilibrated with 0.01 M Tris–HCl buffer (pH 7.2). After applying the enzyme preparation, the column was washed with 100 ml of 0.01 M Tris–HCl buffer (pH 7.2) containing 5 mM mercaptoethanol, then the enzyme was eluted with 300 ml of Tris–HCl buffer (pH 7.2) containing 5 mM mercaptoethanol at a linear gradient between 0.01 and 0.1 M. 5.0-ml fractions were collected.

3. Results

3.1. *Effects of exposure to cold, and of high protein diet*

Chickens were placed in a cold room (2–4°C) for 48 hr, then killed and examined for PEP carboxykinase in the subcellular fractions of the liver. The specific activities of PEP carboxykinase in the soluble fraction and the mitochondrial fraction were 0.20–0.39 and 5.5–6.0, respectively (table 1), which were in the normal ranges [1]. When subjected to the column chromatography, however, the second components of PEP carboxykinase in both subcellular fractions were found to be markedly increased and in turn, the first components were diminished (fig. 1) as compared to the figures obtained with control chickens which were maintained at room temperature (fig. 2). In three out of four chickens treated in the same way, the first components in either subcellular fractions were present in only trace amounts; expt. 1 in fig. 1 represents one of those three experiments. In one of four chickens (designated as expt. 2 in fig. 1), however, the degree of the change in the relative proportion of the first and second components was not so extensive as in the other three chickens, probably reflecting individual variations.

A quite similar change in the relative proportion of PEP carboxykinase as observed after the cold exposure could be observed when chickens were fed on a high protein diet (cheese flake) for several days. In this case, however, usually the reduction of the first components was not as marked as in the chickens

Table 1
Specific activities and relative proportions of the first and second components of PEP carboxykinase in the liver mitochondria from the chickens which were administered with hydrocortisone or isoproterenol

Hormone administered	Chicken number	Specific activity of PEP carboxykinase in mitochondria	PEP carboxykinase activity ([^{14}C]oxaloacetate formed, cpm)		Ratio of II/I
			Peak I fraction	Peak II fraction	
Hydrocortisone	1	6.9	600	350	0.58
	2	15.9	600	730	1.21
	3	7.6	430	570	1.32
	Average ± S.D.	10.1 ± 4.1	543 ± 80	550 ± 120	1.01
Isoproterenol	1	4.3	528	280	0.53
	2	5.4	340	444	1.31
	3	14.0	500	725	1.45
	Average ± S.D.	7.9 ± 4.3	456 ± 83	483 ± 184	1.06
None	Average ± S.D.*	5.7 ± 1.4	742 ± 190	149 ± 39	0.20

S.D.: standard deviation.

* Averages of the values for 5 individual chickens. Hydrocortisone succinate was given to chickens 3 times (one dose, 10 mg/100 g body weight) by subcutaneous injection at 3-hr intervals and the chickens were killed at 9 hr after the first injection. Isoproterenol was injected into chickens twice (one dose, 1 mg/100 g body weight) at 2-hr intervals and the chickens were killed at 6 hr. Conditions of the column chromatography and the assay of PEP carboxykinase in the eluate were the same as in fig. 1. Specific activities were calculated from the values obtained by 2 min incubation. For other explanations see text.

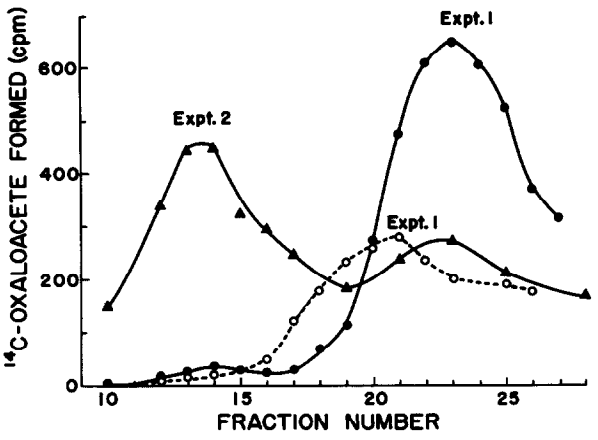

Fig. 1. DEAE-Sephadex column chromatography of PEP carboxykinases from adult chickens exposed to cold. Protein amounts applied were 75 mg for the soluble liver fraction (o—o—o) and 10 mg for the mitochondrial fraction (●—●—● in expt. 1 and ▲—▲—▲ in expt. 2). In expt. 2, only the results with the mitochondrial enzyme are shown. The incubation mixture contained, in a final volume of 1.0 ml: 100 μmoles of imidazole HCl buffer, pH 6.9, 1 μmole of $MnCl_2$, 1 μmole of GSH, 0.8 μmole of GTP, 25 μmoles of $NaH^{14}CO_3$ (0.01 μCi/μmole), 1 μmole of oxaloacetate, and 0.1 ml of respective eluates. Reactions were carried out for 4 min at 30°C.

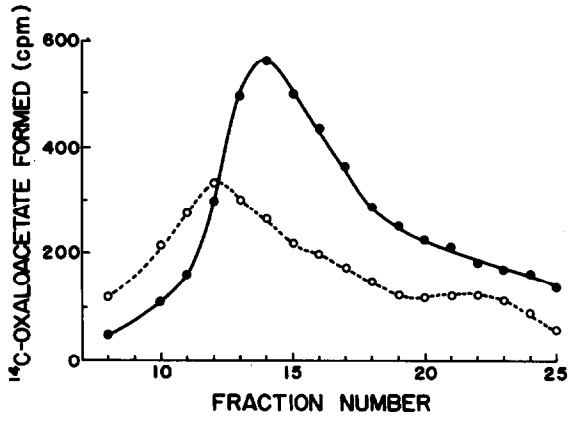

Fig. 2. DEAE-Sephadex column chromatography of PEP carboxykinases from control adult chicken. Protein amounts applied, the assay conditions, and the signs were the same as for expt. 1 in fig. 1.

exposed to cold and the general pattern of the relative proportion of four protein components was quite similar to those for the liver of chick embryo or young chick described in the previous paper [1].

3.2. *Effects of administration of hormones*

The administration of hydrocortisone or isoproterenol to adult chickens also caused the similar change in the relative proportion of PEP carboxykinase in liver. In the chickens treated with either hydrocortisone or isoproterenol, the degree of change in the relative proportion of the enzymes as well as the specific activities of PEP carboxykinase in the soluble liver fraction and the mitochondrial fraction were considerably variable in individual experiments, though the hormone-treated chickens tended to give somewhat higher specific activities than those obtained with the control chickens. The data for the mitochondrial PEP carboxykinases are shown in table 1. Also a typical case with hydrocortisone is depicted by (a) in fig. 3. In Table 1 are presented only the values of the enzymes activities in the peak fractions of the first component (Peak I

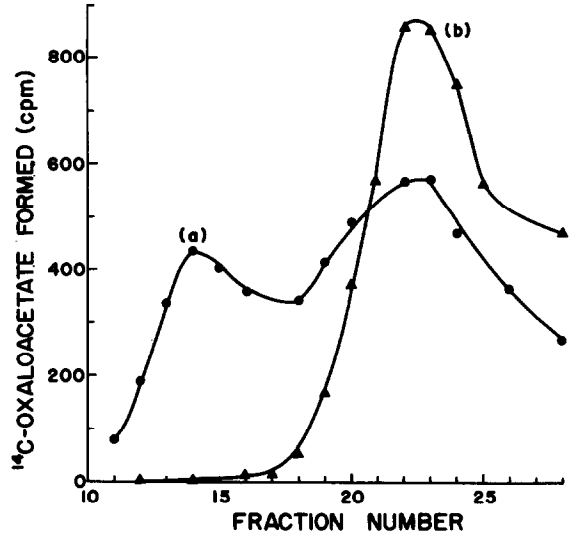

Fig. 3. DEAE-Sephadex column chromatography of the mitochondrial PEP carboxykinase from chickens treated with hydrocortisone alone (expt. (a)) or both hydrocortisone and isoproterenol (expt. (b)). In (a), the chicken was treated with hydrocortisone succinate in the manner as described in table 1. In (b), the chicken was given two doses of hydrocortisone succinate (one doese, 10 mg/100 g body weight) at 0 and 3 hr of the experiment and two doses of isoproterenol (one dose, 1 mg/100 g body weight) at 1.5 hr and 4.5 hr, respectively, and killed at 6 hr after the first administrations of hydrocortisone. Protein amounts applied and the assay conditions were same as in fig. 1.

fraction) and the second component (Peak II fraction), respectively, to give a rough idea as to the effects of the hormones on the relative proportion of the two PEP carboxykinase components, since it was difficult to evaluate exactly the total activities of the respective two enzyme components from the data of the column chromatography. At any rate, these data clearly indicate that both hydrocortisone and isoproterenol exert a profound influence on the relative proportion of the first and second components of the enzyme.

When both hydrocortisone and isoproterenol were given to chickens, the change in the proportion of the first and second components was more marked and practically no activity corresponding to the first component could be detected ((b) in fig. 3). The effects of these hormones seem to be additive. The similar change in the relative proportion could also be observed with the enzymes in the soluble liver fraction although the data are not shown. For the case shown in Fig. 3 (b), the specific activities of PEP carboxykinase in the soluble liver fraction and the mitochondrial fraction were 0.24 and 8.2, respectively.

On the other hand, the relative proportion of the enzyme components was not appreciably influenced by the administration of insulin (1–10 units of insulin in two doses at 2-hr intervals per 100 g body weight and killing the chickens at 4 hr after the first administration) or glucagon (two doses of 150 μg glucagon per 100 g body weight at 3-hr intervals and killing the chickens at 6 hr after the first administration).

4. Discussion

The data obtained with the chickens differ in many respects from the observations with rat and guinea pig. In these animals the gluconeogenic stimuli such as the administration of hydrocortisone [3], exposure to cold [4,5], a high protein diet [6] and starvation [3], bring about a significant increase of PEP carboxykinase but only in the cytosol fraction [7]. In the chicken liver, however, the degree of increase of PEP carboxykinase induced by gluconeogenic stimuli appeared to be relatively small in either the cytosol or the mitochondria and in turn, the gluconeogenic stimuli gave rise to a marked change in the relative proportion of the enzyme components in both the cytosol and the mitochondria. It is unclear at present whether or not the individual four PEP carboxykinases are different proteins (isozymes) and therefore it remains to be clarified whether the observed shift in the enzyme composition is due to an 'on–off' type control of genetic expression at the gene level. On the other hand, Johnson et al. recently reported that (i) the three forms of cytoplasmic tyrosine aminotransferase which could be separated from rat liver by use of CM-Sephadex chromatography were immunologically identical, (ii) the relative proportion of the three forms changed markedly during the hydrocortisone induction cycle, and (iii) the three forms were rapidly interconvertible in vivo [8]. Thus, with respect to the observed change in the relative proportion of the PEP carboxykinase components in chicken liver, the possibility of modification of the enzyme protein which would lead to alteration of physicochemical properties of the enzyme protein should also be considered.

The results of the present study support the view presented in the previous paper [1] that preferential occurrence of the second components of PEP carboxykinase in the chick embryo and young chick may be accounted for by the fact that the growth of chick embryo and young chick depends on an active gluconeogenesis utilizing egg yolk which is poor in carbohydrate [9].

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