IMPAIRMENT OF ALBUMIN SYNTHESIS IN CELL-FREE SYSTEMS FROM RAT LIVER

H. MAENO, G. SCHREIBER, K. WEIGAND, U. WEINSSEN and J. ZÄHRINGER
Biochemisches Institut der Universität Freiburg im Breisgau, 78 Freiburg, Hermann-Herder-Str. 7, Germany

Received 25 November 1969

1. Introduction

Recently, the isolation of radiochemically pure serum albumin from tissue homogenates was described [1]. With the obtained values, the ratio of albumin to total protein synthesis can be calculated. This paper describes the application of the methods to cell-free amino acid incorporating systems.

Microsomes were incubated with pH 5 fraction, or cell sap, from either normal or regenerating rat liver, radioactive leucine and an ATP generating system for 30 min at 37°C. Serum albumin was isolated before and after incubation. The specific radioactivity of protein decreased continuously during purification and finally approached constancy.

Despite a considerable increase of the radioactivity incorporated into total protein, no increase of the radioactivity of albumin was found after incubation. The ratio of the incorporation of leucine into albumin to that into total protein ranged from 0.07% to 0.14%, compared with 3.5% and 1.4% *in vivo* for normal and regenerating liver, respectively. Hence, albumin synthesis was absent or strongly impaired in the cell-free systems.

2. Materials and methods

Procedures for assays of protein and radioactivity, acid hydrolysis of protein, isolation of labelled standard serum albumin, and methods of *in vivo* experiments were described previously [1]. The procedure reported recently for the isolation of radiochemically pure albumin was modified as described in table 1. Partial hepatectomies (70%) were performed according to Higgins and Anderson [2].

For preparation of microsomes and pH 5 fraction, livers were minced with scissors and homogenized in 2 times their weight of 250 mM sucrose, 100 mM tris, 25 mM KCl and 5 mM $MgCl_2$, pH 7.6, with 6 strokes at 500 rpm (1000 rpm for regenerating liver) in a Potter-Elvehjem homogenizer with 0.53 mm clearance between the Teflon pestle and the wall. All preparation steps were performed at 0–4°C. The homogenates were centrifuged at 15,000 × *g* (1000 × *g* for regenerating liver) for 10 min. The upper two thirds

Table 1
Purification of serum albumin from a cell-free system

Purification	Total prot. (mg)	Albumin (mg)	Alb. in tot. prot. (%)	10^{-4} × spec. radioact. of tot. protein (dpm/mg)
None	319	11.8	3.7	33
TCA/Ethanol	16.1	7.2	47.3	12
$(NH_4)_2SO_4$	6.1	5.9	97.0	5.4
DEAE-Seph. A50	3.4	3.5	104	2.0
Electrophor. at pH 9.3	2.3	2.4	102	1.5
Electrophor. at pH 2.7	1.9	2.0	101	1.3

1.7 mC of L-[4.5-$^3H_2$] leucine (60 C/mmole) was incubated with microsomes and pH 5 fraction from normal rat liver, 7.5 mM $MgCl_2$, GTP, ATP and an ATP-generating system in 34 ml at 37° C for 30 min. TCA - Trichloroacetic acid.

of the supernatants were further centrifuged for 1 hr at 105,000 × *g*. The obtained microsomal pellets were immediately frozen in liquid nitrogen, stored at −30°C and resuspended before use in 0.5 ml of 75 mM sucrose, 45 mM KCl, 50 mM tris and 7.5 mM $MgCl_2$, pH 7.5 ("incubation buffer") per gram of original liver. The supernatant was diluted 1:1 with H_2O and the pH was adjusted to 5.1 with 1 N acetic acid. The resulting precipitate was centrifuged and resuspended in 0.5 ml of incubation buffer per gram of original liver. The pH was readjusted to 7.5 with 1 M tris. After centrifugation, the supernatant was stored at −30°C ("pH 5 fraction").

For cell-free amino acid incorporation one ml of incubation buffer contained 50 μC L-[4,5-$^3H_2$] leucine (40–60 C/mmole) or 6.25 μC L-[U-^{14}C] leucine (311 mC/mmole), 50 μg of pyruvate kinase, 10 μmoles of phosphoenolpyruvate, 1 μmole of ATP, 0.5 μmole of GTP, 0.2 ml of microsomal suspension (from 0.45 g original liver) and 0.175 ml of pH 5 fraction (from 0.35 g original liver). After incubation, the mixture was rapidly cooled in ice, and sodium desoxycholate (0.7%), L-[^{12}C] leucine (0.2%), and rat serum albumin (3 mg/ml) were added.

3. Results

An example for the purification of albumin from a cell-free incubation mixture is shown in table 1. After ammonium sulfate precipitation, the purity of albumin was nearly 100% with respect to protein. The combined fractions of the albumin peak after the DEAE-Sephadex step showed the same immunological properties in double diffusion in agar gel and the same speed of migration in analytical disc polyacrylamide gel electrophoresis as standard serum albumin, and so did the final product of the whole purification procedure. However, the specific radioactivity of total protein decreased further from 2.0×10^4 to 1.3×10^4 dpm/mg after electrophoresis on polyacrylamide gel at pH 9.3 and 2.7, suggesting contamination of albumin by trace amounts of non-albumin compounds with very high specific radioactivity. The presence of such contaminants was demonstrated in the polyacrylamide gel electrophoresis at pH 9.3 (fig. 1), where the specific radioactivity of fraction 9, migrating somewhat faster than albumin, was extremely high, but was not precipitable with antialbumin.

Albumin was isolated before and after incubation at various pH's and Mg^{2+}-concentrations. Despite a considerable increase in the specific radioactivity of total protein after 30 min incubation, no significant increase of the specific radioactivity of albumin was

Table 2
Incorporation of radioactive leucine into total protein and albumin under various experimental conditions

System	Incubation conditions				Total activity (A)	Total activity (B)	Specific activity		
	pH	$MgCl_2$ (mM)	Label	Incubation time (min)	Total protein (dpm)	Albumin (dpm)	Total protein (dpm/mg)	Albumin (dpm/mg)	$\frac{B}{A} \times 100$ (%)
Normal liver:									
In vivo (8 rats)			L-[1-^{14}C] leu	10	7.1×10^7	2.5×10^6	3.2×10^3	7.5×10^3	3.5
Microsomes and	7.5	7.5	L-[4,5-$^3H_2$] leu	0	3.1×10^6	1.2×10^5	9.5×10^3	9.3×10^3	4.0
pH 5 fraction				30	1.1×10^8	1.6×10^5	3.3×10^5	1.3×10^4	0.14
Microsomes and	7.0	2.5	L-[U-^{14}C] leu	0	2.4×10^4	–	1.8×10^2	–	–
pH 5 fraction				30	2.6×10^6	2.4×10^3	2.1×10^4	2.8×10^3	0.093
Microsomes and cell sap	7.0	2.5	L-[4,5-$^3H_2$] leu	30	2.6×10^7	1.8×10^4	7.6×10^2	2.4×10^3	0.065
Regenerating liver:									
In vivo (16 rats)			L-[1-^{14}C] leu	10	1.1×10^8	1.6×10^6	5.4×10^3	8.7×10^3	1.4
Microsomes and	7.5	7.5	L-[4,5-$^3H_2$] leu	0	1.4×10^7	1.9×10^5	1.6×10^4	8.9×10^3	1.3
pH fraction				30	2.1×10^8	1.9×10^5	2.3×10^5	8.7×10^3	0.089

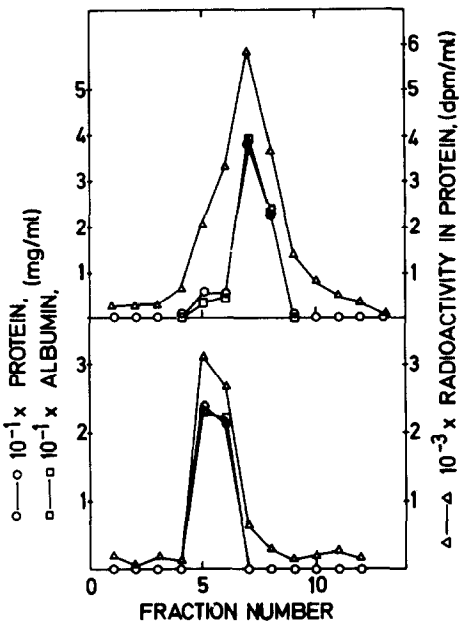

Fig. 1. Top: Electrophoresis on polyacrylamide gel at pH 9.3. Bottom: Electrophoresis on polyacrylamide gel at pH 2.7 of the combined fractions 7 and 8 of the electrophoresis at pH 9.3.

observed (table 2). To be independent of variations in the absolute amino acid incorporating capacity from one system to another, the ratio of the incorporation into albumin to that into total protein was calculated. It was 3.5% for normal liver *in vivo*, whereas *in vitro* under optimal conditions for the incorporation into total protein (pH 7.5 and 7.5 mM $MgCl_2$), only 0.14% was found for the ratio (table 2), demonstrating again the impairment of cell-free albumin synthesis. At pH 7.0 and 2.5 mM $MgCl_2$, which reduced the incorporation of leucine into total protein to one fourth, the ratio was found to be 0.093. Replacement of the pH 5 fraction by cell sap did not influence the ratio.

The incorporation of leucine into albumin was also studied in a cell-free system from regenerating liver (table 2). Despite a striking increase in the specific radioactivity of total protein, again no increase of the specific radioactivity of albumin was observed after incubation. The ratio of leucine incorporation into albumin to that into total protein was 0.09, compared to 1.4 *in vivo* 48 hr after hepatectomy, demonstrating the impairment of albumin synthesis also for cell-free systems from regenerating liver.

Albumin purified from cell-free mixtures incubated for 30 min at 37°C was hydrolyzed in 6 N HCl for 24 hr at 107°C. The obtained amino acids were subjected to high voltage electrophoresis on paper. The majority of the radioactivity was detected on the leucine spot, only a small amount of radioactivity was present at the origin.

4. Discussion

In contrast to our results, rather high ratios of the amino acid incorporation into albumin to that into total protein can be calculated for cell-free systems of other authors, e.g. 25% [3], 30% [4], 7,9% [5], 2,4% [6]. Such variation of the ratios may depend upon the purity of the isolated albumin. Immunological precipitation on chromatography paper discs [7,8], for instance, did not yield radiochemically pure albumin. Radioactivity in the immunoprecipitation lines determined by autoradiography [9,10] is no convincing evidence for cell-free synthesis of albumin, unless it is demonstrated that bound and coprecipitated radioactive compounds have been removed. To establish definitely the cell-free synthesis of serum albumin, the increase of the specific radioactivity of appropriately purified albumin during incubation should be demonstrated.

Acknowledgement

This work was supported by grants of the Deutsche Forschungsgemeinschaft, the Stiftung Volkswagenwerk, Germany and the Anna Fuller Fund, New Haven, Conn., USA.

References

[1] G.Schreiber, H.-M.Rotermund, H.Maeno, K.Weigand and R.Lesch, European J. Biochem. 10 (1969) 355.
[2] G.M.Higgins and R.M.Anderson, Arch. Pathol. 12 (1931) 186.
[3] J.B.Lingrel and G.Webster, Biochem. Biophys. Res. Commun. 5 (1961) 57.
[4] P.N.Campbell, O.Greengard and B.A.Kernot, Biochem. J. 74 (1960) 107.
[5] R.Hirokawa, S.Omori, T.Takahashi and K.Ogata, Biochem. Biophys. Acta 49 (1961) 612.

[6] J.B.Marsh, D.L.Drabkin, G.A.Braun and J.S.Parks, J. Biol. Chem. 241 (1966) 4168.
[7] G.Schreiber and P.Feigelson, Anal. Biochem. 21 (1967) 34.
[8] G.Schreiber, H.-M.Rotermund, E.Dinigen and H.Maeno, Z. Analyt. Chem. 243 (1968) 173.
[9] A.von der Decken, J. Cell Biol. 16 (1963) 471.
[10] M.C.Ganoza, C.A.Williams and F.Lipmann, Proc. Natl. Acad. Sci. US 53 (1965) 619.