Review Letter

THE BIOSYNTHESIS OF SERUM ALBUMIN

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As one who worked on the biosynthesis of rat serum albumin many years ago it is interesting to record the significance of two recent reports of the isolation of a precursor albumin from the microsome fraction of rat liver. There is evidence that the precursor is converted into albumin by limited proteolysis. Judah and his colleagues at University College Hospital, London, have described two methods for the isolation of a 'proalbumin' which differs from albumin in the possession of a hexapeptide at the N-terminus [1]. Schreiber and his colleagues, now at the University of Melbourne, describe the isolation of a 'proalbumin' which differs from albumin in the presence of a pentapeptide at the N-terminus [2]. They have determined the sequence of this pentapeptide which is Gly–Val–Phe–Ser–Arg. This differs from the amino acid composition of the hexapeptide of Judah et al. which contains $(Arg)_3$ $(Phe)_1$ $(Val)_1$ $(Gly)_1$. There is a further report by Russell and Geller and another in press [3].

In spite of some difference in the reported amino acid composition of the additional peptide it seems fair to conclude that serum albumin, at least in rat liver, is first synthesized in the form of a precursor which is presumably converted to albumin before the protein leaves the cell to enter the serum. This discovery is of general interest from two main viewpoints. Firstly, because it is yet another example of a precursor protein and secondly because it explains some puzzling features in the literature about the use of antisera for the identification of specific proteins.

A major reason for a protein being synthesized in the form of a precursor seems to be to allow the correct alignment of two or more polypeptide chains which are linked together in the finished multichain protein. Not only is this so in the case of zymogens, such as chymotrypsinogen, but it also applies to insulin, first synthesized as proinsulin, and to collagen, synthesized as procollagen to allow for correct alignment of the chains of the triple helix. That this is not always the way in which such multichain proteins are synthesized is shown by immunoglobulin where there is no reason to think that there is not a single-chain precursor for IgG. Serum albumin is not, however, a multichain protein, consisting only of a single polypeptide chain of about 575 amino acids, so that there must be another reason for a precursor albumin. One outstanding feature of albumin is that it appears to be a simple protein without carbohydrate; whereas all the other serum proteins synthesized in the liver cell are probably glycoproteins. Could carbohydrate be linked to the additional peptide? From the known amino acid composition of the extra peptide it seems unlikely. One would have expected it to contain asparagine for the linkage of *N*-acetyllactosamine but there are no reports of the presence of this amino acid. Judah reports a serine which could possibly link to carbohydrate but it seems unlikely.

Another reason for a precursor of serum albumin could be to allow for vectorial protein synthesis on the membrane of the rough surfaced endoplasmic reticulum. Thus Milstein and his colleagues [4] have produced evidence for an extra peptide at the N-terminus of the light chain of IgG. They suggested that this was necessary for the attachment of the nascent polypeptide chain on the ribosome to the membrane of the endoplasmic reticulum. Albumin also, of course, is secreted and a similar peptide could be required. It will be interesting to make a comparison of the structure of the two precursor peptides, unfortunately that for light chain is not yet available.

I should like now to turn to the characterization of the 'proalbumin' and albumin. When, in 1960, we first

studied the synthesis of rat serum albumin by the isolated microsome fraction [5] we reported that when such a fraction was incubated with the appropriate factors and [^{14}C] leucine and [^{14}C] valine radioactive albumin was obtained so that we concluded that it had been synthesized. Out method of characterization was by the use of a rabbit antiserum but we also checked the effect of first purifying the serum albumin by use of the trichloroacetic acid–ethanol procedure and a one step electrophoresis on cellulose-acetate. Although these additional procedures reduced the specific radioactivity of the albumin the protein retained significant radioactivity. In the next paper [6] we showed that the [^{14}C] leucine was incorporated into a wide range of albumin tryptic peptides and finally [7] we confirmed that this was so by the application of the Dintzis procedure to the tryptic finger print of albumin. Others confirmed these results by other methods [8]. It was, therefore, concluded that the isolated microsome fraction of rat liver was able to effect the synthesis of serum albumin.

Meanwhile Schreiber and his colleagues, then in Freiburg, were studying the synthesis of serum albumin in the intact animal and in particular were devising extremely sensitive methods for the purification of the protein on a micro scale making use of the improved techniques which were becoming available. They showed [9] that many steps were required to obtain albumin in a state of radiochemical purity and certainly immunoprecipitation was not in itself an adequate criterion. When they applied these methods to albumin obtained after the incubation of isolated microsomes they found that the radioactivity of the albumin decreased throughout the purification and concluded that albumin synthesis was either absent or strongly impaired in the cell-free systems [10].

Judah and his colleagues began their studies on the synthesis of serum albumin in slices of rat liver because they were interested in the relationship between the intracellular [K^+] and the secretion of proteins [11]. I was able to warn them that immunoprecipitation was not a satisfactory criterion of purity for albumin. They carefully followed up this observation and showed [12] that antibody precipitation of albumin from [^{14}C] leucine-labelled rat liver leads to material that is contaminated with a protein or proteins of very high specific radioactivity. In fact only 10–25% of the radioactivity of the antibody precipitate was associated with albumin. They also described a method for the separation of radiochemically pure albumin from antibody precipitates.

As Schreiber [2] points out, the finding of a proalbumin clarifies these apparently conflicting reports. Thus it is probable that we were studying the synthesis of proalbumin rather than albumin by isolated microsomes [5–7] but our methods were not adequate to separate albumin from proalbumin and moreover the finger prints of the two proteins would be virtually identical. It also explains the observation of Judah et al. [13] of a further increase of radioactivity in proalbumin-free albumin after addition of cycloheximide to liver slices incubated with [^{14}C] leucine.

The story has several implications for the study of the synthesis of other proteins. Thus it is becoming increasingly difficult to predict the precise nature of nascent proteins on the ribosomes for it is difficult to know whether any particular protein is synthesized initially in the form of a precursor until one studies its synthesis. It is beginning to seem that one should suspect the presence of a precursor unless one has contrary evidence, especially for secreted proteins. Antisera are now seen to be particularly valuable for the detection of nascent proteins although they must be used with adequate controls. It should be noted that in the case of serum albumin it was the antibody to pure serum albumin that allowed its precursor to be detected. The sophisticated methods of Schreiber [9] removed the proalbumin contamination. In the search for a precursor it is good to examine the antibody precipitate for a precursor protein. Perhaps too it is worth recalling that in considering the size of a messenger RNA for a particular protein one has not only to remember the length of the poly A tail at the 3′ terminus but also to consider the presence of a precursor peptide at the 5′ terminus.

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