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Role of the Polyadenylic Segment in the Translation of Globin Messenger RNA

The translation of native rabbit globin messenger RNA and that of poly(A)-free globin messenger RNA have been compared after injection into *Xenopus* oocytes. The initial rate of translation of poly(A)-free mRNA is close to that found with intact mRNA. However, at longer incubation periods, the rate of globin synthesis with poly(A)-free mRNA is considerably lower than with native mRNA. Similar differences in the template activity of the two mRNA preparations were found with a Krebs II ascites tumor cell-free extract. It is concluded that the presence of the 3′poly(A)-rich sequence in mRNA is required to ensure high functional stability.

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Catalytic and Regulatory Properties of Different Mitochondrial Acetoacetyl-CoA Acetyltransferases from Ox Liver

A control point in ketogenesis at the level acetyl-CoA/acetoacetyl-CoA was deduced from studies in vivo and in vitro on the [^{14}C]carbonyl/[^{14}C]carboxyl ratio of acetoacetate formed from [1-^{14}C]fatty acids[1]. Additional support for this interpretation of the studies in vivo has been obtained by the crystallization of two acetoacetyl-CoA acetyltransferases A and B (EC 2.3.1.9) from ox liver. Both enzymes could be shown to be homogeneous by sedimentation analysis.

The kinetic analysis of the two enzymes in the direction of acetoacetyl-CoA *synthesis* revealed different properties. Enzyme B is nearly 4 times more active than enzyme A. The V values divided by the acetyltransferase activities are 77.5 $nmol \times min^{-1} \times unit^{-1}$ transferase (enzyme A) and 284.5 $nmol \times min^{-1} \times unit^{-1}$ transferase (enzyme B), respectively. The two enzymes also differ in their affinities for acetyl-CoA in the second rate-limiting step (eqn. 2) of the overall reaction (eqns. 1 and 2)[2].

(1) CH_3-COSCoA + Enzyme-SH $\rightleftharpoons$ CH_3-COS-Enzyme + CoASH

(2) CH_3-COS-Enzyme + CH_3-COSCoA $\rightleftharpoons$ CH_3-CO-CH_2-COSCoA + Enzyme-SH

The affinity of acetyl-*S*-enzyme for acetyl-CoA (eqn. 2), as measured in an isotope exchange assay[1,3], gave K_m values of 87.1 μM for enzyme A and 19.6 μM for enzyme B. In assays of the overall reaction (eqns. 1 and 2), however, the K_m values for acetyl-CoA are identical (91 μM) for both enzymes. Acetoacetyl-CoA and CoA both acted as competitive inhibitors of the enzymes A and B and thus exert a negative feedback on acetoacetyl-CoA synthesis.

The higher activity in acetoacetyl-CoA synthesis as well as the high affinity for acetyl-CoA in the exchange assay favour enzyme B as the regulatory enzyme involved in ketogenesis at the acetyl-CoA/acetoacetyl-CoA level.

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G. Jackl and W. Sebald

Identification of Two Products of Mitochondrial Protein Synthesis Associated with Oligomycin-Sensitive ATPase from Neurospora crassa

Soluble mitochondrial ATPase (F_1) was isolated by standard procedures from *Neurospora crassa*. The enzyme protein could be resolved by sodium dodecylsulfate-gel electrophoresis into five subunits with molecular weights of 58000, 55000, 36000, 14000 and 11000. Antibodies prepared against F_1 precipitated 13–15% of the protein of TritonX-100-solubilized mitochondria. The precipitate contained, besides the F_1 subunits, additional polypeptides with apparent molecular weights of 38000, 21000, 19000, 15000, 10000 and 8000. The total polypeptide pattern resembled closely that of oligomycin-sensitive ATPase isolated from yeast[1] or bovine heart[2] mitochondria.

The site of synthesis of the individual polypeptides was investigated by incorporation in vivo of radioactive leucine in the presence of specific inhibitors of mitochondrial (chloramphenicol) and extramitochondrial (cycloheximide) protein synthesis. A cycloheximide-resistant and chloramphenicol-sensitive amino acid incorporation was found in the case of the polypeptides with molecular weights of 19000 and 10000. This shows that these two protein components are translated on mitochondrial ribosomes. Remarkably, a mitochondrial translation product with a molecular weight of 8000, which was found to occur in yeast ATPase[1], could not be detected in the *Neurospora* enzyme.

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H. Jansen and W. C. Hülsmann

Liver and Extrahepatic Contributions to Overall Postheparin Serum Lipase Activity

Heparin releases triglyceridase activity from different organs into the blood. In order to get an insight into the potential clearing rate of triglycerides from the blood, it is important to know the contributions of liver and extrahepatic enzymes to the total lipase activity of postheparin serum. We found earlier[1] that a distinction between liver and extrahepatic lipase activities can be made by immunological methods. Further studies revealed that a number of parameters may influence the relative contributions of liver and extrahepatic lipases. Low intravenous heparin doses (0.5 I.U. hep./100 g body wt.) in rat release almost solely (97%) the extrahepatic lipase activity. When 20 I. U. heparin were injected per 100 g body weight, the liver contributed 63% of the lipase activity. It was also found that under these conditions, blood removed 2 min after heparin injection contained more liver enzyme than blood removed after 20 min. This removal of liver enzyme activity from the blood was accompanied by the removal of liver enzyme antigen, as was shown immunologically, indicating removal of enzyme protein from the blood during heparin degradation rather than denaturation of the enzyme remaining in the blood Therefore it is possible that the enzyme is rebound to liver membranes. These data presented for rat also apply in principle for man. Fat feeding in rats resulted in an increase of heparin-releasable lipase activity in the blood. Earlier[2] this phenomenon was detected employing palmitoyl-CoA instead of trioleate as the lipolytic substrate. Preliminary experiments suggest that the heparin-releasable liver lipase may be identical with heparin-releasable palmitoyl-CoA hydrolase.

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Ph. Janssens de Varebeke

Synergic Effect of $Mg^{2\oplus}$ and Spermine on the Aminoacylation of tRNA of Phaseolus vulgaris

In our last communication, we showed the effect of polyamines on the aminoacylation of the tRNA of *Phaseolus vulg.* for a few amino acids (Arg, Ile, Leu, Tyr, and Val) i. e. the ability of the polyamines to replace $Mg^{2\oplus}$ in the transfer reaction. The various extraction techniques and assay methods were given, too. (Ph. Janssens de Varebeke, 1974). Our recent experiments showed a synergic effect between spermine and $Mg^{2\oplus}$: the addition of spermine (0.005M) to the incubation medium containing a suboptimal concentration of $Mg^{2\oplus}$ (charging at only 5% of the optimal level) restored the charging of the tRNA. We observed a high synergic effect for the following amino acids: Arg, Asp, Gly, Ile, Leu, Met, Phe, Thr, Tyr and Val.

On the other hand no synergic effect was observed for Ala, Asn, His Lys and Ser.

Kinetic studies showed a slower aminoacylation in those experiments where spermine and $Mg^{2\oplus}$ were used together than with $Mg^{2\oplus}$ alone. With methionine, after 60 min of incubation with spermine and a limiting $Mg^{2\oplus}$ concentration, the level of incorporation was 80% of that observed when optimal concentration of $Mg^{2\oplus}$ was used; in this case the plateau was obtained after 30 min at 30 °C.

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D. Jeckel

Binding Constants of Nicotinamide Nucleotides to Native and Modified (Tyrosine Residue) Lactate Dehydrogenase

A satisfactory theory for the mechanism of action of an enzyme should explain the observed pH dependencies of reaction rates and binding constants in terms of the amino acid side chains at the active centre of the enzyme. Stinson and Holbrook[1] investigated the effect of pH on the binding of NADH to lactate dehydrogenase (EC 1.1.1.27) by following the change in fluorescence after addition of nucleotides to the enzyme and found that the affinity for NADH decreases with increasing pH, corresponding to the dissociation of a group with a pK of 9.5, which may be the phenolic hydroxyl group of a tyrosine residue. To examine this presumption we determined the dissociation constants of the binary dehydrogenase-NAD(H)-complexes as a function of pH with lactate dehydrogenase preparations containing a modified tyrosine residue per nucleotide