DISSOCIATION OF HELA CELLS RIBOSOMES BY HEPARIN

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Thé present communication describes the dissociation of ribosomes of mammalian origin, induced by high concentrations of heparin. Heparin, like polyvinyl sulfate is a well known inhibitor of RNAse (Flanagan, 1967). It was systematically used by us in experiments designed to study the variation in the ratio : polysomes - free ribosomes in HeLa cells partially synchronized by thymidine. Recent results with dextran sulfate (Miyazawa & al.), another sulfonated polyanion which depolymerizes ribosomes, prompted us to investigate the effects of heparin.

Methods

Growth of HeLa cells in spinner cultures, preparation of the cytoplasmic extracts and counting the activity of fractions from sucrose gradients, have been previously described (Miller, 1967). Cytoplasmic extracts were analyzed by sucrose gradient centrifugation (15-30% sucrose gradients made up in a solution containing 10^{-2} M Tris pH 7.4, 10^{-2} M NaCl, $1.5 10^{-3}$ M $MgCl_2$, with or without

500 µg/ml heparin : RSB Hep^+ or RSB Hep^- buffers) in the SW 25-1 rotor of the Spinco L ultracentrifuge set at 3-5° for the time and speed mentioned in each caption of the figures.

^3H uridine (760 mC/mM) and ^{14}C l-leucine (161 mC/mM) both randomly labelled were purchased respectively from Amersham Radiochemical Centre (England) and the Centre Nucléaire de Mol (Belgium). Final activities in the growth medium were respectively 1 and 0.1 µC/ml.

Heparin, a product of Hoffman - La Roche & Co Ltd, Basle, Switzerland was used throughout these experiments at a final concentration of 500 µg/ml.

Results and Discussion

Fig. 1 shows the sedimentation pattern of a cytoplasmic extract which has never been exposed to heparin : the 45S peak is barely detectable. Fig. 2A shows that heparin, when added during the extraction steps only, enhances the 60S and 45S peaks considerably. This is the first indication that heparin might

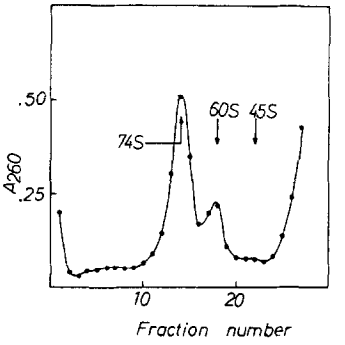

Fig. 1 Sedimentation pattern of a cytoplasmic extract prepared without ever having been in contact with heparin. Centrifugation : 16 hr at 20.000 rpm.

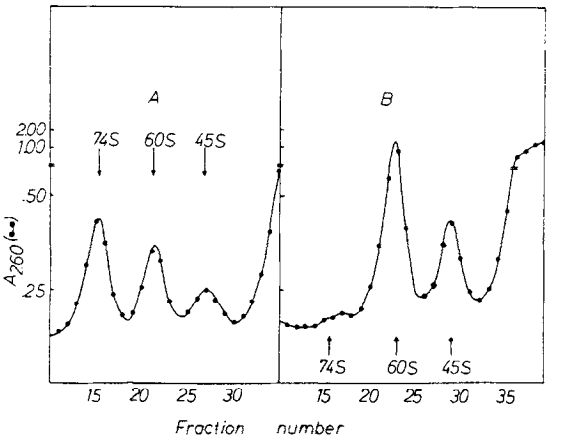

Fig. 2 Effect of time on the dissociation of 74S ribosomes. A cytoplasmic extract is prepared in presence of heparin from approximately $1.5 10^8$ cells. It is carefully halved, each half being centrifuged respectively on a RSB Hep$^-$ (A) or in a RSB Hep$^+$ (B) sucrose gradient. Centrifugation : 16 hr at 20.000 rpm.

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dissociate ribosomes.

Further confirmation is obtained by exposing the extract for a prolonged period to the inhibitor. Ribosomes are seen to disappear completely (Fig. 2B) while the heights of the 60S and 45S subribosomal peaks increase in the same proportion : Careful integration of the surfaces shows the increase in 60S and 45S areas to be quantitatively accounted for by the disappearance of 74S ribosomes.

A more sensitive test for dissociation is to examine single ribosomes from a culture labelled for 24 hr with either ^{3}H uridine (Fig. 3) or ^{14}C leucine (Fig. 4). We should first comment on the presence of slowly sedimenting material tailing from the top of the gradient in these sedimentation profiles (Fig. 4A). Neither ^{3}H uridine nor ^{14}C leucine labels it to a significant extent and

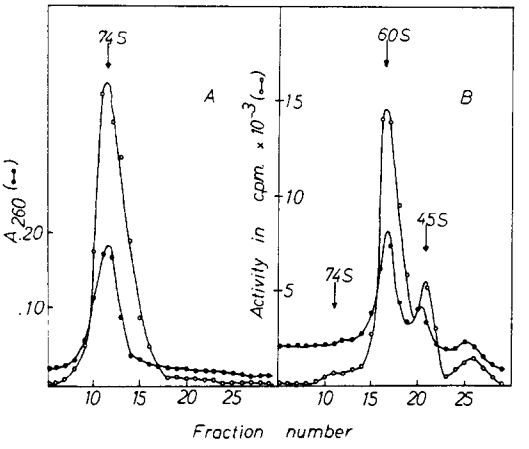

Fig. 3 Sedimentation pattern of ^{3}H uridine labelled ribosomes. About $1.5 10^{8}$ cells are labelled continuously for 24 hr with ^{3}H uridine (see Methods). Further treatment as described in Fig. 2. Centrifugation : 14.5 hr at 23.000 rpm. A : RSB Hep^{-}; B : RSB Hep^{+}.

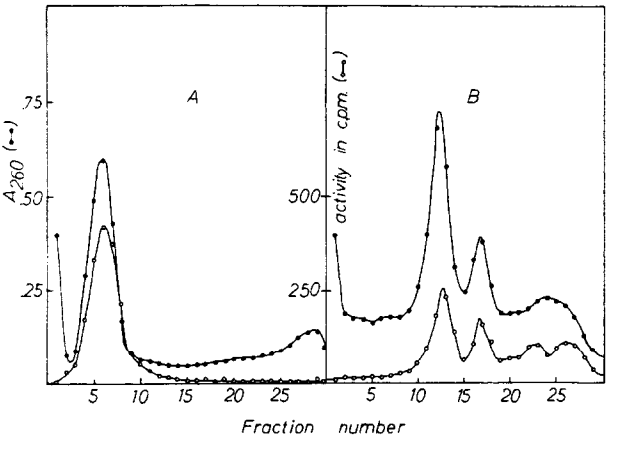

Fig. 4 Sedimentation pattern of ^{14}C 1-leucine labelled ribosomes. About $1.5 10^{8}$ cells are labelled continuously for 24 hr with ^{14}C 1-leucine (see Methods). Further treatment as described in Fig. 2. Centrifugation : 20 hr at 18.000 rpm. A : RSB Hep^{-}; B : RSB Hep^{+}.

it appears in a rather unpredictable way (compare Fig. 3A and 4A). Its very nature remains unknown for the moment. The use of single ribosomes demonstrates in a clearcut way the complete dissociation brought about by heparin : they can not longer be detected by either of the two isotopes (Fig. 3B, 4B). The products formed are 60S and 45S ribosomal subunits together with a heterogeneous sedimenting material to which a nominal value of 30S is given. Summing the absorbance or activity of the 30S peak to that of the 45S gives back the classical 2:1 value for the 60S- to 45S ratio. This suggests but does not prove that a precursor-product relationship might link 30S "particles" and 45S ribosomal subunit. It shows moreover that heparin dissociates only free ribosomes, leaving unaltered those involved in protein synthesis within polysomal clusters. Repeated experiments with ^{14}C leucine show the tracer to be almost equally shared between the two ribosomal subunits. Some radioactivity cosediments however with the 30S material. It does not seem that heparin solubilizes proteins originally present on the 74S ribosome.

The molecular level at which heparin acts is not known. Experiments such as those reported in Fig. 3 and 4 where Triethanolamine buffer was used instead of Tris (Perry, 1966) show that the ribosomes are less easily dissociated in that case. This suggests that the presence of protons coming from the ionizable amino group in the Tris molecule may play a role in that dissociation.

Since heparin dissociates ribosomes so easily it might prove to be a very useful tool in the study of systems containing both ribosomal subunits and unprotected messenger RNA.

Acknowledgments

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