DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

Room 9N-119, Bldg. 10

March 18, 1971

Dr. Debi P. Burma
Department of Biochemistry and
Biophysics
College of Medical Sciences
Banares Hindu University
Varanasi - 5, India

Dear Debi:

First, to take care of your queries.

I no longer have the L. plantarum strain. You could get it from David Logan (who used to be with me):

Department of Biology
York University
4700 Keele Street
Downsview 463, Ontario, CANADA

Tell him I told you to write.

Comments on the lysozyme paper are coming within the week. I also have Ramanand's thesis, from the University.

Millipores enclosed. They are type HA--I hope that that is correct.

RNA (commercial) which Maharani asked for also being sent. A lot of this will also come out of dialysis bag and it should be precipitated with 70% alcohol and dialyzed before use.

The phosphocellulose fractionation must not be tried until after the DEAE column (the first DEAE). This step gets rid of nucleic acids and unless you get rid of them the PNPase doesn't stick on P-cellulose. The other thing is that column must be equilibrated with EDTA, not Mg, or again, enzyme doesn't stick. Also, enzyme should be diluted, prior to application, in enough starting buffer to complex all the Mg AND to lower salt concentration to 0.05 M or below (I use conductivity meter). With that in mind here is procedure, using P-cellulose, P11 (Whatman).

P-Cellulose regenerated by procedure of Bollum, Methods in Enzymology, Volume XIIB, page 593. Then equilibrated with 0.05 M Tris, pH 7.8, 1 mM EDTA, 1 mM β-ME. See Burgess, JBC 244, 6160, 6168 (1969) for easiest way to equilibrate.

Make a column so that you will have 6 phosphorolysis units of enzyme per ml of resin. Wash. Dilute enzyme as above so that conc. equals 5 units per ml. Load on column (cold) at 9-10 ml per hour per cm^2. Wash well with equilibrating buffer, until no more 280 absorbing material comes out.

Then run a linear gradient (total volume of ten times the column volume) from 0 NaCl to 0.3 M NaCl in the equilibrating buffer. Collect in tubes that have enough Mg^{2+} to make final concentration 2 mM in fraction. The enzyme comes out very dilute: we concentrate by ultrafiltration, but a tiny DEAE column should do. Finally, we dialyze against 0.01 M Tris, pH 8.2, 0.2 M NaCl, 1 mM $MgCl_2$, 1 mM β-ME, and store frozen.

I see that you are having a good time with the ultracentrifuge. I'm really delighted to hear that it is in working order. Also, I think the work with the proteins of the 30S subunit is really promising and should lead to interesting experiments. Are you doing this with Salmonella ribosomes or E. coli? What is the explanation for the enhancement of RNase I activity by ribosomes in absence of Mg^{2+}?

Randy Holmes has continued to pursue the RNase V question although it is getting quite tedious. We have checked out the smallest details and even had extracts from Schlessinger himself to test. We find the right level of poly U degrading ability but the activity has none of the properties described for RNase V. If we didn't believe that the whole thing was so important we would have dropped it long ago. Randy has other interesting things to do with T_7--including some lysozyme mutants. In any case, there are now at least five papers published on RNase V--one more confusing than the next--as well as numerous references to it in other papers. Many people have accepted its existence on very scanty evidence. So the notion of knocking down its existence is a very serious one. On the one hand it's not interesting work, but on the other, its important. Well, come June you will see the data for yourself.

Now, here are the thoughts of the boss on your trip. You must make your decision in near future. The lab will manage without you--it is a sad fact of life that they always do manage. In any case, I must know soon in order to arrange the money. You know what Government Red Tape is like. Also, you must soon register for meetings. I cannot say what the money situation will be like if I am not here when you come. I will find out if it turns out to be necessary.

Enclosed are more details on G.R.C. The Nucleic Acid meeting looks excellent for us. There is more enzymology than any other recent conference that I recall. I have almost decided not to go to San Francisco Biochem. meeting myself, and to go instead to GRC--Nucleic Acids, although it is only 2 weeks before our departure. (Enclosed is a note for Maharani.)

That's all for now--we're looking forward to your visit. Don't forget to give me plenty of time to collect any items you require. Also, you never let me know about whether the second year's money came through with no trouble, or not. (Enclosed is a note for Maharani.)

Yours sincerely,

Encls.
MS:peg

Maxine Singer