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J C BOSE MEDAL LECTURE 1980

Bose Institute, Myself and Ribosome

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While receiving the J C Bose Medal of the Academy it is impossible for me to prevent my imagination to flutter its wings and fly back to the good old days. I never met, rather saw Jagadish Chandra Bose. As it was true for most of my sehool-mates, he was one of my several heroes, Rabindra Nath Tagore in literature, Subhash Chandra Bose in politics and Acharya P C Ray as well as Jagadish Chandra Bose in science. In my school days I was fascinated with Jagadish Chandra's 'Abyakta' ('Unexpressed' as expressed in *Bengali*). In what a lucid way he could animate the inanimate objects and make the dumbs speak. I had also read his biography several times; his upbringing in childhood by an ex-dacoit, convicted by his father earlier, was quite thrilling for me. I was so keen to know one such dacoit and hear all the stories of dacoity. Although I was born and brought up in a town, once a while I used to go to my paternal village and heard about the dacoities but those were terrorising and never appealed to me. In 1937 when I was a student of class VII the shocking news of the death of Acharya Jagadish Chandra came. That dealt a death blow to my ardent desire to see one of my heroes with my own eyes. All the time I used to think that I shall go to Calcutta for higher education after finishing my school days and then would have the opportunity of seeing all my heroes to the fullest satisfaction of my eyes as well as heart. Although I went to Calcutta eventually for higher studies, I never saw any of my four heroes. Viswabharati, Rabindra Nath's creation at Santiniketan and the usual place of his stay, was close to Burdwan where I lived but still I had never the opportunity to go to Santiniketan to see him. Subhash Chandra Bose disappeared from India when I was in school, he never came back. Before I entered the University College of Science at Calcutta, Acharya Prafulla Chandra died at the same premises (an extension of the Science College) where he used to work and live. Although it was possible for me to see him yet I missed that opportunity as well. After being a post-graduate student in Chemistry I could get the glimpses of Prafulla Chandra through his disciples, some of whom were my teachers, for example, P C Mitra, P R Ray, P B Sirkar, B C Guha etc. The wing of the Chemistry Department which was constructed by his life's savings was his last abode. This sage without a family and with the most modest

living did not need much. Although Jagadish Chandra and Prafulla Chandra were very intimate friends their modes of living were entirely different. Jagadish Chandra came from a well-to-do family and lived in the Western style.

I could never realise that I shall be intimately connected with the 'Creation' of Jagadish Chandra, the 'Basu Bingan Mandir' (Bose Research Institute), about which I read a lot in my early years. I often dreamt of this temple and later found it not much different from that of my imagination. As a student of the M.Sc. class in Chemistry I had once an opportunity to be inside the premises of the Bose Institute. The day was 30th November, the birthday of Jagadish Chandra and also the foundation day of the Institute. As is the practice every year there was a special lecture on that day by some eminent scientist, I forget, who it was. A few of us went to the Institute to collect the invitation cards which would enable us to attend the function and listen to the lecture in the afternoon. After some pleading we got the cards. But I was not so much interested in the cards than the Institute. The buildings and the set up inside appealed to me. It was a big suspense to find out the beautiful gardens and the calm and quiet atmosphere just beside the noisy main street with the trams, buses and rickshaws plying in a constant flow in both the directions. Even that day I did not realise that I shall be spending long 15 years in that temple of science.

In 1949 I was working as a Lecturer in a private college and frantically searching for a research scholarship as I had the great desire to follow the footsteps of Jagadish Chandra, Prafulla Chandra, C V Raman, Satyen Bose, Meghnad Saha and others. Naturally I was quite thrilled when one of my teachers told me that there was a vacancy at the Bose Institute. They were looking for a physical chemist and he had already recommended my name. I had to meet D M Bose, nephew of Jagadish Chandra and the then Director of the Institute. Although he was a physicist, he was a man of versatile interest in numerous fields including arts and literature. Later I could find out that he devoted most of his time in studies. His scientific life was not actually a great success as the primary object of his career appeared to be to boost up the work of his maternal uncle in biology and specially to support him dogmatically in all his speculations and hypotheses. All the writings of D M Bose clearly point out to this. Thus he forgot about his own career. The interview was over within a few minutes, I got the scholarship, joined the Institute and got stuck there for next fifteen years. Although I eventually left the Institute but quite reluctantly and under very odd circumstances.

The humble work which I am presenting today was initiated after I joined in 1964 this great University founded by Pandit Madan Mohan Malaviya. The work is in the area of biochemistry and molecular biology. Bose Institute, actually speaking D M Bose, transformed me into a biologist from a physical chemist. Throughout my training period in the school, college and university I had no interaction with biology, my first exposure to biology was in this Institute. On the insistence of D M Bose I had to work on 'photosynthesis' in Canada. Intimacy with S P Sen, my microbiologist colleague at the Institute, led me to work on 'nitrogen fixation' in USA. Eventually I had to have the training in 'enzymology' under B L Horecker at the National Institute of Health at Bethesda, Molecular Biology was developing at a slow rate at that time. Like many biochemists I also got involved in Molecular Biology. My involvement became more while working with the Nobel Laureate Severo Ochoa in New York University School of Medicine. Starting with

the career of a pure physical chemist I have ended up in a medical faculty. This is, however, not a big surprise these days.

Practically speaking, I started my career with a drain pipe in order to carry out paper chromatography according to the method of Consden, Gordon and Martin. Through this technique I got interested in Calvin's work on photosynthesis, elucidating the dark reactions following carbon dioxide fixation. But it was in 1955-56 in Bob Burris's laboratory at Madison, Wisconsin that I had my first encounter with the cell-free protein synthesising system. The cell-free extract of *Azotobacter vinelandii* was treated with N^{15}-labelled glutamic acid to find out whether this amino acid was incorporated into protein. Mass spectrometry was used to detect the incorporation of N^{15} (Burma & Burris 1957). At that time Zamecnik, Hoagland and others were developing the cell-free protein synthesising system with the rat liver preparations (cf. Zamecnik 1969). After sometime, following my return to India in 1957 I restarted this work with the help of my wife. We developed a cell-free protein-synthesising system with a particulate preparation of *A. vinelandii* (Chakravorti & Burma 1962). However, we had very little idea about the nature of this preparation. At that time ribonucleoprotein particles attached to the endoplasmic reticular structure of the eukaryotic cells, later named as 'ribosomes', were being recognised as the sites of protein synthesis. When I joined Ochoa's laboratory I presented the work done at Calcutta, at the Federation meeting held at Chicago in 1960 (Burma & Chakravorty 1960). It was an excitement as Zamecnick was the chairman of the session.

My work on protein-synthesising system got interrupted for almost a decade as I became involved in another but related area, nucleic acid synthesis. That started with the attempt in Ochoa's laboratory to isolate the 'true' RNA-synthesising enzyme from the extract of *A. vinelandii* (Burma et al. 1961; Ochoa et al. 1961). Ochoa's earlier work, the discovery of polynucleotide phosphorylase created sensation and led to the tremendous development of Molecular Biology through the synthetic polynucleotides prepared by the catalytic action of the enzyme. But unfortunately this enzyme is not the enzyme responsible for the synthesis of RNA within the cell as it was originally thought to be. Through tremendous effort of several laboratories, RNA polymerase was discovered and it immediately turned out that this is the enzyme responsible for RNA synthesis within the cell. Since that time I have meddled with several enzymes involved in the metabolism of RNA. That was done mainly in the Bose Institute after my return from the second trip to USA and also in this laboratory after joining the university. While handling one such enzyme which degrades RNA (designated as RNase I) I came back to protein-synthesising system again and got directly involved with the 'ribosome', the site of protein synthesis (cf. reviews by Burma 1979a, b). My object is to describe, how this happened and what is the result of this enterprise.

Immediately after joining this university, I had ventured into a new field without much of expertise. The reason was the want of equipments in the new place, for carrying out normal biochemical work. My wife had experience with viruses and I utilised her experience to have my students do some work in biochemistry. I was quite fascinated by a publication of Arthur Kornberg (Kornberg et al. 1959) where he unequivocally demonstrated the induction of a series of virus-specific enzymes following infection of *Escherichia coli* with T2, a virulent phage. I thought of trying my luck with *Salmonella typhimurium*-P22 system with which Maharani was working. I was, however, aware that P22 is a temperate phage unlike T2. Further, it was known that RNA polymerase is responsible

for the synthesis of all kinds of RNA including messenger RNA within the cell. Messenger RNA has a rapid turnover. But even now it is not clear, which enzyme is actually responsible for the degradation of messenger RNA. Once Schlessinger and his coworkers (Kuwano et al. 1970) claimed to have isolated a new RNase (RNase V) responsible for the degradation of mRNA but that was later found not to be true (Holmes & Singer 1971; Bothwell & Apirion 1971). Four more enzymes were characterised at that time in *E. coli* which are responsible for the degradation of RNA, those were designated as RNases I, II, III, and IV. Assuming that one of these enzymes would be responsible for the degradation of mRNA and there will be rapid turnover of mRNA in virus infected cells, we hoped that the level of one or more of these enzymes responsible for the actual turnover will increase following phage infection. We had some preliminary evidences that RNase II is the enzyme (Mukhopadhyaya et al. 1968), but we abandoned the work eventually as the evidences were not too strong. The only enzyme which we could unequivocally demonstrate to be phage-specific was a lysozyme (Koteswara Rao & Burma 1971). Any way, as part of this activity we thought of purifying each and every RNase and characterise those with the great hope that if there is any phage-induced RNase, that will turn out different from host RNases.

Kalpana Chakaburtty who is now settled in USA was busy with the purification of RNase I (Chakraburtty & Burma 1968). She purified it extensively and studied some of its properties but Alok Datta who is also in USA, actually purified it to homogeneous state (Datta & Burma 1972). During the purification procedures it was observed that the activity of RNase I in the crude alumina extract was very low and it increased with the steps of purification. Naturally the suspicion was that there was an inhibitor present in the crude extract which inhibited the enzyme and was removed during the purification. So I asked Amal Mukhopadhyay who is now associated with the 'sister' Department of Physiology, to purify the inhibitor but poor Amal had lot of trouble. After partial purification the inhibitor turned out to be very unstable. It was literally falling apart. Preliminary experiments showed that it was nucleoprotein in nature. That led us to search through the literature and quickly discover that the presumptive inhibitor is ribosome. It was Elson (1958) who first discovered RNase I and located it at the ribosome. As already mentioned, ribosome is the ribonucleoprotein particle attached to the endoplasmic reticular structure of the eukaryotic cells and is the site of protein synthesis. In prokaryotes there is no reticular structure and the ribosomes occur in the free state. In eukaryotes some ribosomes are also found in the free state. Due to Elson's observation, RNase I was believed to be a ribosomal enzyme and suspected to be involved in the degradation of messenger RNA. However, Heppel and his coworker (Neu & Heppel 1964) later showed that RNase I is actually located in the periplasmic space (space between membrane and cell wall) of the bacterium. Since the enzyme has strong affinity for ribosome it gets artificially attached to the ribosome during the breakage of the cells. They further showed that RNase I specifically associates with the smaller subunit of the ribosome of *E. coli*.

Like all ribosomes, *E. coli* ribosome is composed of two subunits, one larger and the other smaller. According to their sedimentation coefficients those are designated as 50 S and 30 S respectively; the intact ribosome has the sedimentation coefficient of 70 S. With the above information available in the literature our work became rather simple. We isolated ribosomes by the classical method of ultracentrifugation,

carried out the hydrolysis of poly A by RNase I and added separately 70 S, 50 S and 30 S ribosomes to the reaction mixture. 70 S and 30 S ribosomes inhibited the hydrolytic reaction whereas 50 S ribosome did not (Datta & Burma 1972). The inhibition was, however, dependent on the concentration of Mg^{++}. This simple inhibition assay is very helpful in determining the cross-contamination of the two subunits and is routinely used in our laboratory for this purpose. The degradation of the subunits by RNase I is equally interesting. At concentrations below 1mM of Mg^{++} both the subunits are degraded at a reasonably fast rate whereas at concentrations of 1 mM or more 50 S ribosome is degraded but 30 S ribosome remains untouched. At very high concentration (20 mM) a part of the structure of 50 S ribosome (10% of total RNA) is degraded and then the remaining structure becomes resistant to the further action of the enzyme. In one of our latest publications (Raziuddin et al. 1979) it has been shown that this structure could be the protuberance (named as L/7L12 stalk, as the proteins L7/L12 are located there) of 50 S ribosome as visualised by immunoelectron microscopy (Strycharz et al. 1978). The treatment of the 50 S subunit with 1M ammonium chloride and subsequent precipitation with 50% alcohol lead to the release of the proteins L7 and L12 from this region (Highland & Howard 1975). It appears from our work that this region is rich in single stranded RNA and therefore more susceptible to the attack of RNase I. Inspite of removal of this region the remaining core particle remains intact and is capable of associating with the 30 S subunit, so this region is not essential for subunit association (Raziuddin 1979). Very recently Byasmuni (unpublished observation in this laboratory) has indication that the core particle derived by the ethanol-ammonia treatment is resistant to the attack of RNase I; this might be due to the secondary or tertiary structures of RNA produced after removal of the proteins. Kinetics of degradation of rRNAs of ribosome by RNase I are now being studied by Dinesh Tiwari with the help of gel electrophoresis technique.

The differential behaviour of the two subunits with respect to RNase I led to other investigations as well. For example, we could show that ribosomes prepared from different microorganisms behaved differently with respect to their degradation by RNase I as well as the inhibitory capacity of the ribosomes towards the hydrolysis of poly A (Das & Das 1977). The ribosomes prepared from the microorganisms belonging to the same species behaved similarly. The method introduced by the Wittmann group to characterise ribosomes depends on the separation of ribosomal proteins by the two-dimensional gel electrophoresis and comparison of the protein maps (Geisser et al. 1973a,b). Due to its simplicity our method is unique and very helpful.

One other approach that is being followed in this laboratory is to study the binding of the intercalating dyes like ethidium bromide and acridine orange to the ribosomal subunits to find out the differences in the overall structural organisation of the two subunits. These dyes are known to intercalate between the base pairs of double stranded DNA and produce distortion therein (Waring 1970). It was shown earlier that these dyes can bind io ribosomes as well as ribosomal RNAs (Stevens & Pascoe 1972, Suryanarayana & Burma 1975). T Suryanarayana, Z Ali, Raziuddin and recently S Chakrabarti studied this phenomenon extensively in our laboratory. Both spectrophotometric and spectrofluorometric methods have been used for the purpose. It has been shown that the binding of the dye leads to the enhanced degradation of ribosome by RNase I (Suryanarayana & Burma 1975). Our working hypothesis is that due to

intercalation of the dye into the double stranded regions the structure of rRNA becomes distorted and the interaction between rRNA and protein is weakened leading to the unfolding of the structure of the ribosome which leads to the enhanced degradation of the ribosome. Later it was shown in another laboratory (Ballesta et al. 1976) that some proteins are released from the ribosome due to the intercalation. This results in the interference with some of the steps of protein synthesis (Ballesta et al. 1976, Ali & Burma 1979).

Our most interesting finding is that the two subunits behave differently to dye binding so far as the effect of Mg^{++} is concerned (Burma et al. 1979). This metal is found to have considerable influence on the binding of the dye to the larger subunit but less so in case of the smaller one. The situation seems to be similar as observed in case of RNase I action. Most probably 50 S ribosome has more flexible regions which are amendable to conformational changes induced by Mg^{++}. Such regions are less or absent in 30 S ribosome. To us the structure of 30 S ribosome appears to be more rigid than that of the 50 S subunit.

When the untreated ribosomes and their subunits are excited at 300 nm, light scattering is observed at 590 nm. On addition of ethidium bromide there is less scattering by 70 S ribosome and 50 S subunit whereas that of 30 S ribosome is enhanced, indicating the difference in the structural organisation of the two ribosomal subunits. When equal amounts of the two subunits are excited at 540 nm in presence of ethidium bromide and the fluorescence intensity is measured at the emission peak (585 nm) the 30 S subunit shows more fluorescence than the equal amount of 50 S subunit whereas the 70 S ribosome shows the fluorescence, intermediate between the two. The binding of ethidium bromide to the two subunits as measured by fluorescence at 585 nm is affected to different extents by varying the concentration of Mg^{++}, the effect being much more in case of the larger subunit than the smaller one.

We are also trying to alter the structure of the ribosome by various treatments and then study the kinetics of degradation by RNase I to find out what effect such treatment produces. Originally, reagents reacting with SH groups of protein (Suryanarayana 1978) or bases of RNA (Das 1977) were used by T Suryanarayana, M Das and others for this purpose (reviewed by Burma 1979a). D Chatterjee and S Ghosh and very recently, B Nag are involved in studies with crosslinking reagents. It is well known that formaldehyde primarily reacts with the free amino groups of the bases of the nucleic acids and the proteins, thereby altering the structure and conformation of both the macromolecules. It is also known to crosslink the two subunits and thus prevent their dissociation (Amons & Moller 1972). It has been shown in our laboratory that the 70 S ribosome of *E. coli* becomes resistant to the hydrolytic action of RNase I on treatment with formaldehyde; the extent of resistance depends on the concentration of Mg^{++} during the treatment as well as during the degradation assay. The 50 S ribosome behaves in the same way as the 70 S ribosome; however, the 30 S ribsome behaves differently. The treated smaller subunit becomes slightly more susceptible to the action of RNase I than the untreated one. This is in agreement with other observations made in this laboratory indicating the differences in the overall structural organisation of the two subunits.

Z Ali used another enzyme, a proteolytic one, in our laboratory to study the overall structural organisation of the subunits (Ali 1978). The tryptic action on the 70 S ribosome and its subunits has been measured by a simple assay method using S^{35}-labelled ribosome as the substrate and determining

the trichloroacetic acid-soluble radioactivity released as a result of the treatment. The 70 S ribosome loses phenylalanine polymerising activity drastically on trypsin treatment although no peptide fragments are released, as determined by the density gradient centrifugation. The structure of 70 S ribosome and its subunits is 'unfolded' on treatment with trypsin but the subunits are more susceptible to tryptic action than the undissociated 70 S ribosome under the same condition of treatment. Again the larger ribosome is more susceptible to tryptic digestion than the smaller one. These results also support the idea that the structure of the 30 S ribosome is more compact than that of the 50 S ribosome. Further, there may be more proteins available on the surface of the small subunit; this leads to the susceptibility to the attack of trypsin and once a few cuts are made in the protein chains the compact structure becomes readily unfolded.

We have also another approach towards the understanding of the differences in the structural organisation of two subunits. M Das and D K Lahiri raised antibodies against 70 S ribosomes as well as 50 S and 30 S subunits in the rabbit. Since an intact ribosome or a subunit is a giant molecule composed of RNAs as well as a large number of protein molecules it was expected that the ribosomes as well as the subunits will be very efficiently precipitated by the antisera. This was not found to be so. 70 S ribosomes and 50 S subunits were very efficiently precipitated but not the smaller subunit (Das 1978, Lahiri & Burma 1980). We prepared the IgG fraction (which is most active in immunoprecipitation of the ribosomes) and measured the precipitation with IgG and had the same results. Gammaglobulins against 70 S and 50 S ribosomes efficiently precipitated both 70 S and 50 S ribosomes. IgG against 70 S or 30 S efficiently precipitated 70 S ribosomes but did not efficiently precipitate 30 S ribosomes. We thought, somehow or other IgG-30 S ribosome-IgG type of complexes are not soluble. So Byasmuni (unpublished observations) carried out the density gradient centrifugation to detect the soluble complexes but he found that the complexes are formed with 30 S subunit no doubt but the amount is comparatively much less. We have been forced to conclude that there is basic difference in the structure of the two ribosomes which leads to this difference. Since the ribosomal proteins of both the subunits are found to be equally antigenic it may be concluded that 50 S ribosomal proteins are more readily available than the 30 S ribosomal proteins for reacting with IgG molecules, or in other words, the structure of 30 S ribosome is more compact than that of 50 S ribosome. Incidentally the extent of precipitation of either subunit is very much dependent on the concentration of Mg^{++}, there is very little immunoprecipitate formed in presence of 0.1 mM Mg^{++} and the precipitation is maximum in presence of 10 mM Mg^{++} (Das 1978). This also shows that the overall structural features of the ribosome are important in immunoprecipitation. From our more recent studies we are inclined to believe that L7/L12 stalk of 50 S ribosome (Strycharz et al. 1978) may play important role in immunoprecipitation. This stalk which is about $100A^{\circ} \times 40A^{\circ}$ is attached to the main body at about 50° and contains all the 4 copies of L7/L12 proteins which are involved in the elongation of the protein chain.

The different studies recorded as above lead to the following characteristics of the two subunits (i) Mg^{++} has more influence on the structure of 50 S ribosome than on the smaller subunit, (ii) 50 S subunit has one or more RNA-rich regions, (iii) some proteins of the 30 S subunits are readily available on the surface, and (iv) the structure of 30 S ribosome appears to be

somewhat more compact that that of 50 S ribosome which appears to be more flexible in structure. The two subunits have evolved for two different purposes. In protein synthesis the 30 S ribosome is responsible to form the initiation complex with the help of *m*RNA, initiator *t*RNA-amino acid complex along with the initiation factors. The subsequent steps of elongation take place after joining of the larger subunit. To carry out these steps the larger ribosome is perhaps more involved that the smaller subunit and therefore its structure has been built up in a more flexible way. Actually the ribosome has to move along the *m*RNA for the elongation of the protein chain. The RNA-rich and protein-poor region (perhaps the L7/L12 stalk as visualised by Lake and his coworkers vide Strycharz et al. 1978, by immunoelectron microscopy) may be responsible for this.

I was associated with the Bose Institute for almost 15 years and left the Institute 15 years ago. My achievement during the three decades has been negligible but that did not deprive me from enjoying research with numerous colleagues, some of whom have been mentioned here due to their involvement in the recent work. Those persons whose names could not be mentioned for obvious reasons, are equally important as they pushed me ahead to receive this honour which I hardly deserve, but am accepting it perhaps more on sentimental than scientific grounds, for which I may be excused.

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