ROLES OF OLIGOPHOSPHATE AND BASE FRAGMENTS IN TRIPLE COMPLEX OF PROTEIN RECA WITH NUCLEOTIDE AND DOUBLE-CHARGED CATION

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The protein recA is the key enzyme of the recombination. All its multiform functions are based on the recA complexes with ATP, ADP and nucleic acids with Mg(+2) participation.

In the NMR and ESR investigation the diamagnetic Mg(+2) is replased by paramagnetic Mn(+2).

We have found that triple complex of the protein recA with ADP and Mn(+2) represents a three-link chain, with the nucleotide in the middle: recA*ADP*Mn(+2). The three-centered construction must be discarded [1].

The binding of the cation by the nucleotide weakly influences the protein-nucleotide interaction. Vice versa, the binding of the nucleotide by the protein does not influence the nucleotide-cation interaction [1].

In the complex of ADP or ATP with the protein recA the oligophosphate fragment appears to be the mane binding group. Nucleozide or the base itself cannot provide a firm binding, but could be responsible for the protein-nucleotide specificity.

EXPERIMENTS

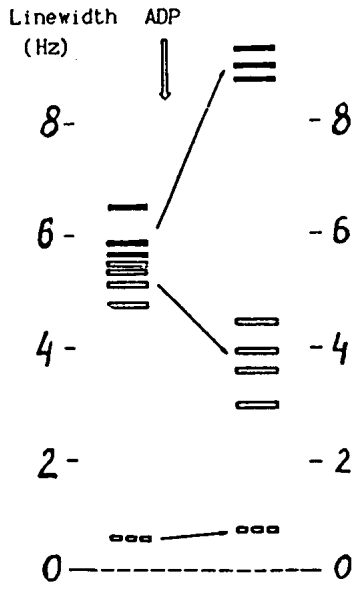

Fig.1. Change of the NMR water line width on effect of Mn(+2) complexes. ■ — the solution contains 0.23 *mM* of the recA and 0.2 *mM* Mn(+2), □ — Mn(+2) only, □ □ □ — recA + Mg(+2) instead of Mn(+2). White arrow: inserting of 0.2 *mM* ADP. Black arrows: the directions of the linewidth change

CONCLUSION. The widening of the line occures only in the presence of the protein, otherwise the narrowing is obserwed. The widening is characteristic for the machromolecular Mn complexes. Hence, triple complex recA*ADP*Mn(+2) does exist.

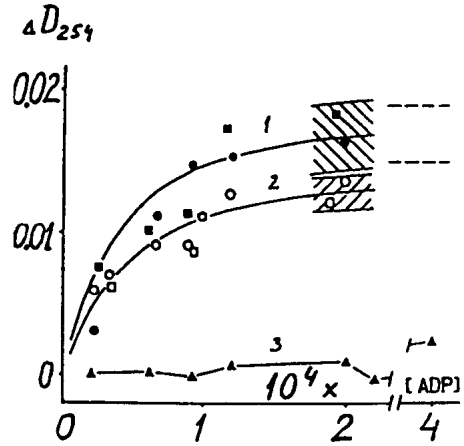

Fig.2. Optical titration of the recA by nucleotides. 0.022 *mM* recA, optical length 1 *mm*.
1 — solutions contain 10 *mM* Mg(+2) ● or Mn(+2) □, *2* — no double-charged cati-○ -titration by ADP, □ — by ATP, *3* — "false" titration curves gives known previously [2] Kdiss = 0.02 - 0.03 *mM*

CONCLUSION. Double-charged cations do not influence the protein-nucleotide complex formation and probably do not participate in direct recA-nucleotide interaction.

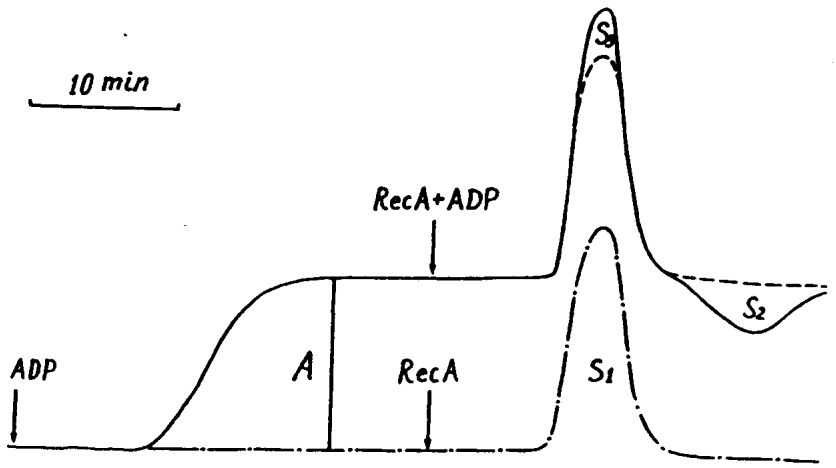

Fig.3. Gel-chromatography of the complex recA*ADP. — — — chromatogramme of protein recA, ——— — recA*ADP with excess of ADP, ——— — the same, with 0.3 *M* pyrophosphate-ion. Sefadex G-50 *M*, coulomn 120X1.8 *mm*, optical registration at 260 *nm*. *A* — optical density of ADP, S1 — area of protein and complex pike, *S2* — area of ADP pit, the quantity taken away with protein complex, *S3* — additional area of ADP in the complex. A, S1, S2, S3 are used for K_{diss} calculation

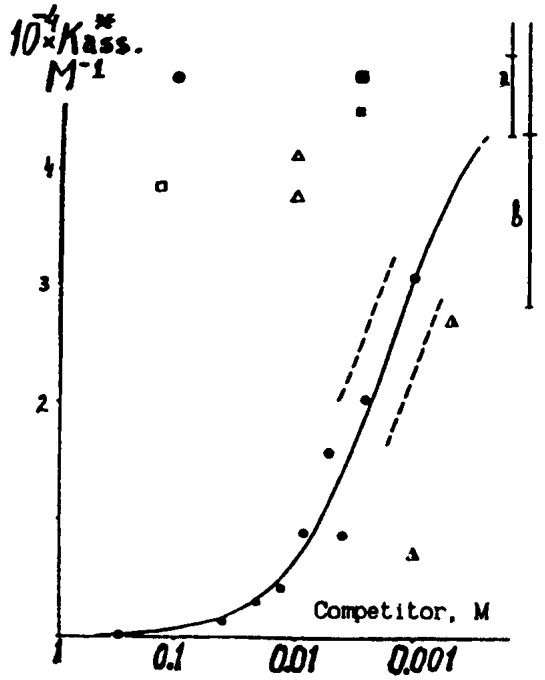

Fig.4. Binding of ADP with recA in presence of various competitors. ● — in presence of pyrophosphate, Δ— ribose-5-phosphate, ▲ — 1-pyrophosphate-5-phosphoribose, ■ — Mn(+2) complex of circular hexaphosphate, ■ — the same + 40-fold excess of EDTA, □ — EDTA only, ◉ — inorganic phosphate. The mean binding constants K_{ass}, calculated on data with pyrophosphate (*a*) and without competitors (*b*) are noted above, on the right

CONCLUSION. The main binding group in the recA-nucleotide interaction is oligophosphate. Neither ribose, nor adenine base does contribute much into the nucleotide-protein interaction.

The $K_{diss} = 1/K_{ass}$ determined for ADP, pyrophosphate and calculated one for adenosine (independent interacton of the groups is emplied) are: 0.02 *mM*, 1.6 *mM* and 710 *mM*, correspondentlv.

The calculated enthalpie of binding of the oligophosphhate and adenosine fragments are 6.1 and 2.5 *Kkal/M*. The 50% variation of the last one leads to 10-fold change of the K_{diss} of the whole nucleotide. I.e., the variation of the weak Van-der-Vaalz interaction of the base with the protein leads to a rather strong change affinity.

REFERENCES

1. Kachurin A.M., Smeliansky A.J., Chernaenko V.M., Achmedov A.T. //Molec.Biology. 1990. V.24. P.621.
2. Knight K.L., McEntee K. //J.Biol.Chem.1985. V.260. P.1077.