ESRF	**Experiment title: A number of proteins froma bacteria to Eukarya and from Antarctic to Volcanic areas**	**Experiment number**: LS-1954
Beamline: ID 14-3	**Date of experiment**: from: 7 May 2001 to: 8 May 2001	**Date of report**: 25 July 2001
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Report: Two proteins have been studied: the retinol binding protein and the Kinase CK2

THE RETINOL RELEASE MECHANISM BY BOVINE RETINOL-BINDING PROTEIN: A pH DEPENDENT HYPOTHESIS BASED ON HIGH RESOLUTION CRYSTAL STRUCTURES OF THE PROTEIN AT DIFFERENT pHs.

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The target transport of non-polar ligands by protein carriers to membranes or membrane receptors involves the release of these ligands on or near membranes. These hydrophobic ligands are often bound inside a deep pocket, as shown by the crystal structures of plasma retinol-binding protein (RBP) from different sources (1, 2). Retinol can be completely released from RBP *in vitro* by treatment with organic solvents, heating or low pH (3). It has been shown that the release of retinol is strongly accelerated by a decrease of pH in the presence of liposomes as well as in their absence. It has also been shown that both *apo* and *holo*-RBP can be transformed at low pH into the molten globule state, which is a conformational state that globular proteins assume under mild denaturing conditions, and it has been suggested and shown experimentally to be involved in protein translocation or insertion into membranes.

In order to test the mechanism of the pH-mediated release of the ligand from RBP, we have exploited the great stability of the crystals of the bovine protein at different pHs and measured high-resolution data from crystals soaked at different pH values. Five data sets were measured, at pH 9, 7, 4, 3 and 2. The crystals at high pH (9 and 7) diffract to about 1.2 Å resolution, whilst the remaining at about 1.4 Å resolution. We were not able to detect the release of retinol from the protein, since the tight crystal packing prevents the drastic necessary conformational change. Nevertheless, we have observed significant changes in the overall pattern of charge distribution at low pH, which is consistent with the original hypothesized mechanism (3). We have also shown the increase of the volume of the cavity on lowering the pH and the loosening of some of the polar interactions which keep the retinol inside the cavity. All these evidences gives interesting clues to explain the mechanism for the release of retinol.

1) Zanotti, G., Berni, R., Monaco, H. L. (1993). Crystal structure of liganded and unliganded forms of bovine plasma retinol-binding protein. *J Biol Chem* **268,** 10728-10738.
2) Cowan, S. W., Newcomer, M. E., Jones, T. A. (1990). Crystallographic refinement of human serum retinol binding protein at 2A resolution. *Proteins* **8,** 44-61.
3) Ptitsyn O. B., Zanotti G., Denesyuk A. N. and Bychkova V. E. (1993). Mechanism of pH-induced release of retinol from retinol-binding protein. *FEBS* **317 (3),** 181-184

DATA COLLECTION AND PROCESSING STATISTICS					
pH	7	2	3	4	9
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions	a=45.91 b=48.67 c=75.28 $\alpha=\beta=\gamma=90°$	A=44.42 b=48.90 c=75.78 $\alpha=\beta=\gamma=90°$	A=44.48 b=48.94 c=75.50 $\alpha=\beta=\gamma=90°$	A=45.29 b=48.77 c=74.61 $\alpha=\beta=\gamma=90°$	A=46.10 b=48.68 c=75.74 $\alpha=\beta=\gamma=90°$
Resolution (Å)	74.54-1.27	44.28-1.48	48.79-1.46	40.82-1.45	40.82-1.09
Total reflections	226144	168138	195663	136920	552856
Unique reflections	42476	26941	28470	28216	65032
Overall completeness (%)	94.3	94.8	96.9	95.7	88.2
Completeness in last resolution shell (%)	94.3	94.8	96.9	95.7	88.2
R_{sym} (%)	5.7	9.4	4.9	6.2	6.7
Multiplicity	3.1	2.7	4.1	3.0	3.8
$I/\sigma I$	5.6	3.3	2.8	5.7	3.1

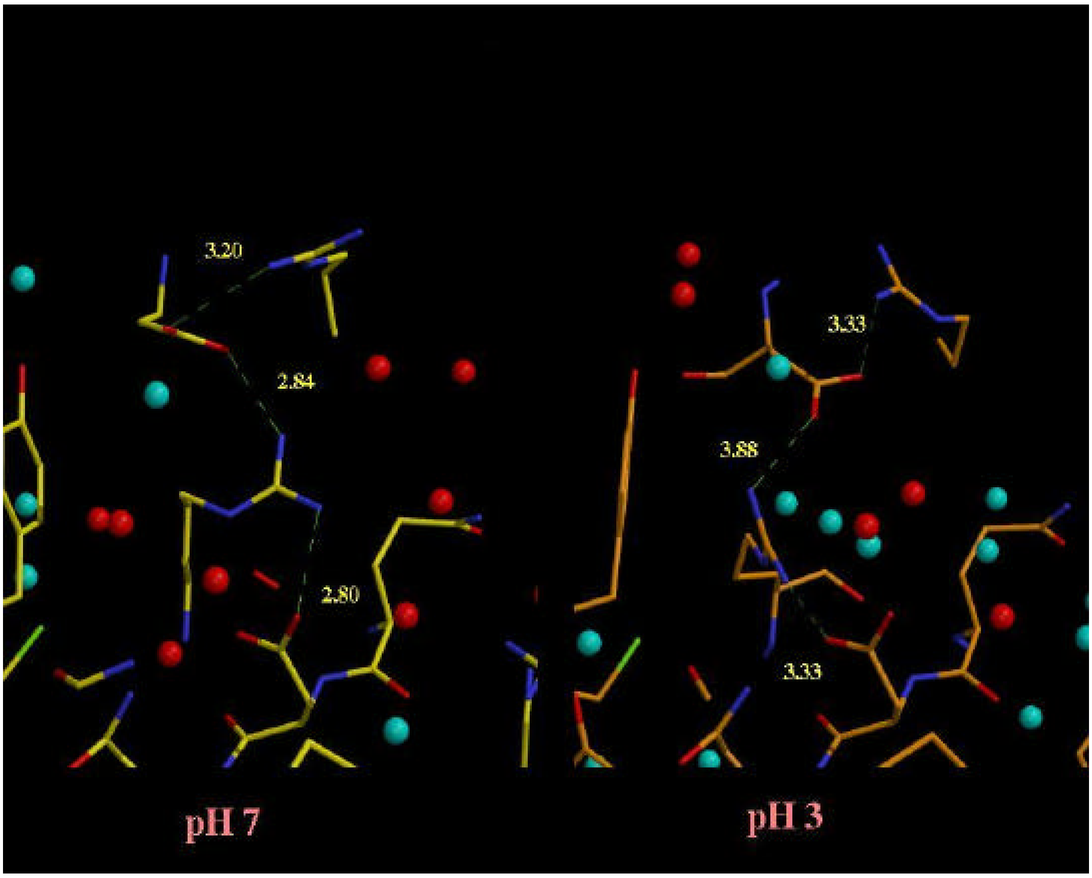

Fig 1. Comparison of potentially charged residues at the protein surface at pH=7 (left) and at pH=3 (right). The neutralization of Asp and Glu destroys the salt bridges and alter the position of side chains.

The complex protein kinase CK2 with the inhibitor quercetin.

Data were measured at line ID14-3, using one crystal and a rotation of 1 degree, for a total of 160 frames.
Statistics on data processing are the following:

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 N 1/d^2   Dmin  Rfac  Rfull  Rcum   Ranom      N   Av_I   SIGMA  I/sigma  sd  Mn(I)/sd Nmeas  Nref  Ncent

 1 0.0277  6.01 0.079  0.017  0.079  0.000      0  142106. 19166.6   7.4  9187. 22.4    1969    721    63  -0.305
 2 0.0554  4.25 0.088  0.141  0.086  0.000      0  189411. 28270.6   6.7 12994. 22.5    4612   1550    84  -0.228
 3 0.0831  3.47 0.088  0.087  0.087  0.000      0  173932. 27035.6   6.4 13270. 20.7    6498   2051    94  -0.220
 4 0.1108  3.00 0.097  0.091  0.089  0.000      0   93644. 15825.5   5.9  9024. 16.6    8056   2480   103  -0.234
 5 0.1385  2.69 0.121  0.086  0.093  0.000      0   43623.  8721.2   5.0  6022. 12.9    9129   2790   105  -0.229
 6 0.1662  2.45 0.153  0.099  0.097  0.000      0   23913.  5773.0   4.1  4959. 10.2   10045   3058   103  -0.217
 7 0.1939  2.27 0.184  0.129  0.102  0.000      0   18540.  5467.8   3.4  5071.  8.3   11068   3355   103  -0.253
 8 0.2216  2.12 0.226  0.140  0.107  0.000      0   13938.  4999.2   2.8  5207.  6.5   11698   3540   105  -0.306
 9 0.2493  2.00 0.277  0.237  0.111  0.000      0    9640.  4197.7   2.3  5102.  4.9   12392   3770   110  -0.298
10 0.2770  1.90 0.375  0.244  0.115  0.000      0    5719.  3368.4   1.7  4700.  2.9    9418   3334    91  -0.169


Overall:        0.115  0.104  0.115  0.000      0   49690.  12448.4  4.0   6671. 10.5     84885  26649 961  -0.235
                Rfac   Rfull  Rcum  Ranom    Nanom   Av_I   SIGMA   I/sigma  sd  Mn(I)/sd  Nmeas  Nref  NceNT


  N  1/d^2    Dmin    Nmeas    Nref   Ncent  %poss  Cm%poss  Mlplcty  AnomCmpl AnomFrc  Rmeas   Rmeas0  Rsy

  1  0.028    6.01      2082     834     95    88.0    88.0     2.5     0.0     0.0     0.100   0.100   0.079   0.102
  2  0.055    4.25      4708    1646    116    97.2    93.9     2.9     0.0     0.0     0.108   0.108   0.088   0.114
  3  0.083    3.47      6574    2127    119    98.9    96.2     3.1     0.0     0.0     0.107   0.107   0.088   0.121
  4  0.111    3.00      8143    2567    129    99.8    97.4     3.2     0.0     0.0     0.116   0.116   0.097   0.134
  5  0.139    2.69      9236    2897    130    99.9    98.2     3.2     0.0     0.0     0.145   0.145   0.121   0.168
  6  0.166    2.45     10165    3178    131    99.8    98.6     3.2     0.0     0.0     0.184   0.184   0.153   0.215
  7  0.194    2.27     11210    3497    129    99.8    98.8     3.2     0.0     0.0     0.220   0.220   0.184   0.262
  8  0.222    2.12     11853    3695    129    99.6    99.0     3.2     0.0     0.0     0.270   0.270   0.226   0.332
  9  0.249    2.00     12567    3945    136    99.4    99.0     3.2     0.0     0.0     0.331   0.331   0.277   0.429
 10  0.277    1.90     10048    3964    114    95.2    98.5     2.5     0.0     0.0     0.468   0.468   0.375   0.630


          Overall     86586   28350   1228    98.5    98.5       3.1        0.0     0.0    0.139   0.139   0.115
                      Nmeas   Nref    Ncent   %poss  Cm%poss   Mlplcty   AnomCmpl AnomFrc  Rmeas  Rmeas0   Rsym
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Report updated on: 25 September 2001