Protein Crystallization

Yan Haoming

October 25
November 1
2024

1 Configuration of Influential Factors

The process of developing protein crystals is influenced by many factors. In this experiment, we changed pH, salt concentration(NaCl) and protein concentration(lysozyme).

The configuration of those Influential factors can be seen in Figure 1. The cells in each row are in the same condition serving as the parallel groups. The first four rows differ in salt concentration and same goes for the last four rows. The pH is different between the first four rows and the last four rows as a whole. In each cell, left drop has small protein concentration while right drop is more condensed.

2 Observations of Crystals

The pictures of crystals discovered by us are shown in Figure 3. And schematic diagram of the distribution of them with the varied conditions can also be seen in Figure 1.

3 Analysis

3.1 Precipitant

Precipitants play an important role in protein Crystallization [2]. In this experiment, we used inorganic salt (NaCl) to be the precipitant. It is commonly said that precipitation ability of salts is mainly affected by anions like Cl^- because the strongly hydrated anions may lead to protein precipitation. Meanwhile, some articles [2] also point out that the cation like (Na^+) would influence the compactness and structure of lysozyme dimer and octamer, and therefore influences the crystallization.

As we had learnt from class, protein has the property of salting out. We increased the salt concentration(Figure 1), there must be a decrease in lysozyme solubility.

As we can see in Figure3, the crystals in D group were much larger than group A and B. Meanwhile, the crystals in G group had a higher success rate than E and F group which were composed of mainly impurities.

I find the observations corresponds well with the theory of salting out and the solubility changing rules both in higher and lower pH.

3.2 pH

The pH influences the process of crystallization mainly via residues [1]. Among the common 20 amino acids, Asp, Glu are negatively charged while Arg, Lys, His are positively charged. The charges are produced by carboxyl ($-COOH$) and amino ($-NH_2$) groups which will be significantly influenced by pH. The logic can be seen in Figure 2.

In our experiment, the Group E to H performed better than Group A to D averagely (larger amount and size with even shape), which means slightly higher pH is more benefit for lysozyme to crystalize.

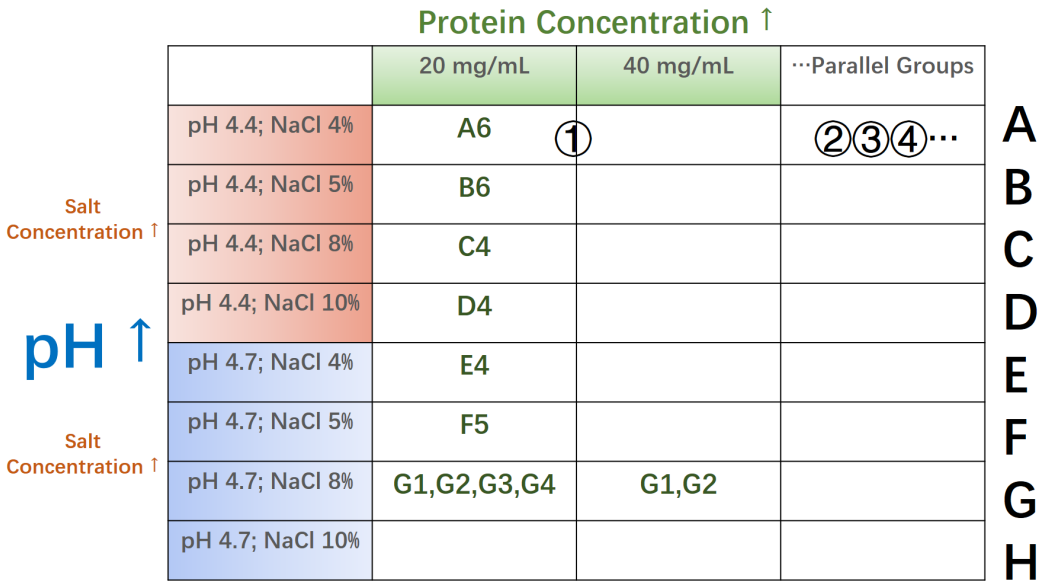


Figure 1: Configuration of Plate and Distribution of Crystals

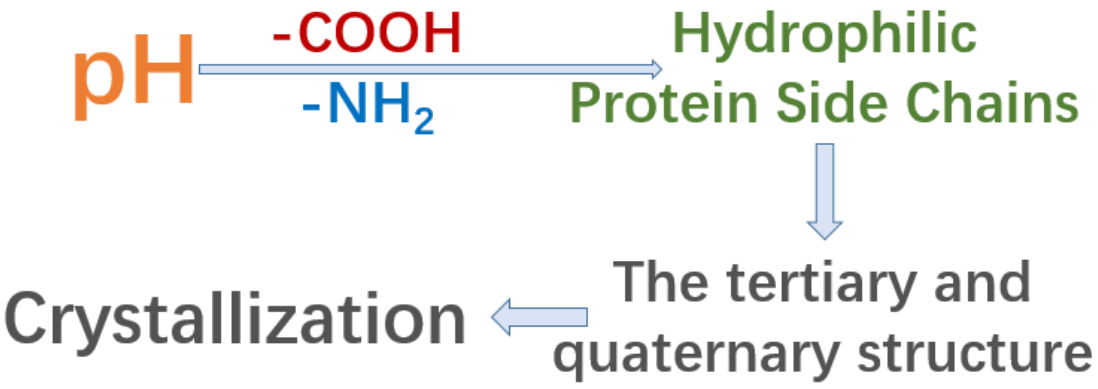


Figure 2: The Influence of pH

3.3 Protein Concentration

In our configuration of the condition, the left cell had lower protein concentration while the right one was higher. However, just G1 and G2 were observed with crystals on the right (higher protein concentration). Thus I suspect relatively low concentration is benefit to crystallization. This view point was confirm by the cited article [2] where the scientists said the transition from non-crystal to single crystal and finally urchin-like crystal corresponds to the increase of both protein concentration and salt concentration.

3.4 Other Aspects

The overall success rate was relatively low, which led to fewer paralleled groups. However, from group G we can conclude that even the experimental parameters were the same, the boundary of crystals can still be different. This phenomenon indicates that the operational factors also contribute to the diverse results significantly.

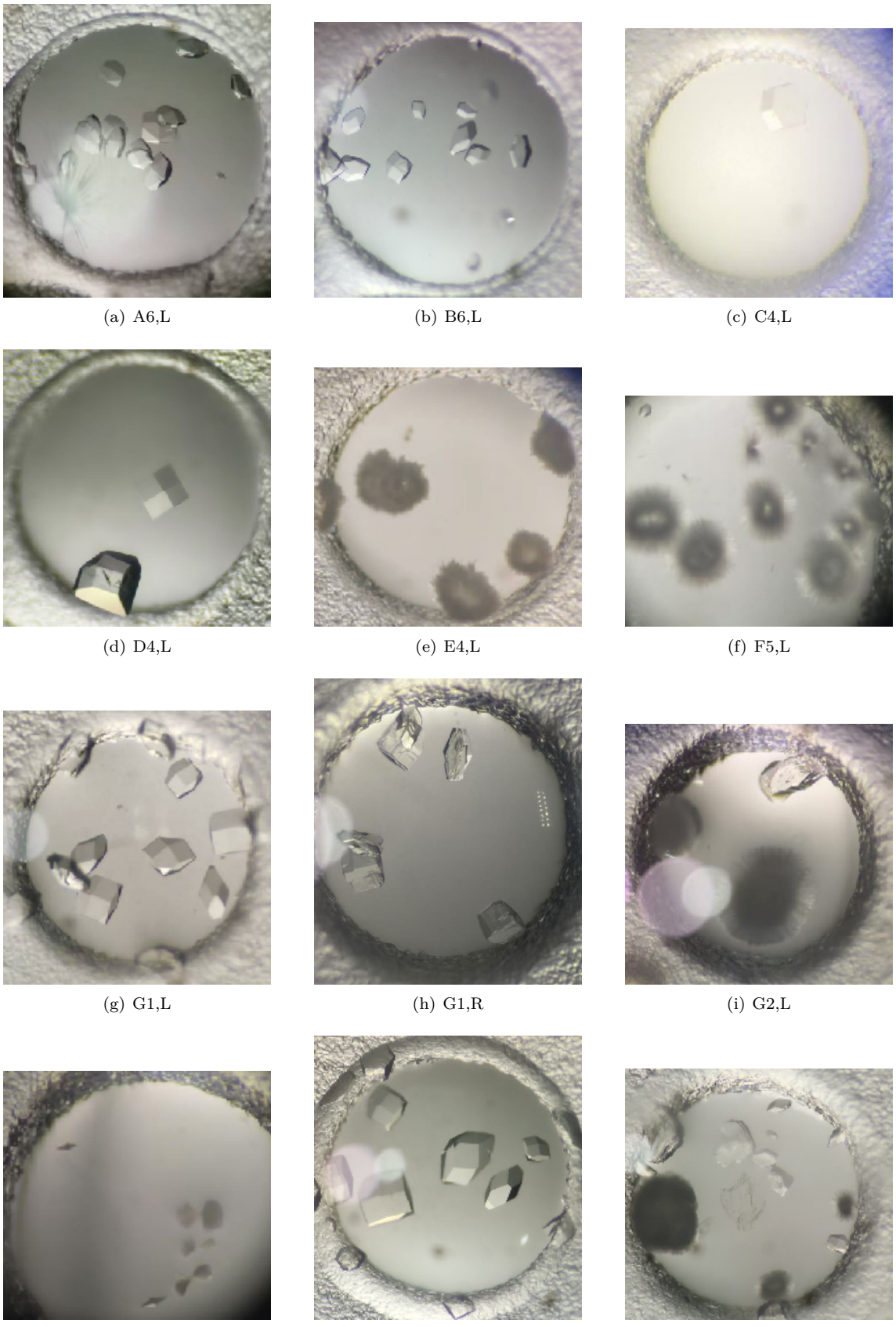
4 Conclusion

In this experiment we studied the protein crystallization process with three influential factors: salt concentration, pH and protein concentration. Relatively speaking, lower protein concentration (20 mg/mL), higher salt concentration (8% to 10%) and slightly higher pH (4.7) should be used for lysozyme in the pursuit of regular single crystals.

References

[1] Wikipedia contributors. Protein crystallization. `https://en.wikipedia.org/wiki/Protein_crystallization`, 2024. Accessed: 2024-11-10.

[2] Xuntao Jiang, Yuxiao Zhang, Xia Wu, Hui Guo, Haozhe Sun, Hua He, and Xiaoxi Yu. The regulation of protein crystallization using choline chloride precipitant and its mechanism. *Journal of Molecular Liquids*, 390:123187, 2023.



(a) A6,L (b) B6,L (c) C4,L

(d) D4,L (e) E4,L (f) F5,L

(g) G1,L (h) G1,R (i) G2,L

(j) G2,R (k) G3,L (l) G4,L

Figure 3: Observations of Crystals