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РОЛЬ ИОНОВ КАЛЬЦИЯ В ПРОЯВЛЕНИИ КАТАЛИТИЧЕСКОЙ АКТИВНОСТИ ФОСФОЛИПАЗЫ D**РАХИМОВ М. М., КАЛЕНДАРОВА Т. Н., РАШИДОВА С. Ш., МАДЪЯРОВ Ш. Р.**

Изучены особенности реакции расщепления мисцеллярного лецитина фосфолипазой D и выявлена роль металла в проявлении каталитической активности фермента. Показано, что Ca^{2+} участвует в образовании каталитически активного промежуточного фермент-субстратного комплекса со строгой стехиометрической соотношением субстрата, фосфолипазы, активатора (DS-Na) и ионов кальция. Исследована кинетика торможения процесса ионами дистата, ионами фтора и ЭДТА. Показано, что торможение реакции в системе лецитин — DS-Na ионы Ca^{2+} — фосфолипаза D ионами дистата обусловлено заменой двувалентного Ca^{2+} трехвалентным La^{3+} в составе промежуточного комплекса. Ингибирующее действие ионов фтора связано с внедрением электроотрицательного иона в координационную сферу промежуточного комплекса и нарушением гидрофобного связывания между лигандами. Ингибирующее действие ЭДТА проявляется на стадии связывания белок — субстрат и не связано с хелатирующей способностью ЭДТА в отношении Ca^{2+} . Показано, что роль ионов кальция не ограничивается его связыванием с фосфолипазой D. Ионы кальция необходимы также для нейтрализации избыточного заряда на поверхности субстратных мицелл, создания необходимой ионной силы раствора и модификации поверхности субстратной фазы на границе раздела фаз. Каталитическая функция кальция выражается в формировании участка «узнавания» активного центра фосфолипазы D.

В настоящее время накопилось достаточное количество данных, свидетельствующих о том, что в проявлении каталитических свойств липолитических ферментов наиболее существенными являются состояние и ориентация липидов и фосфолипидов на границе раздела фаз, а также формы, размеры и физико-химическое состояние поверхности липидных образований. Результаты опубликованных до сих пор работ можно разделить на следующие группы, полученные из экспериментов: 1) с водорастворимыми субстратами [1—5]; 2) с «неорганизованными» липидными субстратами (обычно, водные дисперсии) [2, 6—9]; 3) с ламеллами, образованными в водно-органических смесях [7—10]; 4) с обращенными мицеллами фосфолипидов в органических растворителях [11, 12]; 5) с липосомами, полученными обработкой фосфолипидов ультразвуком [9, 13—15]; 6) с мицеллами липидов и фосфолипидов с детергентами [8, 9, 13—15]; 7) с искусственными бимолекулярными мембранами и мембранами митохондрий, микросом, эритроцитов и т. д. [9, 16, 17]. Естественно, что кинетические закономерности во всех перечисленных выше случаях различны. Наиболее информативные в этом отношении данные получены в опытах с монослоями липидов и фосфолипидов [18], а также в исследованиях с обращенными мицеллами фосфолипидов [11].

Во всех перечисленных публикациях обращается внимание на то, что в проявлении каталитической активности фосфолипаз существенным является наличие в среде ионов металла, преимущественно кальция.

Имеются данные, что одна из основных функций кальция не сводится к простому связыванию с ферментом, а заключается в обеспечении необходимого фазового состояния субстрата [9]. На примере фосфолипазы A_2 показано также, что Ca^{2+} помимо участия непосредственно в каталитическом процессе может выполнять и определенные некаталитические функции, природа которых пока неясна [11].

В настоящей работе приведены результаты по выяснению роли кальция в проявлении каталитической активности фосфолипазы D при изучении системы фосфолипаза D — смешанные мицеллы лецитина и DS-Na .

Rol Ionov Kaltsiia V Organizatsii I Perestroike Tsirkadiannykh Ritmov Endokrinnykh Funktsii

Clemens Wendtner



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